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Estimating social group size of Eurasian badgers *Meles meles* by genotyping remotely plucked single hairs

Thomas L.J. Scheppers, Alain C. Frantz, Michel Schaul, Edmée Engel, Peter Breyne, Laurent Schley & Timothy J. Roper

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Owing to the Eurasian badger's *Meles meles* role as an agricultural pest, its potential role in the transmission of bovine tuberculosis and other management problems, accurate estimation of badger abundance is required. At present, no censusing method exists that is accurate, cost-effective and relatively non-invasive. In this article, we test the feasibility of estimating badger social group and population size by genotyping DNA extracted from remotely plucked hair, obtained using unbaited barbed-wire traps suspended above runs and main sett entrances. Social group size was independently estimated by direct observation. The study was performed on 11 social groups in a population in Luxembourg, and hair samples were collected on alternate days during a four-week period. A total of 332 hair samples was collected, from which 303 single-hair extracts gave rise to a complete genetic profile after a single round of amplification. Of 48 multiple-hair extracts, 23% gave rise to a mixed profile from multiple contributors. Of samples collected from different barbs of the same trap on the same collection day, 53% originated from different individuals. After applying two error-checking protocols, an extended singles filter and a mismatch filter, 55 unique profiles were obtained. Mark-recapture analysis estimated the population to contain 61 badgers, whereas direct observation suggested a population of 49 badgers. By comparison with direct observation, hair-trapping yielded a higher estimate for six social groups, an equal estimate for four groups and a lower estimate for one group. We conclude that hair-trapping by means of unbaited barbed-wire traps, placed at sett entrances and well-used runs, offers a method of censusing badgers that is relatively accurate and precise, comparatively non-invasive, potentially applicable in a variety of habitats and at different population densities, and not prohibitively expensive. We suggest that DNA should be extracted from single hairs, rather than from hairs pooled from a single barb or a single trap, in order to avoid mixed profiles.

Key words: hair DNA, Meles meles, molecular ecology, non-invasive DNA, population size, remote censusing

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Eurasian badgers *Meles meles* are difficult to census accurately because they are nocturnal, semi-fossorial and cryptic (Neal & Cheeseman 1996, Macdonald et al. 1998). However, accurate estimates of badger abundance are required for a variety of management and conservation purposes (e.g. Symes 1989, Griffiths & Thomas 1993, Schley 2000). For example, owing to the badger's possible role as a wildlife reservoir of bovine tuberculosis *Mycobacterium bovis* infection in the UK and Ireland (Dolan 1993, Krebs et al. 1997, DEFRA 2005), information about badger population density is needed to parameterise epidemiological models of bovine tuberculosis (e.g. Smith et al. 1997), to investigate the geographical relationship between badger population density and the incidence of bovine tuberculosis in cattle (Krebs et al. 1997), and to evaluate the effectiveness of control strategies (Wilson et al. 2003a).

Presence of badgers in an area is usually easy to detect because of the burrows ('setts') that badgers dig, and also from the presence of field signs such as latrines and foraging areas (Neal & Cheeseman 1996). In addition, because each social group of badgers usually inhabits a single 'main sett', which can be distinguished by a variety of criteria such as number of entrances and size of spoil heaps (Kruuk 1978, Bock 1986, Thornton 1988), the number of social groups in an area can be estimated by counting the number of main setts (e.g. Cresswell et al. 1989, Wilson et al. 1997, Ostler & Roper 1998). However, the number of individuals per social group varies in different parts of the badger's range (Clements et al. 1988, Neal & Cheeseman 1996, Krebs et al. 1997), so that the number of main setts in an area does not provide a direct indication of population density (Macdonald et al. 1998). What is needed, therefore, is a way of estimating social group size that is accurate, cost-effective to implement and applicable over a wide range of habitats and population densities. Established methods such as capture-mark-recapture (e.g. Rogers et al. 1997, Tuyttens et al. 1999), direct observation or video surveillance of badgers as they emerge from their setts (e.g. Macdonald et al. 1998, Wilson et al. 2003b, Schley et al. 2004), distance sampling (Hounsome et al. 2005), or methods based on field signs such as latrine use or degree of use of sett entrances (e.g. Tuyttens et al. 2001, Wilson et al. 2003a) have so far failed to meet these criteria.

Non-invasive genetic tagging has emerged during recent years as an important tool for the estimation of animal abundance, especially in rare or elusive species, because DNA can be extracted from sources such as faeces or hair follicles that can be obtained without needing to catch the target animal (e.g. Palsbøll et al. 1997, Taberlet et al. 1997, Woods et al. 1999, Mowat & Paetkau 2002, Piggott & Taylor 2003, Wilson et al. 2003b). By using a number of microsatellites, a genetic profile can be obtained that is specific for an individual (Mills et al. 2000), enabling the number of individuals in a given population to be counted. Using this approach, estimates of badger group size have been obtained by generating genetic profiles from badger faeces (Frantz et al. 2003, Wilson et al. 2003b). However, genotyping of faecal samples is unlikely to be cost-effective on a large scale because of the expense of amplifying poor-quality DNA (see Frantz et al. 2003). In addition, it might be difficult to collect sufficient faecal samples from low-density badger populations, where latrines can be hard to find or non-existent (Hutchings et al. 2001, 2002, Frantz et al. 2004).

An alternative approach consists of extracting DNA from remotely plucked hair samples as these samples can contain DNA of sufficient quality and

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quantity to make repeated amplifications unnecessary (e.g. Sloane et al. 2000, Frantz et al. 2004). Using baited barbed-wire enclosures, Frantz et al. (2004) obtained accurate estimates of the size of five social groups of badgers. However, the hair traps required lengthy pre-baiting, which reduced the cost-effectiveness of the technique; and the use of baited traps, insofar as it constitutes a form of artificial provisioning, risks changing the dynamics and behaviour of the target population (Cuthill 1991). Nonetheless, Frantz et al.'s (2004) study shows that genotyping of remotely plucked hair warrants further investigation as a method of censusing badgers.

In this article, we report an attempt to estimate the size of a larger sample of social groups of badgers by genotyping DNA extracted from remotely plucked hair, obtained using unbaited barbed-wire traps. The traps were placed above well-used badger paths and sett entrances. We predicted that the use of unbaited traps would improve the applicability of hair-trapping as a census technique by avoiding problems, such as bait-shyness, associated with the use of baited traps (*cf.* Frantz et al. 2004). On the other hand, unbaited traps could result in an increase in the frequency of mixed hair samples, because several individuals might be expected to pass along the same narrow path, or to use the same sett entrance, during a single night. By contrast, the baited traps used by Frantz et al. (2004) covered a relatively large area so that different badgers could enter the trap at different points. The use of un-baited traps might therefore require a different laboratory protocol, to avoid the necessity of discarding a large number of mixed samples. To summarise, our aim was to test 1) the efficacy of our new trap design as a method of collecting hair samples and 2) whether DNA extractions should utilise single hairs, hairs pooled from a single barb, or hairs pooled from a single trap to provide reliable but unmixed genetic profiles. In addition, we aimed to determine the optimum length of the trapping period needed to obtain a sufficiently accurate and precise estimate of population size. Independent estimates of social group size were obtained by direct observation.

Material and methods

Study site

Our study site was located in the northeast of the Grand-Duchy of Luxembourg, between the villages of Eppeldorf and Medernach. The site covered approximately 13 km^2 and consisted of a mosaic of pasture, arable land and woodland (for further details see Schley 2000). The study site encompassed 11 adjoining main setts (designated setts A-K), previously identified by Schley (2000). Five of these setts were the subject of previous ecological studies in which badgers were live-trapped and radio-tracked (Schley 2000, Frantz 2004), and in which social group size was estimated using baited hair traps (Frantz et al. 2004). Main-sett density in and around the study site was 0.99 setts.km^{-2} with a mean nearest-neighbour distance of 748 m (range: 389-1,154 m).

Collection of hair samples

Hair traps consisted of two metal stakes (6 mm in diameter and 70 cm long), driven about 40 cm into the ground and supporting a single strand of barbed wire about 20 cm above ground level. In a pilot study, adhesive-tape hair traps (Sloane et al. 2000) were also used but were found to be impractical because it was difficult to remove hairs from them without the use of a wash (e.g. Mowat & Paetkau 2002); the adhesiveness of the tape diminished rapidly over time due to exposure to environmental conditions; and the tapes needed to be replaced every time hair was removed from them.

We placed 7-9 traps within 10 m of each of the 11 main setts, either directly above sett entrances or on well-used badger paths ('runs'). Additional traps were placed at or near some of the setts for reasons of impenetrable vegetation cover directly on the main sett, use of annex setts resulting in low trapping success at the main sett, and removal of traps by vandals. Overall, 44 traps were placed at sett entrances and 54 at runs.

Traps were placed at the end of August 2004, 3-4 days prior to the start of hair collection. Hairs were collected every two days, starting on 1 September 2004 and continuing for four weeks. By collecting hairs every two days, we hoped to minimise disturbance to the target setts whilst also minimising environmental degradation of the DNA. A four-week collection period was expected to be sufficient since Frantz et al. (2004), using baited hair traps, were able to capture samples from a high proportion of the population in three weeks.

All snared hairs were collected using tweezers and stored in paper envelopes at room temperature prior to DNA extraction. After each collection,

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both tweezers and barbed wire were flamed using a lighter to avoid cross-contamination.

DNA extraction

Extraction was conducted on the day of collection if possible, and always within three days of collection, in order to minimise degradation of the DNA. A hair sample was defined as all the hair collected from a single barb. Hair samples that did not contain any follicles were discarded.

Because hair can contain relatively little DNA, it has been suggested that the hairs from a sample be pooled during extraction in order to reduce genotyping errors (Goossens et al. 1998). However, because our traps were placed above runs and entrances, it was likely that a single sample contained hairs originating from more than one individual. Therefore, since previous research has shown that a single remotely-plucked hair can provide reliable genetic identification (e.g. Sloane et al. 2000, Banks et al. 2002, 2003, Frantz et al. 2004), we applied a subsampling protocol for the genetic analysis by dividing each sample into two subsamples for extraction: one containing a single hair with a clear root ('single-hair extract') and the other containing all remaining hairs ('back-up extract'). The rationale was that if the single-hair extract did not contain any amplifiable DNA, the extract containing all remaining hairs (potentially more than one) could be used as a back-up. However, we also extracted 48 back-up extracts containing multiple hairs in order to determine the frequency with which such extracts would yield DNA from more than one badger. In addition, we recorded occasions when two or more samples were obtained from the same trap on the same day, so as to be able to determine the likelihood of such samples deriving from different individuals. Note that the term 'sample' refers to the trapping event (namely, all the hairs found on one barb) while the terms 'single-hair extract' and 'back-up extract' refer to the number of hairs from the sample used in the extraction (with ≥ 1 hair in the back-up extract).

To avoid contamination, all extractions and polymerase chain reactions (PCRs) were performed in a separate laboratory that was free of concentrated badger DNA or PCR products, and negative controls were included in each manipulation to monitor for exogenous DNA contamination. Tweezers were ethanol-flamed before handling individual hairs in order to avoid cross-contamination. Hair samples were extracted using a Chelex protocol (Chelex®-100, Bio-Rad, Hercules, CA; Walsh et al. 1991) following Frantz et al. (2004).

PCR amplification and genotyping

Genetic profiles were obtained by amplifying seven microsatellites (Mel-105, Mel-106, Mel-109, Mel-111, Mel-113, Mel-116, Mel-117; Carpenter et al. 2003). The microsatellite loci were amplified in a 25 µL volume, each containing 5 µL DNA extract (approximately $^1/_{40}$ of the extract). The final reaction concentrations consisted of 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.15 µg/mL bovine serum albumin (BSA), 0.01% of Tween, 100 µM of each dNTP, 0.2 µM of primer and 0.6 units of Taq DNA polymerase (ABgene). All PCRs started with a 5-minute denaturation at 96°C. This was followed by touchdown cycles (Don et al. 1991) of 96°C for 45 seconds, annealing at 61-56°C for one minute and 72°C for 45 seconds, decreasing the annealing temperature by 1°C every cycle for six cycles, then 32 cycles of holding the annealing temperature at 55°C. PCRs ended with a final extension at 72°C for five minutes. Reactions were performed using a Bio-Rad iCycler.

Microsatellite fragments were detected on an ABI 310 Prism® automated sequencer (Applied Biosystems) and were analysed and sized using Genescan 3.1 and Genotyper 2.5 software (Applied Biosystems) based on a size standard with bands at least every 50 bp.

Data quality control

Errors associated with non-invasive tagging can lead to the identification in the laboratory of too few or too many individuals. If the genetic markers that form the genetic tag lack the variability to produce unique profiles for each individual that is sampled, two distinct individuals may carry the same profile by chance (the 'shadow effect'; Mills et al. 2000). In contrast, inconsistencies in the profiles obtained from different samples taken from the same individual can result in the creation of false profiles, leading to identification of too many individuals. In order to assess whether the markers used had enough variability to distinguish between siblings, the probability of identity among siblings ($P_{ID\text{-}SIB}$; *sensu* Waits et al. 2001) was assessed using the program GIMLET 1.3.3 (Valière 2002). This value should be < 0.01 if the data are used for population size estimation (Mills et al. 2000, Waits et al. 2001). Several protocols for error-checking non-invasive genetic data so as to detect an excess of individuals

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have been described (e.g. Paetkau 2003, Roon et al. 2005b, McKelvey & Schwartz 2004). We applied two error-checking filters for data quality control as described in Roon et al. (2005b): an extended singles filter and a mismatch filter. For the singles filter all samples with profiles that appeared only once in the data set, after regrouping identical profiles, were reamplified for all loci. Given our subsampling protocol, we extended this filter by genotyping the back-up extract of these samples, if available. Since these back-up extracts were extracted independently of the single-hair extract, a matching profile could be taken as a strong indication of the reliability of the profile. In order to apply the mismatch filter, a pair-wise comparison was carried out between all unique profiles. Profiles that diverged at only one locus (single-locus mismatch) and profiles that diverged at two loci (two-locus mismatch) in a manner that could be attributed to allelic dropout, were identified by the program GIMLET. These profiles were first checked to confirm that the raw data were accurately reflected in the data set (*cf.* Paetkau 2003). If no inconsistencies were detected, the loci causing the mismatch were reamplified.

Estimates of population and social group size

Population size with 95% confidence interval (CI) was estimated using the program CAPWIRE (Miller et al. 2005). This program has been recently developed to maximise the use of DNA-based mark-recapture data and performs well for smaller populations ($N \leq 100$) with substantial capture heterogeneity (Miller et al. 2005). The program has two models to estimate population size, based on the absence ('even capture probability model') or presence ('two innate rates model') of capture heterogeneity. Selection of the appropriate model can be defined by a likelihood-ratio test or by the user itself as the test performs poorly with small sample sizes. As the estimator assumes a closed population, it is important that the size of the population remains constant during the study period. We assumed demographic closure on the basis of the relatively short sampling period, while geographical closure was maximised by placing the hair traps in the proximity of the main sett, well within the territorial boundary of a social group. In order to remove pseudo-replicates, as suggested by Miller et al. (2005), samples collected from the same trap on the same day that originated from the same individual were considered as a single observation. To determine the optimal length of the sampling period needed to obtain a good estimate of population size, the CAPWIRE estimate was calculated after sampling periods of 9, 19 and 29 days.

To assess the minimum sampling effort needed to identify all group members, group size could be estimated and compared to the number of profiles obtained for each group. However, mark-recapture analysis was unlikely to produce meaningful results for the estimation of social group size owing to the small number of individuals per group. Instead, we plotted the cumulative percentage of 'captured' animals (as calculated from the hair-trapping estimate) against sampling days for each social group. The point at which the resultant curve reached 100% was taken to indicate the minimum sampling effort needed to identify all group members. Note that while plotting the percentage of captured animals might incorrectly suggest that all animals have been caught by the end of the sampling period, it removes the effect of group size variation and so gives a better idea of when new animals were caught.

Direct observations

To obtain an independent census of each social group, we carried out direct observations at each main sett in the month preceding hair-trapping and during the first two weeks of the sampling period. Since badgers show little sexual dimorphism and individuals are rarely distinguishable on the basis of morphological differences, we were only able to estimate minimum social group size, as indicated by the maximum number of badgers seen at a sett at any one time (Kruuk 1989, Neal & Cheeseman 1996).

Observations began approximately two hours before sundown and continued until one hour after sundown, using night-vision equipment when necessary. We aimed to carry out observations until a consistent census was obtained for each sett, but this was not achievable at setts H, I and J owing to large group sizes in combination with difficult topography and dense vegetation cover. Overall, we carried out an average of five observation sessions per sett.

Results

Collection of hair samples and genotyping success

Hair trapping resulted in 332 hair samples that were suitable for extraction (i.e. that contained at least one hair with a clear follicle). From these, 303 sin-

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gle-hair extracts (91.3%) gave rise to a complete profile after a single round of amplification. Of the single-hair extracts, 29 (8.7%) did not contain any amplifiable DNA, but for 19 of these samples a back-up extract was available. Of these 19 back-up extracts, five contained only one hair while the remaining 14 back-up extracts contained 2-12 hairs. Of the single-hair back-up extracts only one gave rise to a complete profile. All 14 multiple-hair back-up extracts gave rise to a complete profile, but four of these extracts were excluded because they yielded DNA from more than one individual, identified by having more than two alleles at, at least, one locus. The 10 remaining multiple-hair back-up extracts yielded profiles that matched single-hair profiles and were therefore judged to originate from one contributor. Thus, the total number of profiles obtained was 314.

Profiles were grouped if all alleles were identical at all seven loci. After regrouping identical profiles, 10 profiles appeared only once in the data set. Since the raw data supported the obtained profiles, the samples were reamplified for all loci in order to confirm the profile. For seven of these samples a back-up extract was available and was also genotyped. The reamplification of the 10 single-hair extracts confirmed the original profile. Of the seven back-up extracts, five confirmed the original profile while one gave rise to a mixed profile (in which all alleles from the single-hair extract profile were present) and one, containing only one hair, gave rise to another profile from the data set (which mismatched at four loci, one not suggestive of allelic dropout). As the 10 profiles were confirmed by reamplification, and five profiles also by the back-up extract, we judged these profiles to be reliable.

The program GIMLET identified three single-locus mismatches and 13 two-locus mismatches, but only three of them in a manner that could be attributed to allelic dropout. No genotyping errors were detected during the reamplification of the mismatched loci. To summarise, the 304 profiles obtained from non-invasively collected single-hair DNA and the 10 profiles obtained from multiple-hair DNA contained no obvious genotyping errors after applying the two error-checking filters.

To determine the probability of obtaining hairs from more than one individual on the same barb, 48 multiple-hair extracts were genotyped. Of these, 11 (22.9%) gave rise to mixed profiles by having three or more alleles at one or more loci. All other profiles matched single-hair extract profiles, minimising the probability that a profile with one or two alleles per locus is in fact a mixture of two or more profiles. Pair-wise pooling of all unique profiles was simulated in order to test the possibility of obtaining mixed profiles that did not have three or more alleles at, at least, one locus. This showed that five mixed profiles could theoretically resemble a legitimate profile. While one of these mixed profiles matched a profile of the data set, the remaining four would result in the creation of a new profile. For four of the five mixed profiles, both contributors were members of the same social group.

On 79 occasions, two or more samples were collected from the same trap on the same day. Of these, 42 (53.2%) gave rise to profiles from different individual badgers.

Estimates of population and social group size

Overall, 55 reliable unique profiles were obtained. Using seven microsatellites the $P_{ID\text{-}SIB}$ value was 6.60×10^{-3}, showing that sibling badgers could be distinguished with $> 99\%$ certainty. Therefore, we conclude that the 55 profiles corresponded to 55 individual badgers. This compares to an estimated population size of 49 badgers based on direct observation (Table 1) and a CAPWIRE estimate of 61 (CI: 55-67; Fig. 1).

Comparison of profiles between setts showed that seven badgers were trapped at more than one sett. Of these, four were trapped on multiple occasions at one sett and only once at the other sett, so these badgers were assigned to the sett at which they

Table 1. Estimated social group sizes based on direct observation, and on direct enumeration from genotyping of remotely plucked hair, respectively. Setts where direct observation was difficult, owing to dense vegetation and large social group size, are marked with an asterisk. A badger that was hair-trapped once at sett C and once at sett G was assigned a membership of 0.5 to each of these groups.

Social group	Observation estimate	Hair-trapping estimate
A	2	2
B	3	3
C	4	5.5
D	2	2
E	7	7
F	5	5
G	4	5.5
H	7*	7
I	6*	8
J	7*	10
K	2	0
Total	49	55

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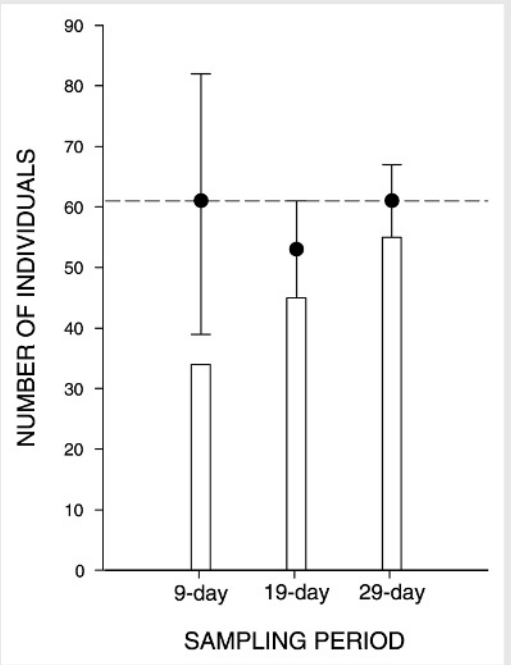

Figure 1. CAPWIRE estimated population size with 95% confidence interval after a 9-day, 19-day and 29-day sampling period. The number of unique profiles is indicated by the open histogram bars. A horizontal dashed grey line indicates the final estimated population size of 61 individuals.

were trapped more often. Two badgers were trapped on multiple occasions at two setts (setts F and G in both cases), but previous data from a pilot hair-trapping study suggested that both were members of group F. Consequently, both were assigned to group F. One badger was trapped once at sett C and once at sett G, and was therefore assigned a membership of 0.5 to each of these groups (see Table 1).

After assigning profiles to their most likely social group, hair-trapping and direct observation yielded the same estimated group size in six of the 11 groups (see Table 1). In four groups, the number of profiles exceeded the number of badgers observed, while in one group two badgers were observed, but no hair samples were obtained. Genotyping of these two badgers, for one badger using freshly shed hair (confirmed by several extracts and reamplification) and the other one using freshly plucked hair collected outside the sampling period, showed that these two badgers were not observed at other setts during the sampling period. Therefore, the combined estimate from hair-trapping and direct observation was 57 badgers, comparing well to the CAPWIRE estimate of 61 badgers.

Sampling period

There was considerable variability in the ease with which badgers were 'captured' at different setts (Fig. 2). For example, for setts A, B, F, G, I and J, the cumulative curve of captures against time reached 100% within the first three weeks of hair collection, suggesting that all members of those groups were captured. By contrast, new badgers were still being captured at setts C, D, E and H during the fourth week of trapping. At setts D and E, reliable estimates were obtained by direct

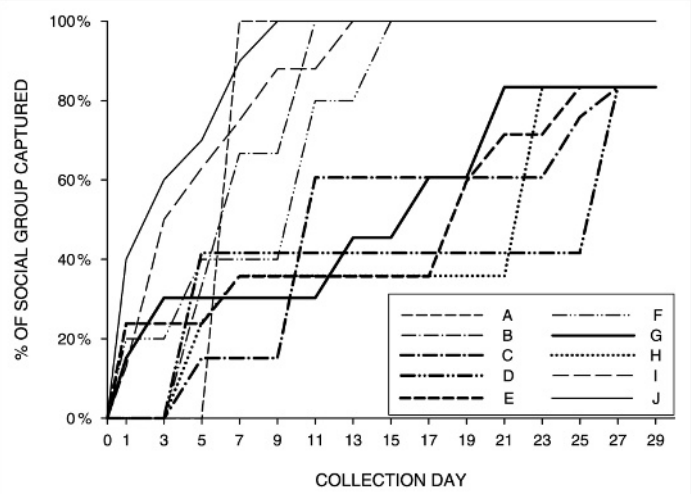

Figure 2. Cumulative plot of number of animals 'captured' from each social group (A-J), expressed as a percentage of the total number captured, over successive collection days. Please note that social group K is not included as no samples were obtained during the sampling period.

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observation and the observation estimates matched the hair-trapping estimates (see Table 1), so it is likely that all members of these groups were captured. At sett H, direct observation was difficult but the two estimates of social group size nevertheless matched, again suggesting that all animals were captured. At sett C, additional animals might have been captured had the sampling period been longer. To summarise, it is likely that the four-week collection schedule, with samples being collected on alternate days, resulted in trapping of all members of at least nine of the 11 groups. Alternatively, according to the CAPWIRE 95% confidence interval, a four-week collection schedule allowed us to obtain a DNA-sample from 82-100% of the population. Therefore, our methodology allowed us to sample a very high proportion of the population and only a few animals might have been missed.

Besides the variability in the ease with which badgers were captured, there was also considerable variability in the number of hair samples obtained from each individual badger (mean: 5.71, range: 1-21; Fig. 3) and in the number of collection days yielding samples from each badger (mean: 3.69, range: 1-11). Thus, the traps were not visited equally often by all members of the groups in question.

As regards the length of the sampling period needed to estimate the population size, the CAPWIRE estimates obtained over sampling periods of 9, 19 or 29 days gave estimated population sizes of 61 (CI: 39-82), 53 (CI: 45-61) and 61 (CI: 55-67) animals, respectively (see Fig. 1). For these estimates we used the 'two innate rates model' (TRIM), as capture heterogeneity was demonstrated (see above). The 'even capture probability model' (ECM) was rejected by the likelihood-ratio test of CAPWIRE for the 19- and 29-day periods, but not for the 9-day period. Nevertheless we selected TRIM as recommended by the authors when working with small sample sizes where capture heterogeneity is suspected (Miller et al. 2005). Considering the 29-day estimate as the final and most accurate estimate, population size was estimated correctly after nine sampling days, but underestimated after 19 days (though it was encompassed in the 95% CI). Increasing sampling effort resulted, as expected, in a decrease in the CI width (see Fig. 1).

Discussion

Collection and genotyping success

Our results show that unbaited hair traps, placed at sett entrances and well-used runs, constitute an effective non-invasive method of obtaining badger hair samples for genotyping purposes. Single hairs

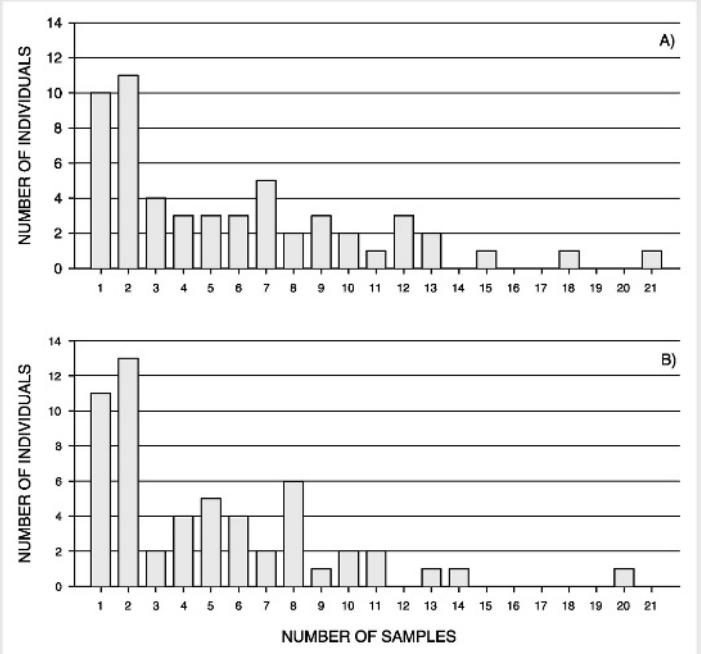

Figure 3. Frequency distribution of the number of times different individuals were sampled including (A) and excluding (B) pseudo-replicates.

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yielded reliable genetic profiles in a high percentage (91%) of cases, which is important to the success of the method because when multiple-hair extracts were genotyped, 23% were found to contain hair from more than one individual. Thus, had all the extractions been done on pooled hairs rather than on single hairs, almost a quarter of them would have had to be rejected because they would have contained DNA from more than one badger. Pooling all of the hairs from a single trap would have resulted in rejection of an even higher proportion of samples. We therefore suggest that in future studies, two single guard hairs, each with a clearly visible follicle, should be extracted separately from each sample. Under this protocol, if the first extraction were found not to contain DNA, the second would be available as a reserve.

As genotyping errors can create new individuals and lower the recapture rates, proving that the data set is error-free is essential for the generation of accurate and defensible population estimates (McKelvey & Schwartz 2004). By applying an extended singles filter and a mismatch filter, we have demonstrated the reliability of genotyping single hairs. According to Paetkau (2003), a match in profiles between two samples can be taken as strong evidence that the samples in question are from the same individual, and that neither profile contains errors. Thus, the fact that 304 of the 314 samples (96.8%) produced profiles that were observed in at least one other sample provides further evidence for the reliability of the data.

While some previous studies have also obtained reliable profiles using single hairs (e.g. Sloane et al. 2000, Banks et al. 2002, 2003, Frantz et al. 2004), others have reported high error rates (e.g. Gagneux et al. 1997, Taberlet et al. 1997, Goossens et al. 1998, Morin et al. 2001). Factors underlying the success of genotyping single hairs include the use of freshly plucked hair rather than shed hair, optimal collection, storage and extraction procedures, and optimal PCR conditions (Sloane et al. 2000, Morin et al. 2001, Banks et al. 2002, 2003, Roon et al. 2003, Frantz et al. 2004). Inter-species variability in hair DNA yield may also be relevant (Goossens et al. 1998, Taberlet & Luikart 1999, Woods et al. 1999, Banks et al. 2002).

When the optimal conditions cannot be met, pooling multiple hairs in a single extraction can increase DNA yield, consequently improving genotyping success (e.g. Goossens et al. 1998, Mowat & Paetkau 2002, Alpers et al. 2003). However, when samples are collected without observing the individual, pooling hairs risks mixing different individuals. Nevertheless, it is predicted that these mixed samples will easily be detected by the presence of more than two alleles per locus (Alpers et al. 2003, Roon et al. 2005a). In our study, a simulated pair-wise pooling of all unique profiles resulted in five mixed profiles going undetected using this rule. As in the case of four mixed profiles, both contributors were members of the same social group, and so it is likely that extracting multiple hairs collected using our trap design would have resulted in the creation of new individuals. Therefore, the use of single hairs for remote censusing should not only be restricted to populations with low genetic diversity (Alpers et al. 2003), but also to social groups with high levels of relatedness.

Accuracy and precision

Ideally, any method of estimating population density needs to be accurate and precise, cost-effective and not detrimental to the species. As regards accuracy, hair trapping identified 55 individual badgers and allowed the population to be estimated at 61 badgers, whereas direct observation yielded a lower estimated population size of 49. This is consistent with other studies suggesting that direct observation tends to underestimate the number of animals present (Macdonald et al. 1998, Tuyttens et al. 2001). At the level of individual social groups, hair-trapping performed as well as, or better than, direct observation for 10 of the 11 groups, in the sense that it detected at least as many individual members of those groups. However, complications arose in the process of assigning individual profiles to social groups, owing to the fact that seven animals (13% of the profiles) were 'captured' at more than one sett. Extra-territorial visits by badgers can therefore reduce the accuracy of hair-trapping as a method of determining the size of individual social groups, and could also lead to overestimation of the size of small populations owing to the inclusion of visitors from outside the population. Direct observation, on the other hand, will be less affected as only diurnal visits potentially result in an overestimation. Given that our study took place in September, which is known to be a time of increased mating activity in badgers (Cresswell et al. 1992), the extra-territorial visits that we detected may have occurred for mating purposes (Christian 1994, 1995, Rogers et al. 1998, Carpenter et al. 2005). If this is so, then the problem of extra-territorial visits could

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be reduced or avoided by censusing badgers outside the mating season.

No direct comparison is possible between the accuracy and precision of hair-trapping and the accuracy and precision of other methods, such as live-trapping or genotyping of faecal samples. However, the mathematical models used to analyse mark-recapture data require high capture probabilities and frequent trappings if they are to provide accurate and precise population estimates (Otis et al. 1978, White et al. 1982, Rosenberg et al. 1995), so that the accuracy and precision of different methods can be assessed by comparing their capture success. Frantz et al. (2003) genotyped faecal samples collected on 10 consecutive days from the territories of three social groups of badgers that had a long history of intensive live-trapping. Faecal genotyping captured 59-71% of the population, while live-trapping yielded a capture success of at most 85% (for further details see Frantz et al. 2003, Wilson et al. 2003b). As hair-trapping has a capture success of 82-100%, it is therefore likely to provide more accurate and precise population estimates than either live-trapping or faecal genotyping. Furthermore, live-trapping may be impracticable over much of the badger's geographical range owing to low capture success (e.g. Schley 2000, Do Linh San 2004).

Cost-effectiveness

As regards cost-effectiveness, collection of faeces or hair samples using unbaited traps is less labour-intensive than either live-trapping or hair-trapping with baited traps which need to be pre-baited. On the other hand, methods based on genotyping involve significant laboratory work; but the cost of this is less for hair genotyping than for faecal genotyping because multiple amplifications are not required (Frantz et al. 2004). By comparison with direct observation, hair-trapping is more expensive in terms of both labour and materials; in our study, direct observation involved approximately 90 hours of evening observations, by comparison with about 400 hours of work for hair collection and genotyping. However, direct observation is often difficult or impossible, for example where setts are concealed by vegetation, where badgers are easily disturbed or where the number of individuals in the social group is large; and direct observation suffers from problems of inter-observer reliability and unconscious observer bias (Macdonald et al. 1998, Wilson & Delahay 2001).

One aim of our study, relevant to the issue of cost-effectiveness, was to determine the minimum trapping period necessary to census a population. On the basis of a previous study (Frantz et al. 2004), we expected a four-week collection period to be sufficient. However, new animals were still being 'captured' at some setts during the fourth week, and a few animals, known from direct observation to be present, were never 'captured'. Nonetheless, an estimate of population size obtained by applying mark-recapture analysis, based on data collected over four weeks, was precise and apparently accurate since it matched fairly closely with the combined estimate based on hair-trapping and direct observation. A longer sampling period would increase the precision of the estimate, and the duration of the optimal trapping period might differ with population density.

Animal welfare

Finally, with respect to welfare, our method is clearly less invasive than live-trapping. However, the placing of traps at sett entrances apparently led to the temporary abandonment of two setts, suggesting that the novelty of the traps may have initially deterred badgers. Trapping at runs may therefore be preferable, on welfare grounds, to trapping at sett entrances; and it may be necessary to habituate badgers to the presence of traps when censusing populations that are especially sensitive to human interference. Nevertheless, no indication of a negative trapping response was observed, and the fact that hair samples were still plucked near the end of the sampling period indicates that badgers do not suffer from the hair snagging event itself as they could easily avoid the traps.

Conclusions

To summarise, hair-trapping by means of unbaited barbed-wire traps, placed at sett entrances and well-used runs, offers a method of censusing badgers that is relatively accurate and precise, comparatively non-invasive, potentially applicable in a variety of habitats and at different population densities, and not prohibitively expensive. The setting of traps and collection of hair samples could be carried out by personnel with little prior training, and the laboratory procedures required for extraction and genotyping are standard. DNA should be extracted from single hairs, rather than from hairs pooled from a single barb or a single trap, in order to avoid mixed profiles.

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