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Estimation of badger abundance using faecal DNA typing

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Summary

1. Wildlife management and conservation programmes often require accurate information on population density, but this can be difficult to obtain, particularly when the species in question is nocturnal or cryptic. Badger populations in Britain are of intense management interest because they are a wildlife reservoir host of bovine tuberculosis (TB). Attempts to manage this infection in badgers, whether by population control or vaccination, require reliable methods of estimating population size. In addition, such estimates are also required to support research into badger ecology and TB epidemiology. Currently, the most accurate estimates of local badger population size are obtained from labour-intensive and time-consuming mark–recapture studies.

2. In recent years, DNA has been successfully extracted from the faeces of certain mammals, and used to generate a genetic profile of the defecating individual. Here we report on an application of this technology to estimate badger abundance.

3. Faecal samples were collected on 10 consecutive days from every freshly deposited dropping at latrine sites close to occupied setts in three badger social groups. Badger DNA was extracted from 89% of samples, and 20 different individuals were reliably identified. The genotypes derived from the faecal samples were compared with those obtained from blood or samples from badgers live trapped at the same setts.

4. The faecal genotypes from badgers with known trap histories revealed that latrines were used equally by males and females, and by badgers ranging in age from cubs (< 1 year old) to 9 years old. Individual badgers used the latrines on between one and six different nights. Rarefaction analysis produced abundance estimates that closely matched those obtained from live trapping.

5. *Synthesis and applications*. Systematic sampling and genetic typing of fresh faeces from badger latrines can provide data that can be used to estimate abundance accurately. This approach requires considerably less human resources than repeated live trapping and mark–recapture. The technique may be valuable for future badger research and management in relation to bovine TB, where accurate estimates of abundance at a local scale are required.

Key-words: molecular scatology, population size, survey technique.

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Introduction

The estimation of population size has a central role in wildlife management and research. Intervention and active management of animal populations is carried out for a variety of reasons, such as disease control, conservation and pest control. Estimates of abundance are generally required, to various degrees of accuracy depending on the nature of the issue. Species that are difficult to observe directly, for example those with nocturnal or cryptic habits, may require trapping-based methods that can be highly labour intensive and time consuming. Methods based on quantifying field signs have also been employed, but these can be strongly influenced by sources of error associated with environmental factors (reviewed in Wilson & Delahay 2001).

Over much of southern Britain, European badgers *Meles meles* L. live in communal social groups, at

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moderate to high densities (Kruuk 1978; Neal & Cheeseman 1996; Wilson, Harris & McLaren 1997). Badgers are currently the focus of intense research interest as they are thought to be the main wildlife reservoir of bovine tuberculosis (TB), which is a serious problem in cattle herds in parts of Britain (Krebs 1997). Current research includes the experimental manipulation of badger densities (Krebs 1997) and multivariate analyses of farm management, incorporating environmental and ecological factors (including badger density) considered likely to contribute to the risks of infection in herds. Probable future options for the management of badgers in the context of TB include vaccination, targeted culling and identification of potential risk areas. Knowledge of local badger abundance would be required in order to proceed with any of these as a policy.

At present, the most reliable method for obtaining accurate estimates of badger abundance is live trapping and mark–recapture analysis (Rogers *et al*. 1997a, 1999; Tuyttens *et al*. 1999) but for logistic reasons this is impractical at anything other than a local scale. Badger field signs have also been used as crude, indirect measures of density. For example, repeat surveys for badger setts were carried out in a random sample of 1-km squares throughout Britain to monitor national change in populations over a 10-year period (Cresswell, Harris & Jefferies 1990; Wilson, Harris & McLaren 1997). This methodology has also been used at a regional scale (Ostler & Roper 1998). In another study, the number of latrine sites encountered per unit distance of transect walked was found to correlate with badger density (Tuyttens *et al*. 2001). However, neither method could be used to estimate group size accurately.

Communal latrine sites are believed to be sources of information transfer via scent marking (Roper, Shepherdson & Davies 1986, Roper *et al*. 1993). In moderate- to high-density badger populations, individuals use latrine sites throughout their territories (Kruuk 1978). Hinterland latrines (i.e. those within the core of badger territories, away from the territorial boundary) appear to be used equally by males and females (Roper *et al*. 1993) and may be important for intragroup communication. In contrast, boundary latrines (i.e. those at the edges of territories) are used more often by males (Roper *et al*. 1993) and may be more important for intergroup communication. The deposition of droppings at hinterland latrines may also serve as a defence of the sett against interlopers (Roper *et al*. 1993), a strategy that would be best served where a high proportion of the resident individuals made deposits. Given their significance in communication between badgers, latrines may provide a means to estimate the size of badger populations.

Recent developments in molecular techniques have made it possible to obtain species-specific DNA from faecal samples collected in the field, thus providing a method of sampling animals without trapping or observing them (Kohn & Wayne 1997; Palsboll 1999). Epithelial cell material shed from the gut of the animals with the faeces contains host DNA that can be isolated and analysed. In the last decade, molecular scatology has been applied in a number of studies of mammalian biology and ecology. Hypervariable microsatellites are regions of short-sequence repeats of nuclear DNA that can be highly polymorphic. By assaying several of these a 'genetic fingerprint' can be produced that is unique to individual animals (Palsboll 1999). For species that are difficult to trap, hard to observe or rare, this can be a powerful technique and diverse information can be collected without ever having to capture, or even see, the animal. For example, population size can be estimated using DNA from faeces in more than one way. Faeces can be collected in a standardized manner, for example along transects, and each genotype established can be considered as a 'capture'. By carrying out repeated, temporally distinct sampling visits, a data set of genotypes can be collated and treated as mark–recapture data (Banks *et al*. 2002). Alternatively, by plotting the number of faeces sampled against the cumulative number of new genotypes, a regression curve is produced that asymptotes at the estimated population size, as successfully applied to coyotes in California, USA (Kohn *et al*. 1999).

A comprehensive suite of 39 microsatellite markers for badgers has been created, using samples taken from animals trapped at Woodchester Park in Gloucestershire, UK (Carpenter *et al*. 2003). We hypothesized that systematic sampling and genotyping of faeces deposited at communal latrines near badger setts would enable us to estimate the number of resident individuals. Here, we describe a field study to estimate badger numbers by the systematic collection of fresh faeces from latrines followed by faecal genetic typing. The study was carried out at Woodchester Park, where a long-term, intensive study of the resident badger population provides robust independent estimates of badger numbers for comparison, plus complementary data on individual life histories (Cheeseman *et al*. 1987; Rogers *et al*. 1997a; Delahay *et al*. 2000a).

Materials and methods

BASELINE BADGER POPULATION DATA

Since 1975 the resident badger population in an 11-km^2 area in and around Woodchester Park, Gloucestershire, south-west England, has been the subject of an intensive ecological and epidemiological study (Rogers *et al*. 1997a, 2000; Delahay *et al.* 2000a). The present study involved three of the 25 badger social groups (Parkmill, Nettle and Kennel) previously identified in the more intensively studied core of the site. We decided to carry out this study on a group-by-group basis, as a badger social group is likely to be the smallest scale at which badger abundance is ever likely to be required. However, we also assessed the success of the technique for the three-group study population as a whole, ignoring the territorial boundaries.

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In all the core groups badger territories were delineated by 'bait marking' during spring when territorial scent marking was at its peak (Delahay *et al.* 2000b). This involved distributing portions of a highly palatable bait mix comprising indigestible, coloured plastic beads, syrup and peanuts around each occupied sett for 10 days. Droppings from badgers that had consumed the bait were laced with the undigested plastic beads. A subsequent survey of badger latrines for marked faeces provided information on the territorial configuration of each social group. The entire study area was also subject to seasonal live trapping using steel mesh box traps, and every captured individual was permanently marked with a unique tattoo (Rogers *et al.* 1997a). At each individual's first capture, a sample of blood or hair follicles was archived for use in genotyping of the trapped individuals. In addition, at each capture clinical samples were taken to determine TB infection status, and the sex, age, location of capture, weight, body length, physical and reproductive condition of the animal were also recorded (Rogers, Cheeseman & Langton 1997b). The trapping success is high at Woodchester Park. For example, from 1987 to 1997, the number of individual badgers trapped in each year was within 10% of the minimum number alive estimate, calculated retrospectively using data from subsequent years (G. Wilson, unpublished data). Also, the proportionally low numbers of unmarked badgers amongst those found dead in the study area suggests that the trap catch in any one year is likely to be close to the true population size.

COLLECTION OF FRESH DROPPINGS

Surveys were carried out to locate all the latrines in the proximity of all setts with signs of occupation within the hinterland of the territories of the three chosen social groups. An arbitrary radius of 30 m around each sett was searched systematically. A total of 14 discrete latrines was located and monitored. Limiting the search to this distance reduced the likelihood that these latrines were visited by non-resident badgers from adjacent social groups. This probability is likely to be greater for latrines close to the territorial boundaries.

A light dusting of builder's chalk (Stanley Tools, Connecticut, USA) was used to mark the droppings at latrines each day, so that new deposits could be identified easily the following day. Every morning for 10 consecutive days, small samples of approximately 1 g of faecal material from the surface of each fresh dropping were transferred directly into 2-ml screw-cap microfuge tubes containing 70% ethanol. Faeces were often deposited in separate dung pits within the same latrine. In cases where faeces were close together in the same dung pit, care was taken to take the samples from parts of the deposits that were furthest from the contact point of any other dropping. Samples were collected at first light, to minimize environmental degradation of faecal DNA. This also maximized the length of time available to allow any human scent left at the latrines to disperse before the following night.

REMOTE VIDEO SURVEILLANCE

During the 6 months prior to the faecal collection period, all adult badgers captured during routine trapping operations in the three chosen social groups were given unique fur-clips (Stewart & Macdonald 1997a). The contrast between the dark-coloured outer guard hairs and the lighter under-fur allowed the identification of these individuals by video surveillance. Over the 10-day latrine monitoring period, the sett that appeared to be most active (Wilson *et al.* 2003) in each of the three social groups was monitored using video surveillance equipment. The immediate area around each sett was illuminated using an infra-red light (Tracksys Ltd, Nottingham, UK), to which badgers appear to habituate quickly (Stewart & Macdonald 1997b). A monochrome video camera (Sanyo VCB-35721RP; Sanyo Electric Co. Ltd, Basel, Switzerland) sensitive to infra-red light was positioned to maximize observations of badgers in the immediate proximity of each sett. The cameras were connected to time-lapse video recorders (Sanyo TLS-9168P; Sanyo Electric Co. Ltd) set for 12-h recording. Each complete unit was powered by two 12-V dryfit batteries (Sonnenschein A500; CMP Batteries Ltd, Stevenage, UK) that ran the systems for two nights before recharging was required. The systems ran from 19:30 to 06:00 each night during the sampling period. The resulting video tapes were viewed, and data were collated on all badger observations.

TYPING OF DNA

Non-invasive sampling of animals using faecal DNA has potentially serious limitations resulting from the degraded nature of the genetic material. These must be fully addressed before embarking on applied studies, or the genotyping errors may lead to misleading results (Taberlet & Luikart 1999). Considerable preliminary research was carried out in order to establish the most appropriate methodology to generate accurate molecular tags from badger faeces. A full description of the genotyping protocols is presented in Frantz *et al.* (2003). These are presented here in summarized form.

Faeces were stored in 70% ethanol and extracted using the GuSCN/silica method (Boom *et al.* 1990; Hoess & Pääbo 1993). DNA extractions from faeces were performed in a laboratory that was free of concentrated badger DNA or polymerase chain reaction (PCR) product, and negatives were included in all PCR and extractions. Seven microsatellite loci were used to genotype each faecal sample (*Mel* 102, *Mel* 105, *Mel* 106, *Mel* 109, *Mel* 111, *Mel* 113, *Mel* 117; Carpenter *et al.* 2003). A modified version of the multiple-tubes approach (Taberlet *et al.* 1996) was developed for this study, to reduce the number of amplifications while

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Table 1. The number of badgers that were live trapped in 2001 and/or identified as having used the monitored latrines by analysis of faecal DNA. Estimates of group size from faecal samples are presented, showing the median asymptote value from 1000 rarefaction curves where the sample order was randomized each time, and the interquartile range

				Totals			
Group	Trapped and visited latrine (a)	Trapped but did not visit latrine (b)	Visited latrine but not trapped (c)	Faecal genotypes (a + c)	Trapped badgers (a + b)	Faecal genotypes and trapped badgers (baseline estimate) (a + b + c)	Rarefaction curve estimate (interquartile range)
Parkmill	7	6	3	10	13	16	15 (13–18)
Nettle	4	5	1	5	9	10	9 (8–10)
Kennel	5	3	0	5	8	8	–
All	16	*14	4	20	30	34	36 (32–42)

*Of the 14 badgers that were trapped but did not use the latrines, remote video surveillance showed that eight had remained in the area during the period of latrine monitoring.

retaining sufficient power (Frantz *et al*. 2003). Each locus was amplified an average of 3·4 times per sample. This method had full safeguards for detecting false alleles and allelic dropout, with sufficient replicates performed to achieve 95% reliability. The probability of identity among siblings (*sensu* Waits, Luikart & Taberlet 2001) was assessed using profiles obtained from individuals previously trapped at this study site. The markers employed in this study were sufficient to distinguish between siblings with 99% certainty (Frantz *et al*. 2003).

Reliable genetic profiles were produced from blood or hair samples taken from all badgers caught in the target groups in 2000 and 2001, and were compared to the genotypes obtained from the faecal samples.

ESTIMATION OF GROUP SIZE

Baseline estimates of group size were calculated from trapping records from 2001, during which time four trapping events at each of the target social groups were carried out. Due to the small number of faecal samples collected within each social group, mark–recapture analysis was considered unlikely to produce abundance estimates with acceptable confidence intervals. Consequently, rarefaction analysis (Krebs 1989) was carried out on the faecal genotype data collected over the 10-day period to generate estimates of badger social group size. The cumulative number of genotypes was plotted against the number of faecal samples collected. Using the program PROC NLIN (SAS Institute Inc. 2000), a non-linear function of the form $y = ax/(b + x)$ was fitted to the plot (where y = cumulative number of genotypes, x = number of faeces sampled, a = asymptote, b = rate of decline of curve) and group size was projected as the asymptote a. Because the order in which the samples are listed affects the shape of the curve and hence the value of the asymptote, the sample order was randomized for each of 1000 iterations, and the regression repeated at each step (Kohn *et al*. 1999).

Results

FAECAL TYPING

Badger DNA was extracted from 47 (89%) of the 53 droppings deposited in the monitored latrines over the collection period. All seven loci could be amplified in 39 (74%) samples, and for a further eight it was possible to score reliably at least the most informative locus (the locus that was most variable between individuals). The complete molecular tags revealed that the faecal samples represented 20 different individuals. Comparison with the reference set of genotypes derived from the live trapped badgers showed that 16 of these faecal tags corresponded with known resident badgers (Table 1). Four faecal samples produced genetic profiles that did not match any of those from trapped badgers, and a further 14 that had been trapped were not detected (Table 1). Despite not being represented in the latrines, eight of these 14 were confirmed to be resident during the faecal sampling as they were recorded by video surveillance. The remaining six badgers that were trapped but did not use the latrines were not positively identified on video.

LATRINE-USE PATTERNS

The sex ratio of those badgers that had visited latrines and whose identity was known from trapping records was 1 : 1 (eight males, eight females), as it was for those badgers that had been trapped at the setts in question but had not deposited faeces at latrines (seven males, seven females). Of those badgers for which age data were available (i.e. those which had been originally trapped as cubs) there was no significant difference in the mean ages of those that did and did not deposit faeces at latrines (Mann–Whitney $U_{13,15} = 135$, $P > 0{\cdot}05$), and the latrines were used by badgers ranging from cubs of that year to one that was 9 years old. Individual badgers deposited faeces at latrines on between one and six separate nights during the 10-day collection period (Fig. 1).

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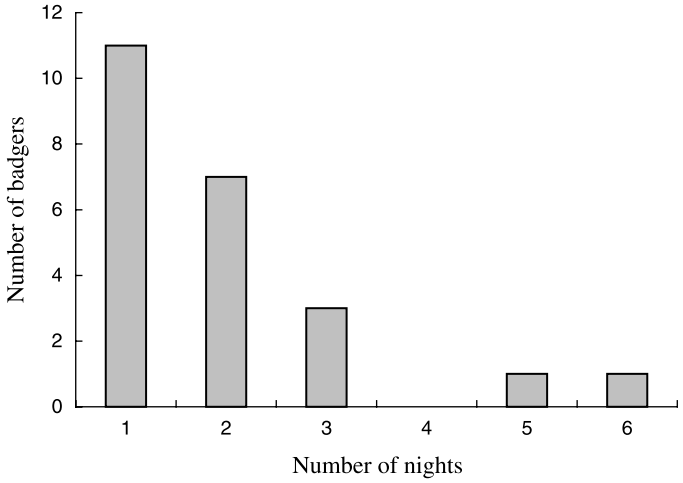

Fig. 1. Frequency distribution of the number of nights on which badgers used the latrines during the 10 days of monitoring.

ESTIMATION OF BADGER GROUP SIZE

Estimates of group size based on live trapping records were higher than the number of genotypes obtained from monitoring latrines adjacent to active setts (Table 1). In addition, typing faecal DNA identified some badgers that were resident in these groups but remained untrapped (see above). Consequently, a combination of trapping records and genotypes was considered to provide the most complete baseline estimate of group size (Table 1).

Rarefaction analysis of genotypes produced estimates of group size for Parkmill (Fig. 2a) and Nettle (Fig. 2b) that were comparable with the baseline estimates. Unfortunately, analysis of data from the Kennel social group did not produce consistent results; 20% of the regression curves reached asymptotes with unrealistically high values, indicating that too few samples were collected. When the data for all three social groups were combined, the analysis again produced a median asymptote (Fig. 3) that was in close agreement with both the baseline trap-derived estimates of group size and the estimates based on trapping and genotypes (Table 1).

Discussion

The badger's habit of predictable latrine use means that collection of fresh samples for DNA analysis is relatively straightforward. Marking and collecting samples in the way described here ensures that they remain exposed to the elements for a very short time prior to collection, thus maximizing the chance of successfully producing a genotype (Taberlet, Waits & Luikart 1999). The latrines monitored in our study were used equally by badgers of both sexes and of a wide range of ages. Also, there were no sex or age differences between the resident badgers that used the latrines and those that did not. This suggests that the method did not incur any inherent age- or sex-related bias at the time of year that samples were collected. This finding is consistent with the observation of Roper *et al.* (1993) that hinterland latrines were used equally by males and females. These attributes combine to provide a reliable method for estimating badger population size.

Only four of the genotypes obtained from faecal samples did not match those from trapped animals. Genotyping of blood and hair samples from trapped badgers only took place in 2000 and 2001, so it is possible that these four unmatched badgers were resident in the area during these years but were not trapped. Alternatively they may represent post-trapping immigrants, transient animals or untrappable individuals. Consequently, the technique may provide a means by which to estimate the proportion of the population that is untrappable.

Several of the trapped badgers from the target social groups did not use the latrines over the 10 days, despite being confirmed on camera as being in the area. Presumably these badgers used alternative latrines in the group territory. Given that only a small number of the total number of latrines in each group territory was monitored in this study, it seems likely that had we expanded our search outwards from each sett, we would have found more latrines and potentially sampled a higher proportion of the resident badgers. However, this may lead to an increased possibility of sampling badgers from neighbouring territories. In the case of the badgers that were trapped but did not use the latrines, and were not seen on camera during the sampling period, there are three possible explanations: (i) they were still in the area but their fur-clip had grown out so they could not be identified on camera; (ii) they were resident in an alternative sett to that covered by the camera; (iii) they had moved away from the territory subsequent to being trapped and prior to this study.

The estimates of social group size using the genotype data from Nettle and Parkmill were very close to those derived independently from the trapping data. However,

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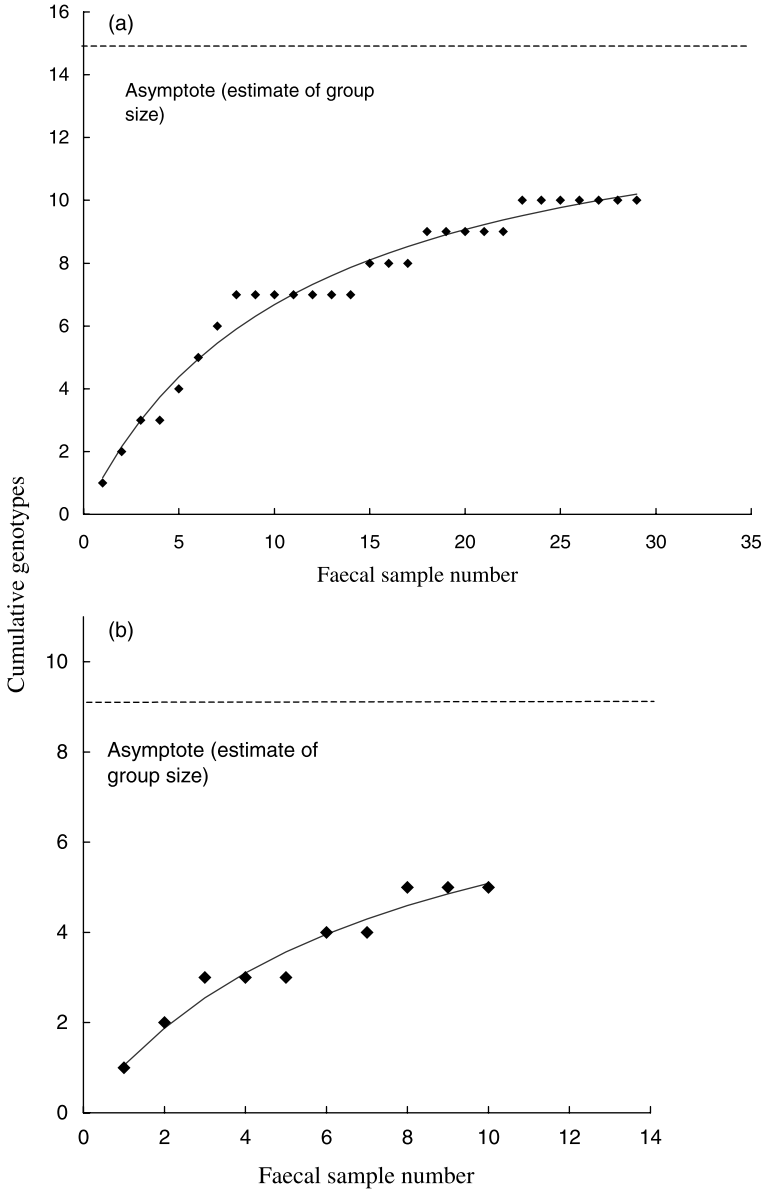

Fig. 2. (a) Rarefaction curve giving the median asymptote value for the Parkmill social group from 1000 iterations of the regression, with the sample order randomized each time. Extrapolation to the asymptote indicates an estimated group size of 15 individuals. (b) Rarefaction curve giving the median asymptote value for the Nettle social group, from 1000 iterations of the regression, with the sample order randomized each time. Extrapolation to the asymptote indicates an estimated group size of nine individuals.

there was considerable variation in the social group size estimates (i.e. the asymptotes) resulting from the 1000 randomizations (Table 1), and the samples from Kennel gave no consistent estimate, with many of the randomizations producing curves that reached unrealistically high asymptotes. This situation could potentially be improved by sampling more latrines per territory and/or monitoring for longer. Rarefaction curves would then be more likely to reach consistent asymptotes, and thereby produce social group size estimates with greater confidence. Additionally, rates of faecal accumulation at these latrines may be highest in mid-winter (Hutchings, Service & Harris 2001; G.J. Wilson and R.J. Delahay, unpublished data), when badgers are less active and remain near the sett for longer each night (Cresswell 1988; B.T. Garnett, unpublished data). Therefore, a higher proportion of social group members may be detected within less time if faecal DNA sampling is carried out in winter.

Encouragingly, the estimate of population size for the three groups combined agreed closely with the trap-derived value. This suggests that the method could be applied successfully for the estimation of badger numbers over larger areas, with no prior knowledge of social group territories.

This paper describes the first reliable method of estimating badger numbers without the use of live trapping. Collecting faecal samples is much less laborious and disruptive than trapping, marking and recatching badgers. Furthermore, the principal advantage of the

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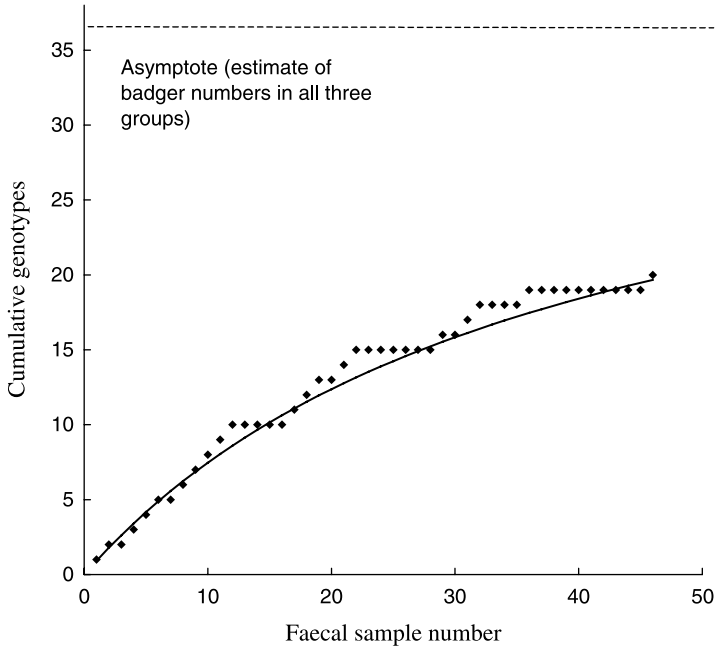

Fig. 3. Rarefaction curve giving the median asymptote value for the three groups combined, from 1000 iterations of the regression, with the sample order randomized each time. Extrapolation to the asymptote indicates an estimated population size of 36 individuals.

faecal DNA approach over methods based on field signs (Wilson, Harris & McLaren 1997; Tuyttens *et al*. 2001) is that the genotype data are essentially direct counts of individual badgers. This method is unlikely to be confounded by extrinsic factors, for example seasonal variations in weather, which can have a strong influence on field sign indices (Wilson *et al*. 2003). Due to the genotyping error rates, which are common when working with faecal DNA (Frantz *et al*. 2003), several PCR reactions per sample are required in order to achieve a reliable genotype. Laboratory time per sample is therefore expensive and will ultimately limit the scale of any study using this approach. However, by investigating the effect on population size estimates of varying the number of latrines monitored, and duration of sampling period, a protocol can be developed that maximizes the efficiency of the method.

An accurate method of determining badger density is likely to be highly relevant in the context of future research and management programmes into the control of bovine TB. The current randomized culling trial (Krebs 1997) seeks to assess the importance of badgers as wildlife reservoirs of TB, and is one of a range of research projects aimed at producing a successful policy for the control of TB in cattle. In the future there are a number of possible scenarios where estimation of badger numbers will be required. Should limited culling be necessary, pre- and post-cull estimates of badger numbers will be required in order to assess the efficacy of any culling operation. Furthermore, monitoring of recolonization over time in these areas will also be necessary, ideally using non-invasive but accurate methods such as the one described here.

Vaccination using treated bait distributed in the environment has been used elsewhere to control wildlife disease (Stohr & Meslin 1996) and remains a possible future management option for badgers in the UK. A successful vaccination programme requires that a sufficient proportion of the population is treated in a given period of time. Therefore it is necessary to assess the size of the target population in advance, in order to plan a cost-effective vaccine delivery programme (Delahay *et al*. 2003). The faecal DNA approach would provide an efficient means to do this, which would avoid disruptive and laborious, repeat, live trapping.

It has been proposed that a potentially counterproductive effect of culling badgers is the disruption of the social organization of the population, leading to increased contact rates between badgers and subsequent increases in numbers of infected animals (Swinton *et al*. 1997; Tuyttens *et al*. 1999). By identifying individual genetic profiles from faeces, it may be possible to carry out 'non-invasive tracking' and thereby quantify the disruptions in movement patterns.

From a conservation perspective, the badger is listed on the UK biodiversity action plan as a species that should be monitored (Macdonald, Mace & Rushton 1998). Although the badger population in Britain increased nationally throughout the 1980s and 1990s (Wilson, Harris & McLaren 1997), continued monitoring is essential to ensure that any significant reverse of this situation does not go unnoticed. Although too expensive in laboratory costs to carry out at a national scale, the method described here may be useful in validating other, index-based measures of abundance that can be used over larger areas (Sadlier *et al*. 2003).

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