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REVIEW

Use of lure sticks for non-invasive genetic sampling of European wildcat populations: lessons learnt and hints for future insights

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ABSTRACT

1. Non-invasive genetic sampling is an increasingly common approach in wildlife research. It allows the gathering of first-hand data on wild mammalian populations without capturing or handling individuals. For this reason, it has proved to be particularly useful when applied to elusive species living at low population densities and/or hard to identify in the field.
2. The European wildcat represents an interesting case study in this respect.
3. Several papers have been produced in the last decades, in which non-invasive genetic sampling has been applied. Nevertheless, evidence from different case studies presents a complex scenario, where the efficiency of the method can vary considerably.
4. This paper aimed to analyse possible interpretations of such differences and to identify potential drivers and barriers. 20 papers on the subject have been reviewed and compared, although differences in several details reported in the examined papers limited an in-depth comparison.
5. The review showed that the overlap of the study period with the reproductive season does not affect the final results of lure stick hair sampling research on the European wildcat. Moreover, valerian lure sticks generally provided positive results in the Continental ecoregion, whereas, in the Mediterranean and Atlantic regions, outcomes were absent or very scarce.
6. Most of the other working hypotheses remain still plausible, despite not yet being definitely provable. Setting up future wildcat monitoring schemes based on effective non-invasive genetic sampling in different biogeographical regions of Europe is certainly a scope to be pursued.
7. Some suggestions are provided in this respect (e.g. the set of parameters needed to allow further comparisons; the need to test other types of attractants, to make the application of the method possible where the use of valerian was proven to be inefficient or scarcely efficient, in order to allow a better comparison of future results).

INTRODUCTION

The European wildcat is a medium-sized carnivoran included in 'Annex IV' of the European Habitats Directive (92/43/CEE) and in 'Annex II' of the Bern Convention, and classified as Least Concern on the IUCN Red List of Threatened Species (Gerngross et al. 2022). However, it is considered a protected or 'strictly protected' species by most European countries' national laws. The main threats to species conservation are the loss of suitable habitats (Klar et al. 2009, 2012), hybridisation with the domestic cat (*Felis catus*; Mattucci et al. 2013, 2019), road kills and other human-related mortality (Bastianelli et al. 2021). Wildcat distribution is currently expanding in different parts of its European range (Sordello 2012, Gilles 2015, Catello et al. 2021, Gerngross et al. 2021), whereas some populations still show decreasing trends (Gerngross et al. 2022).

The resemblance of the European wildcat to the domestic striped tabby cat, the occurrence of the latter in the wild, together with possible crossbreeding events that generate fertile hybrids, make an identification based only on phenotypic characters (Ragni & Possenti 1996, Kitchener et al. 2005) potentially misleading. A recent paper (Sforzi & Lapini 2022) suggests a method to classify wildcat observations from pictures or videos from the visual documentation most commonly available.

Non-invasive genetic sampling may overcome the limitations of phenotypic identification, potentially providing genetic insights on the studied populations while simultaneously increasing the number of C1 observations (i.e. those that combine wild phenotypic traits and genetic confirmation; Sforzi & Lapini 2022). This set of methods has the main advantage of gathering information on a wild population with no need to capture or handle individuals (Hupe & Simon 2007), and it has proved to be particularly useful for rare species and for taxa that are difficult to identify in the field (Burki et al. 2010), such as the European wildcat.

Originally, Hupe and Simon (2007) set up a hair sampling method using wooden sticks and valerian lures in Lower Saxony, Germany, to attract wildcat individuals, stimulate rubbing behaviour and collect hairs to be genetically analysed. The same method was then further developed by Steyer et al. (2013) in the Kellerwald-Edersee National Park (Germany) and, since then, an increasing number of researchers in different countries have carried out non-invasive monitoring programmes on the species to investigate its presence, distribution and genetic status.

As pointed out by Velli et al. (2015), the simultaneous use of lure sticks, camera traps and hair genetics (microsatellite, SNPs analysis and mtDNA sequencing from hair samples) can considerably increase the efficiency of wildcat detection and the quality of collected data. The use of camera traps associated with lure sticks allows the observation of wildcat behaviour towards the lure, to possibly recognise individual specimens that stay longer in the visual field and to obtain images of individuals not interested in the attractant, which could not be detected by means of hair sampling alone. Pictures or videos taken during the rubbing behaviour provide evidence of the phenotypic traits of the sampled individuals, allowing a match with genetic results. Furthermore, the subsequent genetic work is facilitated by the possibility of immediately discarding any samples belonging to non-target species.

Therefore, this combination of methods is a sensitive tool, which not only enables the detection of wildcat presence but also potentially allows individual identification, providing insights into the conservation status and population dynamics of the species (Velli et al. 2015). Overall, a comprehensive non-invasive wildcat sampling method can therefore be considered as composed of three different elements: camera traps, lure sticks and genetic analyses.

Camera traps are flexible and increasingly powerful tools widely used in the field. In particular, after the development and diffusion of modern digital camera traps since the mid-2000s, they became a standard instrument, professionally used for scientific research and ecological inference in a constantly growing number of studies (Rovero & Zimmermann 2016, Wearn & Glover-Kapfer 2017).

On the genetic side, highly informative molecular markers combined with advanced statistical approaches have been set up to reliably investigate the evolution of European wildcat populations in Europe and their conservation status (Mattucci et al. 2013, 2019). Recent studies showed that the employment of thousands of markers might help to unveil previously undetectable backcrosses, estimate the timing from admixture events (Galaverni et al. 2017) and allow researchers to better understand the dynamics of anthropogenic hybridisation. The limited success of genetic analysis conducted on hair samples collected in the field can pose some constraints on the wide application of the method, being dependent on several factors that might influence genotyping success, such as environmental factors degrading hairs' DNA or contamination of samples.

To maximise genetic sample collection, the use of lure sticks takes advantage of the chemical properties of valerian (*Valeriana officinalis*), a perennial herbaceous plant native to Europe and Asia, commonly used in herbal medicine for its calming properties. Valerian is known to exert an attractive effect on both wildcats (Monterroso et al. 2011) and domestic cats (Bol et al. 2017); this response is thought to be due to a high concentration of actinidin, an iridoid that can cause effects similar to that of nepetalactone contained in catnip (*Nepeta cataria*; Tucker & Tucker 1988, Patočka & Jakl 2010, Bol et al. 2017). Although its mechanism of action has not been clarified yet, it is believed that the smell of valerian imitates glandular secretions of some carnivores, potentially attracting mesocarnivores (Ferreras et al. 2018). In susceptible wildcat individuals, valerian stimulates a rubbing behaviour (with head, neck, but also the entire body) on the treated object, according to some authors, especially during the reproductive period (Hupe & Simon 2007). This distinctive feature has been used to obtain hair samples to be genetically analysed with no need to physically capture animals, thus allowing easier population monitoring (Steyer et al. 2013).

The employment of valerian lure sticks for non-invasive wildcat hair sampling shows wide variability in its effectiveness and applicability. Several papers on the subject (e.g. Hupe & Simon 2007, Steyer et al. 2013, Kilshaw et al. 2014, Velli et al. 2015, 2021) provided quite different results, with a level of efficiency variable from 100% to almost 0.

This paper aims to identify the underlying factors causing those differences, through the identification and analysis of the main variables involved. Individual variation in the response to valerian lures is known, but to our knowledge, factors affecting the efficiency of the method have never been investigated so far. As a matter of fact, despite general differences in the outputs of the different studies being known among the community of specialists in Europe, a detailed comparison of the available bibliography, looking at possible drivers behind those differences, has never been performed. This paper represents the first attempt to focus on a set of possible explanatory hypotheses and to produce recommendations for a standardised sampling protocol aimed at a more extensive application of the method in the future, including the environmental contexts where it has not yet produced valuable data.

Working hypotheses

The response of wildcats to the attractant varies in different contexts. In some areas, mainly in Central Europe, the success rate is generally high, whereas in other areas, the use of valerian associated with lure sticks has not given the expected results. The application of the method in Scotland (Kilshaw & Macdonald 2011, Kilshaw et al. 2014), Sicily (Anile et al. 2012) and the Iberian Peninsula (Monterroso et al. 2014) did not apparently produce any valuable results. In another environmental context, specifically in the Casentinesi Forests National Park (Italy), quite different results were obtained in the same area in two subsequent studies, which recorded positive responses to the attractant, respectively, by 20% and 69% of the specimens observed by camera trapping (Velli et al. 2015, 2021). This highly variable effectiveness in different studies calls for an explanation. Starting from the diverse set of information currently available, the following hypotheses were developed:

H1: Some populations of European wildcats might be genetically more 'predisposed' to respond to valerian, whereas others would be almost insensitive (Anile et al. 2012, Kilshaw et al. 2014). While for valerian there are no specific studies on the mechanism behind its attractiveness, it could be similar to catnip in domestic cats, where the ability to respond to the attractant is known to be inherited as an autosomal dominant gene expressed in two-thirds of domestic cats (Todd 1962, Tucker & Tucker 1988).

H2: The effectiveness of valerian as an attractant could be limited to the breeding season, as reported in literature (Hupe & Simon 2007), so investigations carried out outside this period would be less successful.

H3: Climatic factors could influence the outcome of the study. For example, in contexts with a Mediterranean climate, valerian tincture or other valerian-based solutions could quickly evaporate, significantly limiting the duration of the possible attractive effect, whereas in colder areas, characterised by a Continental climate (e.g. Switzerland and Germany) with higher humidity, this aspect could play a minor role or be negligible.

H4: The sampling success with valerian lures could depend on the population density of the studied wildcat population. Hartmann et al. (2013) showed a high success rate in an area characterised by a known high population density, whereas in a different area with a lower population density, limited success was reported, albeit greater than the results obtained by Steyer et al. (2013) in a recently colonised area (where the population could be possibly considered even smaller).

H5: The type of valerian-based attractant used (e.g. formulation and/or concentration) could influence the result (e.g. >concentration may imply >efficacy). In most of the analysed papers, 70% concentrated valerian tincture was employed, whereas in some cases, different formulations or concentrations were used.

H6: The density of hair traps (lure sticks) in the field could influence the sampling success: Hupe and Simon (2007) and Weber et al. (2008) suggest using 0.2–0.5 traps/km^2 and 3 traps/km^2 respectively. This value is rather variable in other studies (Table 2).

MATERIALS AND METHODS

A detailed literature search on non-invasive wildcat genetic sampling in Europe was performed using Web of Science and Google Scholar databases, with the following combinations of keywords: '*Felis silvestris* valerian', 'European wildcat valerian' and 'European wildcat hair traps'. No constraints on location, language, publication date or type were applied to the search. Papers, reports and posters were selected that involved the application of the field method based on valerian lure sticks for the collection of wildcat hair samples. The papers of Belaud et al. (2021) and Viviani et al. (2024), which used valerian on a different support (metal brushes) in France and Italy, respectively, were also added to the sample.

From each scientific paper, information was extracted concerning geographical area, study period, sampling effort and type of attractant used (Table 1). In all those cases where some details were not directly reported in the publications, the authors were contacted by email to receive clarifications and additions.

In order to test the different hypotheses described above, concerning the varying efficacy of valerian as an attractant, an attempt was made to fit a Tweedie generalised linear model with a log

Table 1. Parameters extrapolated from the examined papers. These details are needed for a comprehensive overview of the non-invasive sampling method implementation and to enable more in-depth comparisons in the future

Variable category	Parameter	Description
Geographic descriptors	Ecoregion	Biogeographical region where the study area is located (European Environment Agency 2016)
	Latitude	Mean latitude of the study area
	Altitude	Mean altitude of the study area (m.a.s.l.)
Genetic descriptor	Biogeographic group	Genetically defined biogeographic group to which the studied population belongs (sensu Mattucci et al. 2016)
Sampling design	Lure type	Type of valerian-based attractant used
	n lure sticks	Number of lure sticks used
	Area	Extension of the study area (km^2).
	Distance	Average distance between lure sticks (km)
	Trap density	Density of stakes in the study area (*n*/km^2)
Temporal descriptors	Period	Study period (months)
	Semester	Differentiation of the study period according to the season (cold, mixed and warm)
	Mating/not mating	Study period in reference to the breeding season of the European wildcat (mating, not mating or both)
	Trap nights	Total trap nights, calculated as (*n* study nights * *n* traps)
	Trap nights CTs	Total nights-traps as far as camera traps are concerned, in cases where camera traps have been used together with the hair camera traps
Main results	*n* samples	Total number of wildcat samples obtained (morphologically identified by researchers from the camera trap captures)
	Wildcat capture success	Hair capture rate [(*n* capture events * trap nights)/100].
	n samples genotype	Total number of hair samples of *Felis silvestris silvestris* from which it was possible to perform genetic analyses and obtain the genotype.
	Positive stations	Percentage of lure sticks that performed a wildcat hair capture
	N images	Number of wildcat photos or videos obtained
	Image capture success	Photo/video capture rate

link function using glmmTMB function (glmmTMB package, Brooks et al. 2017) in R 4.3.2 (R Core Team 2023). Tweedie error distribution combined with log link function can be used when the response variable is continuous and non-negative, such as densities or rates (Klatt et al. 2020, Pack et al. 2022, Fattorini et al. 2023). Hair capture success rate was used as response variable and several explanatory variables were identified (i.e. Biogeographic group, mating/non-mating period, ecoregion, semester, lure type and trap density) based on the working hypotheses to be tested.

RESULTS

A total of 20 papers were identified, dealing with non-invasive genetic sampling of European wildcats. Their year of publication ranged between 2007 and 2022. Study areas covered nine different European countries (Italy, Spain, France, Scotland, Luxembourg, Switzerland, Germany, Austria and Romania, Fig. 1). The total duration of field sampling ranged between 48100 and 500 trap nights; study areas also varied in size, between 3719km^2 and 4–5km^2. Table 2 summarises data collected and used for the subsequent analyses. In some publications, the success rate was calculated considering only the number of hair samples from which the genotype was obtained (e.g. Kéry et al. 2011, Steyer et al. 2013, Velli et al. 2015). To overcome this lack of data, authors of the papers were contacted to integrate the dataset according to the specific gaps to be filled. Table 2 summarises the data collected from the literature and the information gathered from the authors used to integrate initial gaps.

Preliminary analyses of the collected data suggest that the examined method generally gives positive results in the Continental ecoregion, whereas in the Mediterranean and Atlantic regions, outcomes are usually negative or with few positive results (Fig. 2).

DISCUSSION

The general picture

Various studies in Europe have applied non-invasive genetic sampling to wildcat monitoring with different objectives, including (a) estimating population density using capture–recapture models (Kéry et al. 2011), (b) studying the use of wildlife bridges for crossing highways (Pir et al. 2011), (c) analysing the effect of potential ecological barriers on the local population (Hartmann et al. 2013), (d) monitoring the introgression of domestic genes into wild populations (Nussberger et al. 2014) and (e) studying the distribution and spatial overlap between wildcats and domestic cats

Fig. 1. Geographical distribution of the papers taken into account in the current review.

(Beutel et al. 2017). In addition, it was possible to detect wildcat presence in a recently colonised low-density area (Catello et al. 2021) and to identify a breeding population of European wildcats where the species was considered extinct (Gerngross et al. 2021).

However, even though the cited studies had different final aims, the core structure of the hair sampling method was similar, hence allowing a comparison between different studies. Minor differences have been taken into account and described above.

Critical evaluation of our hypotheses

Although one of the original aims of this work was to test the different hypotheses described in the introduction section, the overall limited amount of available data and the difficulty of gathering uniform information made this objective hardly reachable. Despite the statistical analyses were generally not sufficiently robust to extrapolate accurate results, in the case of H2, our findings clearly indicate that the hypothesis is not supported.

Valerian is generally thought to exert its attractive effect on cats mainly during the breeding season (Hupe & Simon 2007, which is the basis for H2). Nevertheless, in several cases, the results found in the literature contradicted this statement. Dietz et al. (2016) in Germany successfully collected samples until the end of April and Pir et al. (2011) in Luxembourg obtained a high number of samples between the end of March and the end of May, as Velli et al. (2015) did in the Casentinesi Forests (Italy). Also, Gerngross et al. (2021) in Austria showed a peak in hair sample captures between the end of April and May. Moreover, the study period of Belaud et al. (2021), in Southern France, ranged between September and November, with quite a high hair sampling success. Therefore it is plausible to conclude that H2 cannot be supported. The overlap of the study period with the reproductive season does not apparently affect the final results.

The results shown in Fig. 2 seem to suggest that climate affects the effectiveness of valerian lures (hypothesis H3). On one hand, a warmer and drier climate, such as the Mediterranean climate, could make the valerian-based solution evaporate faster in comparison with the climate typical of Continental Europe, with the consequence that the odour will fade more quickly, resulting in a lower attractive effect. On the other hand, more frequent rainfall typical of the Atlantic climate could quickly wash the attractant away, resulting in a similarly lower attractive effect compared with the Continental region. Detailed weather information associated with future studies might help to shape this hypothesis further.

Nevertheless, a difference in outcomes among biogeographical regions (Fig. 3) might also suggest that the ability to respond to valerian is genetically determined (hypothesis H1). The populations of Continental Europe, genetically distinguishable from the others (Mattucci et al. 2016, Velli et al. 2023),

Table 2. Information extracted from selected papers. Numbers in blue were calculated from the information available in the papers or personally communicated by the authors

Reference	Eco region	Biogeographic group	Latitude	Lure_type	Semester	Mating/ non- mating	Trap_nights	*N*_samples
Hupe (2007)	Continental	4	52	Valerian_tincture	Mixed	Both	7680	24
Weber et al. (2008)	Continental	4	47	Valerian_tincture	Mixed	Both	48100	525
Pir et al. (2011)	Continental	4	49	Valerian_tincture	Mixed	Both	11638	122
Anile et al. (2012)	Mediterranean	2	37	Valerian_tincture	Cold	Non-mating	1080	0
Steyer et al. (2013)	Continental	3	51	Valerian_oil	Mixed	Both	35300	33
Hartmann et al. (2013)	Continental	4	50	Valerian_oil	Cold	Mating	17100	68
Kilshaw et al. (2014)	Atlantic	NA	57	Valerian_tincture	Warm	Non-mating	500	0
Littlewood et al. (2014)	Atlantic	NA	57	Valerian_tincture	Cold	Both	7499	12
Monterroso et al. (2014)	Mediterranean	5	38	Valerian+Lynx_ urine	Mixed	Both	NA	0
Nussberger et al. (2014)	Continental	4	47	Valerian_tincture	Cold	Both	9450	91
Carotenuto et al. (2014)	Mediterranean	2	42	Valerian_tincture	Mixed	Both	6200	0
Velli et al. (2015)	Continental	2	43	Valerian_tincture	Mixed	Both	8415	19
Brix et al. (2016)	Continental	NA	46	Valerian_extract	Mixed	Both	8200	95
Dietz et al. (2016)	Continental	4	50	Valerian_tincture	Cold	Both	11500	49
Beutel et al. (2017)	Continental	3	48	Valerian_oil	Cold	Both	880	9
Belaud et al. (2021)	Continental	4	43	Valerian_oil	Cold	Non-mating	2128	17
Cascini et al. (2021)	Mediterranean	2	39	Valerian_tincture	Mixed	Both	1416	0
Catello et al. (2021)	Alpine	1	46	Valerian_tincture	Cold	Mating	727	6
Gerngross et al. (2021)	Continental	3	48	Valerian_tincture	Mixed	Both	5725	32
Velli et al. (2021)	Continental	2	43	Valerian_tincture	Warm	Non-mating	4170	39
Viviani (2022)	Continental	2	44	Valerian_oil	Cold	Both	1370	0

could be more predisposed to be attracted. Thus, separate phylogeographical groups may react differently due to both climatic reasons and genetic predisposition.

The results of Littlewood et al. (2014) in Scotland introduce further uncertainties in support of H1 hypothesis. Although Kilshaw and Macdonald (2011), in their previous study, had not obtained any results with the use of valerian and hypothesised that the Scottish wildcat population was not able to react to it, Littlewood et al. (2014) managed to obtain some hair samples using the same method. However, in this particular case, the response to attractants should be considered with caution since the current wild-living cat population within Scotland consists of a genetic continuum between wildcat and domestic cat (Senn et al. 2019).

The response to valerian-based solutions could also vary on an individual basis, as it was proved for domestic cats. Bol et al. (2017) found that only 40% of examined domestic cats showed an intense response to valerian and 7% a weak response, whereas 53% expressed no interest at all. Similarly, Velli et al. (2015) found that in 51.7% of cases, wildcats that were observed through camera trapping showed no reaction to valerian tincture. Therefore, each population may not be entirely sensitive or entirely insensitive to valerian, but only a certain percentage of individuals within the population itself may be attracted to it. However, testing this hypothesis, especially in field conditions and on a solitary species that normally lives at low population densities, poses objective difficulties.

Population density is another important factor that likely affects the results of the study. One can postulate that in a densely populated area, sampling is expected to be more successful (hypothesis H4). Indeed, Hartmann et al. (2013) in Germany achieved a high success rate in two regions where the population density was high, whereas the success was lower in another area where the abundance of wildcats was probably lower, albeit with better results than those of Steyer et al. (2013) in a recently colonised area, where wildcat population density was considered to be very low. A lower efficiency of valerian-based sampling has also been observed in southern Switzerland where, according to the results of the 2020 national wildcat monitoring, the population density was lower than in the northern areas of the country (B. Nussberger, personal communication). Nevertheless, an estimate of wildcat population density is not always available and it is often calculated using different methods (e.g. snow tracking, trapping, radio telemetry, camera trapping, etc.), making a comparison between the obtained values very difficult. Hence, the estimates may show a high variability between different areas and periods.

Furthermore, the type of valerian-based attractant used in the analysed papers was also variable. This reflection was the basis of H5. In most cases, the attractant used was valerian tincture (70%), produced by various pharmaceutical companies and generally readily available. However, Steyer et al. (2013) and Beutel et al. (2017) used a 'valerian oil', while Kéry et al. (2011) used an unspecified 'valerian

Wildcat_ capture_success	*N*_samples_ genotype	*n*_lure_sticks	Area_km^2	Distance_km	Traps_density (/km^2)	Positive _ stations	Trap_nights_ CTs	*N*_images	Image_ capture_success
0.31	NA	39	187	NA	0.2	NA	0	NA	NA
1.09	136	132	66	0.5	2	32%	NA	268	NA
1.05	21	51	5	0.035	10	31%	0	NA	NA
0	0	18	10.9	1	1.6	0%	1080	44	4.1
0.09	25	71.2	43.6	0.5	1.7	6%	0	NA	NA
0.40	34	190	430	1.5	0.4	NA	0	NA	NA
0	0	20	20	1.15	1	0%	1960	8	0.4
0.16	8	356	712	1.4	0.5	2%	7499	58	0.8
0	0	67	120	1.4	0.5	0%	NA	NA	NA
0.96	23	405	3719	5	0.04	11%	0	NA	NA
0	0	18	41	NA	NA	0%	NA	NA	0.08
0.23	8	45	28	0.53	1.6	11%	819	25	3.1
1.16	26	48	80	1	0.6	NA	0	NA	NA
0.43	30	92	51	0.5	1.8	34%	0	NA	NA
1.02	8	10	53	2.4	0.2	20%	31107	19	0.06
0.80	NA	19	19	1	1	NA	2128	57	2.7
0	0	9	448	8	0.02	0%	1416	1	0.07
0.83	1	10	4	0.6	2.5	10%	641	5	0.8
0.56	32	28	12	NA	2.3	46%	5537	136	2.5
0.94	12	21	20	1	1.05	14%	3220	99	3.1
0	0	10	20	1	1	0%	1370	10	0.7

solution'. The study carried out in the Iberian Peninsula made use of a combination of valerian and lynx urine (Monterroso et al. 2014). Finally, Belaud et al. (2021) and Viviani et al. (2024) used a solution of valerian combined with sunflower oil (Belaud et al. 2021). Therefore, the variability of chemical formulation is quite high; differences in the attractiveness of valerian in different contexts might also arise from differences in the composition and/or concentration of the attractant being used.

Hypothesis H6 postulated that the density of hair traps (lure sticks) in the field could influence the sampling success of wildcats. Hupe and Simon (2007) in Germany and Weber et al. (2008) in Switzerland were the first ones who described and applied the method. In both publications, the authors report some guidelines to follow while applying the method to obtain a high probability of success, which were derived from the two cited works and previous studies carried out by the same authors in the two study areas. However, the data are partly discordant. For example, Hupe and Simon (2007) recommend the use of 0.2–0.5 sticks/km^2 in areas where a stable presence of the wildcat is known and 0.6–1.5 sticks/km^2 in potential new colonisation areas, while Weber et al. (2008) recommend using 3 sticks/km^2 to ensure an effective survey. A high density of traps in the study area should allow for easier detection of wildcats passing by and, therefore, increase the chances of success. The density of traps used in the papers that were analysed varied from 10/km^2 (Pir et al. 2011) to about 0.02/km^2 (Cascini et al. 2021). Indeed, the success rate is very high in the first case and equal to 0 in the second. However, the same difference could also be determined by many other collateral factors, such as the time elapsed between successive checks (therefore, the renewal rate of the attractive substance on the lure sticks), as well as climatic and/or genetic factors.

Further comments

Although olfactory communication plays an important role in the social life of felids, their sense of smell is not as well developed as that of other animals such as canids (Sunquist & Sunquist 2002). Therefore, increasing the density of traps means increasing the likelihood that an individual, even accidentally, will pass by one of them, at a sufficiently short distance to detect the valerian smell to be attracted by it. For the same reason, a fairly frequent renewal of the lure is arguably necessary to maintain a strong attractive effect.

In the study carried out by Cascini et al. (2021) in the Pollino National Park, Southern Italy, the lures treated with valerian were very far from each other and the time intervals between operators' visits to lure sites were quite far apart. Therefore, it cannot be excluded that the apparent lack of interest from wildcats was due to the way the method was applied in the field. A similar comment applies to the failure of the valerian sampling method in Sicily (Anile et al. 2012). The attractant, in this case, was presented using a piece of cork impregnated with valerian tincture and inserted in an untreated wooden stake. Owing to the poor absorption capacity of the material used, the released odour may not have been sufficiently intense to attract passing wildcats. In fact, these authors did not observe any reactions even from foxes and martens, which responded quite intensely and in a variety of ways in other areas of Italy (Viviani et al. 2024), Switzerland (Burki et al. 2010) and Greece (D. Migli, personal communication).

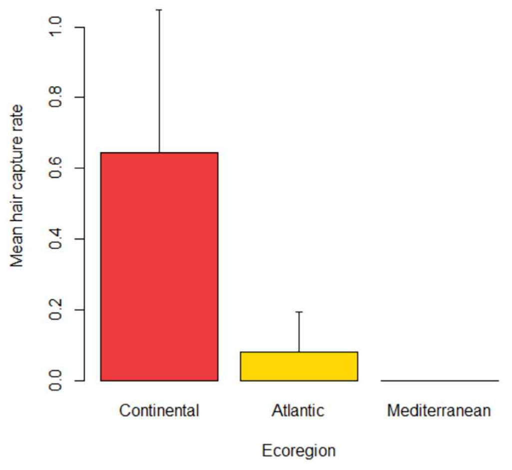

Fig. 2. Average hair capture success per biogeographic region. Alpine ecoregion is not shown since it consists of only one case study. The error bars indicate SD.

Finally, another bias could be at play. Studies with positive results are much more likely to be written up and published, whereas studies with negative results are much less likely to be published. From this perspective, the comparison between all the studies carried out proves to be less exhaustive and drawing conclusions on the reasons behind the different outcomes is much more complex. Remarkably, problems which could provide useful insights to avoid failures in other contexts are usually not totally disclosed (Patkó et al. 2016). As a matter of fact, most of the publications relating to the non-invasive genetic sampling of the European wildcat come from central Europe, where the method is generally particularly effective. Some studies carried out in other contexts mention tests carried out with the originally described method, with little or no results (e.g. Belaud et al. (2021) in the French portion of the Pyrenees, Migli et al. (2021) in northern Greece and Gil-Sánchez et al. (2020) in southern Spain), but since details of those negative results have not been published, it was not possible to include them in the analysis and subsequent comments.

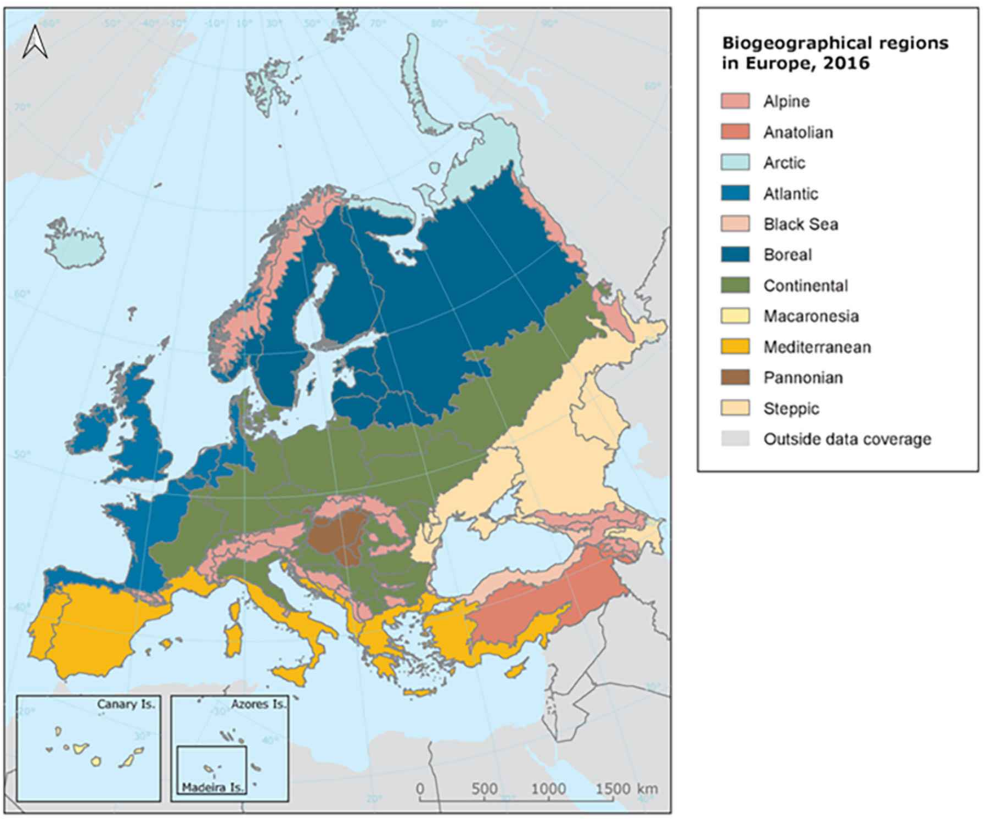

Fig. 3. Biogeographical regions in Europe (EEA 2016).

Final remarks

In conclusion:

1. The overlap of the study period with the reproductive season possibly does not affect the final results of lure stick hair-sampling research on the European wildcat. This indicates that the lure stick method can be potentially used in any season;
2. Preliminary analyses of the collected data suggest that valerian lure sticks generally give positive results in the Continental ecoregion, whereas in the Mediterranean and Atlantic regions, outcomes are usually absent or very scarce. Further data are needed to statistically confirm this assertion;
3. Most of the other working hypotheses that were set up and tested (e.g. on genetic factors, population density and type of attractant) remain still plausible, despite not yet being definitely supported, paving the way for a future more robust dissertation on the topic across Europe.
4. The comparison of the existing literature on the subject highlighted several details that need to be addressed. In order to allow more in-depth analyses, future studies on the topic are encouraged to provide by default the complete set of parameters listed in Table 1.
5. Given the negative results reported by studies carried out in some European geographical regions (e.g. the Mediterranean region), other types of attractant need to be tested (also recording the same parameters cited above) to allow the application of non-invasive genetic sampling where the use of valerian proved to be inefficient.

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CONFLICT OF INTEREST

Both authors declare that there are no potential sources of conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not appropriate for this paper. We do not plan to make the data underlying our paper available in the public domain because they have been already published by other authors.

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