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EFFICACY OF PASSIVE HAIR-TRAPS FOR THE GENETIC SAMPLING OF A LOW-DENSITY BADGER POPULATION

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ABSTRACT - A hair-trapping survey was carried out in the western River Po plain (NW Italy). We aimed to test whether barbed wire hair snares in combination with DNA profiling might represent an effective tool to study a low-density badger population. Traps were placed above the entrances of twelve badger setts between 15 February and 30 April 2010. Trapping effort was expressed as the number of trap-nights required to pluck a hair sample and the trend in the number of genotyped individual over time was analysed by regression analysis. Forty-three hair samples were collected, with an overall trapping effort of 54.8 trap-nights per one hair sample. Twenty-eight samples yielded reliable genotypes, allowing the identification of nine individual badgers. The length of storage period (1-3 months) before DNA extraction did not seem to affect genotyping success. According to the regression model, trapping effort allowed to sample 75% of the overall population. Our results suggest that the efficacy of passive devices is affected by population density.

Key words: trapping effort, genotyping success, non-invasive sampling, *Meles meles*

RIASSUNTO - ***Efficacia delle hair-traps passive per il censimento genetico di una popolazione di tasso a bassa densità***. Per verificare se i lacci di filo spinato possano rappresentare un mezzo efficace per lo studio di popolazioni di tasso a bassa densità, nel periodo 15 Febbraio - 30 Aprile 2010 è stato condotto un test preliminare di trappolaggio nella Pianura padana occidentale. Le trappole sono state posizionate di fronte agli ingressi di 12 tane. Lo sforzo di cattura è stato espresso in "notti-trappola" necessarie per il prelievo di un campione di pelo, mentre l'andamento delle catture è stato analizzato tramite regressione lineare. Sono stati prelevati 43 campioni, per uno sforzo di cattura di 54,8 notti-trappola per campione. Ventotto campioni sono stati genotipizzati con successo, per un totale di 9 diversi individui identificati. La durata del periodo di conservazione dei campioni prima dell'estrazione del DNA non ha influito sul successo di genotipizzazione. In base al modello di regressione, è stato campionato il 75% della popolazione. I risultati ottenuti suggeriscono che la densità della popolazione della specie target influisce sull'efficacia delle *hair-traps* senza esca.

Parole chiave: sforzo di cattura, successo della genotipizzazione, censimento non invasivo, *Meles meles*

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INTRODUCTION

Non-invasive genotyping is a popular tool to study elusive or rare animals for which live-capture poses methodological or ethical challenges. The most commonly used sources of non-invasive DNA are faeces and hair samples, as they can be abundant and relatively easy to collect in the field (Piggott and Taylor, 2003). However, as a result of DNA degradation, working with non-invasively collected material is problematic because genotypes will be subject to allelic dropout and false alleles (Taberlet *et al*., 1999). Error rates tend to be particular high for faecal DNA samples, at least compared to DNA obtained from remotely plucked hair samples (e.g., Frantz *et al*., 2004). In the last decade several snagging devices have been specifically designed to obtain remotely plucked hair for molecular analyses. The effectiveness of each device is likely to vary among species, depending on their behaviour and size, as well as hair length and texture.

Hair samples from mesocarnivores, especially foxes and mustelids, have mainly been collected by baited devices. However, while effective these methods can influence the behaviour of the target species (Kendall and McKelvey, 2008). Passive methods, such as various kinds of snares strung across animals' travel routes or natural rub objects, do not have this disadvantage and should thus be more effective for assessing habitat use. Generally, these traps have also the advantage of being cheap and easy to move from one route to another (Beier *et al*., 2005). Barbed wire snares have been used to collect hairs from bears (*Ursus* spp.; Beier *et al*., 2005; Haroldson *et al*., 2005; Mowat *et al*., 2005) but also proved successful for wolves (*Canis lupus*; Clevenger, 2006) and Eurasian badgers (*Meles meles*: Scheppers *et al.*, 2007; Huck *et al.,*2008).

The Eurasian badger generally lives in mixed-sex, communally breeding social groups, with relatively large differences in the size of social groups and group ranges throughout its range (Woodroffe and Macdonald, 1993; Johnson *et al*., 2002). While badger demography and social structure have been well documented for high-density populations in the British Isles (Cheeseman *et al*., 1987; Evans *et al*., 1989; Rogers *et al*., 1997; Carpenter *et al*., 2005; Huck *et al*., 2008), information is still lacking for medium- (Frantz *et al*., 2004, 2010; Scheppers *et al*., 2007) and low-density population on the continent. In central Italy the species has been described as solitary (Pigozzi, 1987), but the evidence supporting this claim has been heavily criticised (see Roper, 2010).

The efficacy of travel route snares is likely to be influenced by the population density of the target species (Kendall and McKelvey, 2008). To test whether barbed wire snares may represent an effective tool to investigate the social behaviour of low-density Italian badger populations, we carried out an explorative hair-trapping survey in a study area in the western River Po plain, where mean sett density is rather low at 0.21 setts/km^2 (Remonti *et al*., 2006a). We analysed trapping success in relation to trapping effort and the trend in the number of genotyped individual over time. We predicted that low

badger density would curtail the trapping success of passive devices.

STUDY AREA

The study area included a Natural Reserve ('Garzaia di Valenza', Piedmont region, NW Italy, 45° 01' N, 8° 64' W; hereafter: NRGV) and its surroundings, covering about 11 km^2 on the left bank of the River Po (Fig. 1). The entire area was flat, and extensively covered by maize crops, rice fields and poplar *Populus* sp. plantations. Woods (ca. 7.5% of the study area) consisted of willows *Salix cinerea* and *S. alba*, oak *Quercus robur*, poplars *P. alba* (and various hybrids), alder *Alnus glutinosa* and black locust *Robinia pseudoacacia* bordering an abandoned river meander and three naturalised artificial lakes. About 5% of the study area was wooded hills up to 260 m above sea level on the right orographic side of the River Po. Oak *Q. pubescens*, wild cherry *Prunus* sp., smooth-leaved elm *Ulmus minor*, black locust and black elder *Sambucus nigra* were the main tree species. Two villages and a few rural farms were scattered throughout the area. The climate was sub-continental temperate, with mean yearly temperature of 12.4°C and 1000 mm of rainfall.

METHODS

1. Hair collection

Hair traps were placed at a total of 12 setts (Fig. 1), previously identified by Remonti *et al*. (2006a). Setts consisted of 1-12 entrances (mean ± SD: 5.6 ± 3.9; min-max: 1-12) and were grouped ("Casone", "Argine lungo", "Argine crollo", "Frascarolo", "Montevalenza", "AFV"; Tab. 1) according to the presence of badger paths that clearly showed that they were used by the same badgers.

Traps consisted of two iron rods (5 mm in diameter and 80 cm long), supporting a sin-

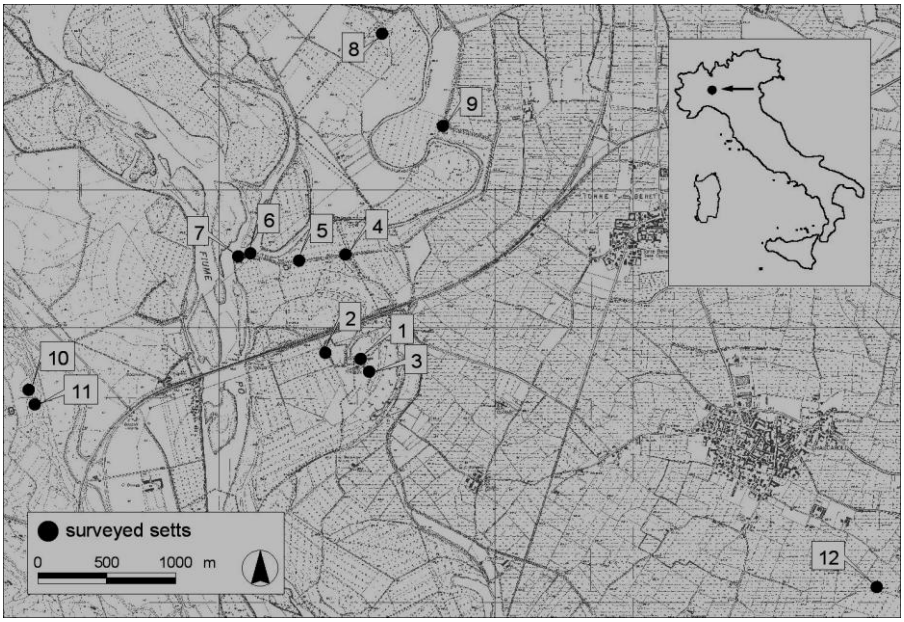

Figure 1 - Study area showing the location of badger setts. Numbers (N) correspond to those in Table 1.

Figure 2 - Barbed wire snare strung in front of one entrance of a badger sett.

gle strand of barbed wire - with four prongs per set of barbs and 10 cm spacing between barbs -, about 20 cm above ground level (Scheppers *et al*., 2007; Fig. 2).

Between 15 February and 30 April 2010, traps were placed both directly above sett entrances and on well-used paths connecting them. As disturbance caused badgers either to emerge from different holes or to abandon temporarily some small setts, both the number and location of snares were varied opportunistically throughout the study period. Hairs were collected every 3-4 days using mono-use latex gloves and temporarily stored in polythene bags. A hair sample was defined as all hair collected on a single barb, regardless of the number of hairs present. Each hair sample was moved within 3-4 hours into a microfuge tube containing 99% ethanol and stored at -20ºC until DNA extraction, which was carried out in mid June 2010 for all samples. We tested for the effect of the length of storage period before DNA extraction, by splitting the samples according to the month of collection and testing for variation in genotyping success (i.e.: number of genotyped samples / total number of samples analysed) using chi-squared (χ^2) test for contingency tables.

Trapping effort was expressed as the number of trap-nights (i.e.: number of traps x number of working nights) needed to pluck a hair sample. The number of trap-nights was plotted against the correspondent cumulative number of identified individuals and regression analysis was used to find the model that best fitted the resulting growth trend over time.

2. Molecular analyses

Hair samples were extracted using a Chelex protocol (Chelex®-100, Bio-Rad, Hercules, CA; Walsh *et al*., 1991). After incubating the root portion of the hairs at room temperature for 30 min in 1 mL doubly-distilled H_2O, 200 µL of 5% Chelex was added to the root and mixed well. This was followed by incubation at 56 °C for 30–45 min, mixing the samples occasionally. After checking that the hairs were immersed,

the Chelex solution was boiled for 8 min. After centrifugation for 3 min at 13 000 *g*, the supernatant was removed and placed in a sterile tube.

Samples were genotyped at nine microsatellite loci: *Mel*-104, *Mel*-106, *Mel*-107, *Mel*-109, *Mel*-111, *Mel*-113, *Mel*-115 (Carpenter *et al*., 2003), *Mel*-1 (Bijlsma *et al*., 2000) and *Mel*-14 (Domingo-Roura *et al*., 2003). All loci were amplified in one multiplex PCR, using the Qiagen multiplex kit (Qiagen, Hilden, Germany). Each multiplex reaction contained 1 × Qiagen multiplex master mix, 0.2 µM of each primer and 0.5 × Q-solution. After drying 1 µl of DNA for 15 min at 52°C in a 384-well PCR plate (Greiner Bio-One, Stonehouse, UK), the multiplex PCR was performed in a total volume of 2 µl (Kenta *et al.,* 2008). Following reaction times described in the multiplex kit manual, a touch-down profile was used, starting with a 15-min denaturation at 95 °C, followed by denaturation at 94 °C for 30 s, annealing at initially 61 °C for 90 s and extension at 72 °C for 1 min. The annealing temperature was reduced by 1 °C per cycle for five cycles, then kept at 55 °C for the remaining 29 cycles. Final incubation was at 60 °C for 30 min. Reactions were performed using a DNA Engine Tetrad thermocycler (MJ Research, Waltham, USA). PCR products were separated using an ABI 3730 automated DNA sequencer (Applied Biosystems, Warrington, UK) and the data were analysed using GENEMAPPER version 3.7 (Applied Biosystems).

We followed the modified multiple tubes approach (Frantz *et al*., 2003) to ensure that our genetic profiles were reliable. In some instances, a genetic profile was accepted as reliable if it matched another consensus genetic profile, even if not every locus was genotyped in duplicate.

RESULTS

The total number of trap-nights was 2358. Forty-three hair samples were collected at six setts (Table 1). Tracks revealed that "AFV A" was abandoned soon after the placing of the traps. Considering only the setts yielding at least one hair sample (1701 trap-nights), trapping effort was 39.6 trap-nights per one hair sample. Trapping effort varied among setts, ranging from 20.2 to 61.4 trap-nights (Tab. 1). Also trapping trend varied, with some setts ("Casone A" and "Montevalenza A") yielding most hairs soon after the start of the trapping period and some ("Argine crollo A" and "Frascarolo") needing up to three weeks of trapping to pluck the first sample.

Out of 40 analysed samples, 28 (70%) yielded amplifiable DNA, corresponding to 72.6 trap-nights per sample. We generated a total of 656 genotypes to deduce 250 consensus genotypes (*sensu* Frantz *et al.*, 2003). Seven genotypes contained spurious alleles and 21 genotypes allelic dropouts, giving rise to an overall per genotype error rate of 4.3%.

Genotyping success did not vary among months of hair collection (February: 0.57; March: 0.76; April: 0.67; $\chi^2 = 1.0$, $p = 0.61$, 2 d.f.).

A total of nine different individuals were identified (Tab. 1). On average, 17 ± 11.2 (SD) nights (min-max: 5-40) or 136.3 ± 114.8 (SD) trap-nights (min-max: 42-417) were needed to obtain a hair sample from each individual badger. Each individual was sampled 2.78 ± 1.72 (SD) times (min-max: 1-6). The growth trend in the number of genotyped individuals followed a sigmoid curve ($R^2 = 0.992$, $F = 752.9$, $p < 0.0001$), with equation: $\ln y = 2.49 + (-530.38/x)$ and $y \rightarrow 12$ when $x \rightarrow \infty$ (Fig. 3).

Table 1 - Trapping effort, expressed as the number of trap-nights needed to obtain one hair sample, and results of molecular analyses for 12 badger setts sampled in February-April 2010. In brackets: values obtained including only the setts positive for badger hairs.

N	Sett		N° of entrances	Trap-nights	Hair samples	Trapping effort	Genotyped samples	N° of individuals
1	Casone	A	10	401	16	25.1	10	3
2		B	2	26	-	-	-	-
3		C	1	45	-	-	-	-
4	Argine crollo	A	8	568	12	47.3	6	2
5		B	4	154	-	-	-	1
6	Argine lungo	A	4	62	-		-	-
7		B	3	81	4	20.2	4	1
8	AFV	A	12	280	-	-	-	-
9		B	1	38	-	-	-	-
10	Montevalenza	A	9	344	6	57.3	5	1
11		B	3	52	-	-	-	-
12	Frascarolo		10	307	5	61.4	3	1
	Total			2358 (1701)	43	54.8 (39.6)	28	9

Seven out of nine (77.8%) individuals were identified in the first half of the trapping period (31 nights, 773 trap-nights). Genotypes from more than one individual were obtained in two of the five setts.

DISCUSSION

In the last decade, hair trapping has been used to investigate the distribution (e.g. Karamanlidis *et al*., 2007), relative abundance (Mowat and Paetkau, 2002), density (Mowat *et al*., 2005; Scheppers *et al*., 2007), reintroduction success (De Barba *et al*., 2010) and population genetics (Morin *et al*., 1994) of several mammal species. Most studies have reported and discussed the percentage of hair samples successfully amplified, whilst, to our knowledge, both hair trapping and genotyping success have been rarely related to trapping effort, although these relationships represent a non-negligible aspect of the cost-effectiveness of non-invasive sampling techniques.

In our study area, hair-trapping success varied largely among setts. Heterogeneity in trapping success is a common result to several surveys, as a consequence of individual variation in behaviour and trapping response (Conner and Labisky, 1985; McCullough and Hirth, 1988; Corn and Conroy, 1998). Accordingly, variability in both the number of hair samples plucked from each individual and in the trapping effort needed to sample each badger suggest that traps were not visited by all badgers with the same frequency (see also Scheppers *et al*., 2007). Also bad-

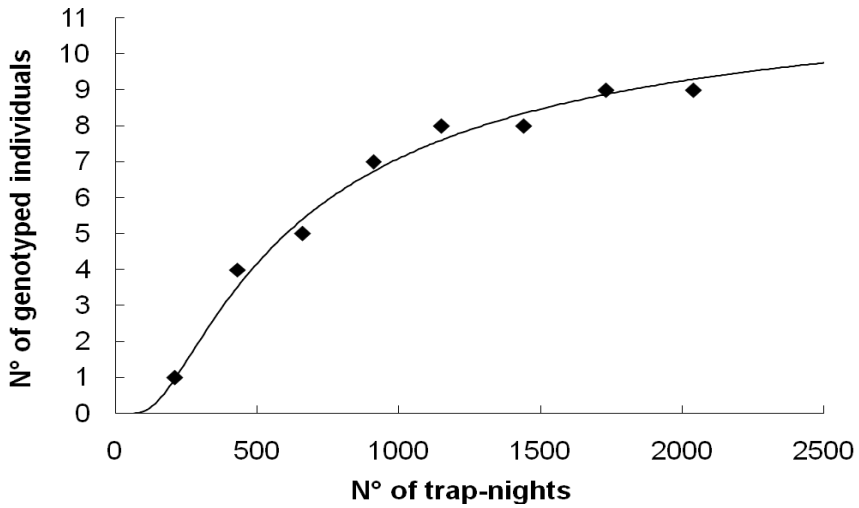

Figure 3 - Growth trend in the number of genotyped individuals in relation to the number of trap-nights.

ger response to disturbance varied among setts, in one case the placing of snares causing the abandonment of a large sett.

According to our prediction, overall trapping effort was about six-fold higher than for a medium-density (sett density = 0.99 sett/km^2) badger population in Luxembourg, where barbed wire snares yielded one hair sample per 8.3 trap-nights (calculated from rough data in Scheppers *et al*., 2007). Still focusing on passive devices, between 3.6 and 156.6 trap-nights were necessary to pluck one hair sample from river otters (*Lontra canadensis*) by modified body-snares and foot-hold traps (Depue and Ben-David, 2007), confirming that the density of the target species plays a major role in determining the success of non-invasive trapping.

Genotyping success was similar to those reported for bear hairs plucked by passive devices (67.9%-70%; Beier *et al*., 2005; Haroldson *et al*., 2005), whilst badger hairs have been reported to have a higher success (91%-95%; Scheppers *et al*., 2007; Huck *et al*., 2008).

Our genotyping error rate is slightly higher than those reported by Frantz *et al*. (2004) and Scheppers *et al*. (2007) for plucked badger hairs but lower than the 8% reported by Huck *et al*. (2008). Overall, while care is required to avoid spurious genotypes, the overall quality of the DNA obtained from remotely plucked badger hair is good enough for non-invasive genotyping to be used as a research tool at a local and possibly regional, scale.

Similarly to Roon *et al.* (2003), the length of storage period before DNA extraction did not seem to affect genotyping success, our result may depend on the higher time interval to hair collection (3-4 *vs.* 1-2 days) with respect to previous studies. Otherwise, passive snares could pluck hairs from two or more individuals more frequently than

baited corrals (see Kendall and McKelvey, 2008), resulting in a higher occurrence of mixed genetic profiles, as reported by Scheppers *et al*. (2007).
According to the regression model, trapping effort allowed to sample 75% of the overall population, while a four-week collection period, which is sufficient to sample medium-sizes populations (Frantz *et al*., 2004), allowed to identify only about 60% of badgers.
Currently further trapping surveys are being carried out to outline more accurately badger distribution in the study area as to assess population density and group size. Nevertheless our initial results seem to suggest that badgers in Italy are not, or not always, solitary as reported by Pigozzi (1987). Low-density badger populations are very difficult to census also by traditional methods (Balestrieri *et al*., 2006; Remonti *et al*., 2006b): as a touchstone, 1350 trap-nights were necessary to capture four adult badgers (337.5 night-traps per individual) in the same study area (Remonti *et al*., 2006b). Moreover body snares need to be checked daily, increasing the costs of trapping.
Considering the ethical implications of invasive procedures such as telemetry (Darimont *et al*., 2008), although the efficacy of barbed wire snares proved to be affected by population density, passive devices may still represent an effective alternative method for the remote censusing of low-density badger populations.

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