Published paper

Dugdale, Hannah Louise, Davison, Dan, Baker, Sandra E, Ellwood, Stephen A, Newman, C, Buesching, Christina D and Macdonald, David W (2011) *Female teat size is a reliable indicator of annual breeding success in European badgers: Genetic validation.* Mammalian Biology - Zeitschrift fur Saugetierkunde . ISSN 1616-5047 (In Press)
http://dx.doi.org/10.1016/j.mambio.2011.06.001

FEMALE TEAT SIZE IS A RELIABLE INDICATOR OF ANNUAL BREEDING SUCCESS IN EUROPEAN BADGERS: GENETIC VALIDATION

HANNAH L. DUGDALE*[+†], DAN DAVISON[‡], SANDRA E. BAKER[+], STEPHEN A. ELLWOOD[+], CHRIS NEWMAN[+], CHRISTINA D. BUESCHING[+], AND DAVID W. MACDONALD[+]

[+]*Wildlife Conservation Research Unit, University of Oxford, Oxford, UK*

[†]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK*

[‡]*Department of Statistics, University of Oxford, Oxford, UK*

*Corresponding author: Hannah Dugdale, Tel: +44 (0)114 222 0112. Fax: +44 (0)114 222 101, E-mail: h.dugdale@sheffield.ac.uk

Running title: Inferring female breeding success from teat size

Word-count = 5,875

Abstract

Assessing which females have bred successfully is a central requirement in many ecological field studies, providing an estimate of the effective female population size. Researchers have applied teat measurements previously to assess whether females, in a variety of mammalian species, have bred; however, this technique has not been validated genetically. Furthermore, several analytical techniques are available to classify individuals, but their misclassification rates have not been compared. We used 22 microsatellite loci to assign maternity, with 95% confidence, within a high-density population of European badgers *Meles meles*, as plural and subterranean breeding means that maternity cannot be inferred from behavioural observations. The teat lengths and diameters of 136 females, measured May–July 1994–2005, from social groups in which all offspring were assigned a mother, were reliable indicators of recent breeding success. A Generalised Linear Mixed Model (GLMM) classified both breeding and non-breeding females with lower error rates than discriminant analyses and crude teat-size criteria. The GLMM model *logit probability = -20 + 1.8 month + 1.6 mean teat length + 1.0 mean teat diameter* can be applied quickly in the field to assess the probability with which a female badger should be assigned maternity. This is a low-cost measure which, after validation, could be used in other badger or mammalian populations to assess the breeding success of females. This may be a particularly useful welfare tool for veterinary practitioners, especially during badger culls.

Keywords: *Meles meles*, Discriminant analysis, Generalised Linear Mixed Model, Genetic validation, Reproductive success

Introduction

Understanding how reproduction is distributed among individuals is a key requirement in many studies, such as those on social evolution (Vehrencamp, 1979) and population dynamics (Oli and Dobson, 2003). Teat measurements have been suggested as a tool for identifying which females have bred in a wide variety of mammalian species (Petrides, 1950; McCravy and Rose, 1992). This low-cost methodology has been implemented in primates (Garber et al., 1993), carnivores (Fissipedia: Brooks, 1997; Macdonald and Newman, 2002; Pinnipedia: McKenzie et al., 2007), Chiroptera (Hoyle et al., 2001), insectivores (Jackson, 2006) and rodents (Branch et al., 1993; Vernes, 2004); however, very few studies have validated this method. Teat size, in combination with behavioural observations, has been demonstrated to indicate reproductive maturity reliably in New Zealand fur seals (*Arctocephalus forsteri*, McKenzie *et al.* 2007) and to discriminate between females that have, and have not, bred within a given year in both captive wolves (*Canis lupus*, Mech, Meier & Seal 1993) and wild black bears (*Ursus americanus*, Brooks 1997). Post-mortem examinations have also shown that teat size can be used to predict current breeding success in fishers (*Martes pennanti*, Frost *et al.* 1999) and seven species of small mammals from Canada (McCravy and Rose, 1992); however, the use of post-mortem to assess maternity may lead to the inclusion of females who lost late-stage foetuses or dependent young among breeders.

Validation in other species is lacking and no published studies that we are aware of have applied molecular genetic techniques in combination with long-term field data to assess the accuracy of teat measurements as indicators of annual breeding success. This is surprising given the wide-scale application of this methodology across mammalian species, and its potential practical use by field researchers and veterinary practitioners. In particular, genetic validation is important for plurally breeding species where behavioural inferences of breeding status are difficult to make (e.g., nocturnal or subterranean species) or where reproductive failure may occur after behavioural inference of breeding status (e.g. palpation of pregnant females, Cant, 2000). Additionally, several analytical techniques are available for assessing the breeding status of individuals based on traits, such as crude field criteria (e.g. cut-off teat-size criteria to identify breeders, Macdonald and Newman, 2002), discriminant analyses (that cluster individuals into groups based on traits) and generalised linear (mixed) models (that predict the probability of group membership [e.g. breeder] based on traits). The misclassification rates of these different techniques, however, have not been compared, which will be useful for future studies wishing to classify or predict group membership.

Assessing, with confidence, which females have bred successfully has not been possible previously in group-living European badgers *Meles meles* using field-diagnostics (Carpenter et al., 2005; Dugdale et al., 2007). This is because more than one candidate mother is generally present in a group and although females give birth once a year, around February, they do so underground, hindering observation (Dugdale et al., 2010). Mothers lactate until their cubs are weaned at around 12 weeks of age (Roper, 2010); however, for welfare reasons badgers are not trapped during this cub suckling period. When trapping is resumed after weaning, lactogenesis has ceased, and so expression of milk / teat palpation is not a pertinent diagnostic, however, teat size acts as an indicator. Lactating female badgers have been inferred previously as those with teats > 5 mm in diameter and > 2 mm in length before August (Macdonald and Newman, 2002), by measuring the teats of females that were diagnosed as pregnant in January using ultrasound (after Woodroffe and Macdonald, 1995). The accuracy of this approach for identifying females that go on to give birth to viable offspring, however, has not been validated, which is important given that failure is common throughout the female badger's reproductive cycle (reviewed in Yamaguchi et al., 2006).

Our study assesses whether teat size is a reliable indicator of breeding success in a medium-sized mammal, the European badger, compares the misclassification rates of three analytical techniques, and discusses potential applications of this low-cost methodology. We assess whether: 1) teat size can be used to distinguish females that were assigned maternity with 95% confidence (through the use of 22 microsatellite markers) from those that were not assigned maternity; 2) parity in the previous year compromises this result; and, 3) the misclassification rates of analytical techniques (field criteria used to distinguish breeders from non-breeders (Macdonald and Newman, 2002), Generalised Linear Mixed Models, and discriminant analyses) differ.

Materials and methods

We conducted our study in Wytham Woods, Oxford, UK (01° 19'W, 51° 46'N), a 4 km^2 area of deciduous woodland (Kirby and Thomas, 2000) surrounded by mixed arable land and permanent pasture. Badgers included in our analyses were trapped between June 1987 and November 2005. Trapping was usually undertaken at least four times a year, over one week in January and two weeks in each of May/June, July/August and November. Further details of the trapping methodology are provided in Macdonald and Newman (2002). Briefly, badgers were sedated by an intra-muscular injection of approximately 0.2 ml / kg ketamine hydrochloride (McLaren et al., 2005; Thornton et al., 2005). We identified individuals through a unique number tattooed on their inguinal area (Cheeseman and Harris, 1982), classified them as a cub (< 1 year old), yearling (≥ 1 to < 2 years), or adult (≥ 2 years), and sexed them. Teat lengths (from base to tip) and diameters (at the base) were then measured to the nearest millimetre for those females trapped May–July 1994–2005, inclusive. Teat lengths and diameters vary slightly according to their position on the female with the anterior and middle teats being slightly longer and wider than the posterior teats (Supplementary Figure 1). Measurements were made at each of the six teat-positions (except for three females that were missing data from one teat, on one occasion, and two females that were measured on two occasions but only had five teats), and the means were calculated. The mean teat lengths or diameters were recorded on 272 occasions from 136 females (70 as breeders only, 47 as non-breeders only, and 19 with data as breeders and non-breeders). All analyses accounted for females that were measured on multiple occasions (see statistical analyses section for details).

Genetic analyses

Published parentage data (based on 915 badgers, trapped 1987–2005 in Wytham Woods, and genotyped at 16–21 microsatellite loci) are available for 630 cubs born 1988–2005 (Dugdale et al., 2007). We used a sub set of these data; 524 cubs born 1994–2005 (the years in which data were also available on female teat size). Of these cubs, 233 (54% of those genotyped, or 44% of those trapped) were assigned maternity with 95% confidence. We defined breeding females as those females that were assigned offspring with 95%

confidence. Reproductive failure is common at all stages of pregnancy in badgers and pre-independence cub mortality also occurs (Yamaguchi et al., 2006; Dugdale et al., 2008). We therefore classified a breeding attempt as successful if the cub survived the period of maternal investment (12–15 weeks of age) - the first point currently at which we can quantify breeding success throughout our study population through trapping (post-weaning). Non-breeders were restricted to females from groups in which all cubs were assigned a mother with at least 95% confidence (to prevent inclusion of females that bred but were not assigned maternity).

The parentage methods and results have been published previously (Dugdale et al., 2007). Briefly, candidate mothers and fathers were selected for parentage analyses according to biological rules and trapping data. Candidate mothers were defined as adult females present in the cub's social group in the year the cub was born. Candidate fathers included all adults and yearlings present in Wytham Woods in the year before the cub was born (i.e. males born the year before the cubs were excluded, Dugdale et al., 2007). As badgers may be present, but not trapped (95% of the inter-trap intervals were within 525 days, n = 6193; Dugdale *et al.* 2007), adults and yearlings were included for two years after their last date of capture, and cubs for one year. Additionally, badgers first trapped as adults with tooth wear of 4–5, were judged to be at least 2 years old, otherwise they were judged to be at least 1 year old (da Silva and Macdonald, 1989). Badger groups contain relatives, including full-siblings (Dugdale et al., 2008); on average, full-siblings will be assigned parentage over the true parent (Thompson, 1976). CERVUS (Kalinowski et al., 2007) is the only parentage software, that we are aware of, that enables inclusion of relatives of the offspring among the candidate parents to account for this. CERVUS 3.0.1.8 (Kalinowski et al., 2007) was therefore used to assign parentage.

Parentage runs were conducted by year (1994–2005), with the following range of parameters: candidate mothers (number = 7–9, proportion sampled = 0.81–0.94, proportion of relatives among the candidates = 0.85–0.89), candidate fathers (number = 106–180, proportion sampled = 0.76–0.93, proportion of relatives = 0.04–0.06), relatedness to offspring = 0.11–0.22 (estimated using RELATEDNESS 5.0.8, Queller and Goodnight, 1989), proportion of loci typed = 0.98–0.99 (calculated from all of the genotyped badgers, per year), genotyping error = 0.005, and 100,000 offspring simulated. The number of candidate fathers was much larger than that of candidate mothers, as candidate mothers must reside in their cub's natal group in order to raise their cub to independence, whereas 50% of cubs are sired by extra-group males (Carpenter et al., 2005; Dugdale et al., 2007), with these males residing up to 5 social groups or 2.0 km away (Dugdale et al., 2007). The proportions of candidate parents that were sampled were estimated as the number of candidate parents that were genotyped for at least 16 loci, as a percentage of the actual number of candidate parents in the population, which was calculated from detailed trapping records (Macdonald and Newman, 2002; Dugdale et al., 2007). The proportion of loci that were typed incorrectly was estimated by re-genotyping 5% of the population (Dugdale et al., 2007). Of the 823 single-locus genotypes compared, three allelic dropouts were observed, each at different loci, and one false allele, giving an estimate of 0.005 loci typed incorrectly, which was entered in the parentage analyses (Dugdale et al., 2007). For further details of the parentage analyses, see Dugdale et al. (2007).

Statistical analyses

All analyses were run in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). General or Generalised Linear Mixed Models (GLMMs) were run with Laplace likelihood approximation method, Gaussian or binomial (logit link) error distribution and containment degrees of freedom method (Littell et al., 2006).

We first ran a GLIMMIX procedure with Gaussian error distribution to test whether the mean teat sizes of breeders and non-breeders differed; residuals were tested for normality using the Kolmogorov-Smirnov test and homogeneity of variance was tested by plotting the residuals and fitted values. Mean teat lengths (272 records from 135 females) or diameters (244 records from 123 females) were fitted as the response, month as a continuous fixed effect, and breeding success (0 = not assigned maternity; 1 = assigned maternity) as a categorical fixed effect. Badger identity was included as a random factor, to account for repeated measures. We then tested whether the teat lengths (167 records from 89 assigned mothers) and diameters (165 records from 87 assigned mothers) of breeders varied with the number of cubs that they were assigned maternity of. We repeated the previous analysis, but we only included females that were assigned maternity, replacing breeding success with the number of cubs to which they were assigned maternity.

Secondly, we ran a paired t-test to assess whether the mean teat size of adult females differed between years when they were not assigned maternity (but were sexually mature and were assigned to groups where all cubs were assigned maternity with 95% confidence), and an earlier year when they were assigned maternity with 95% confidence.

These data were available for ten adult females, and one had two data entries, so one was selected at random (our results were unaffected by the record that we selected). Deviations from normality were tested for by the Kolmogorov-Smirnov test. Means are provided ± their 95% confidence interval.

Thirdly, we generated a GLMM to predict the probability that a female bred on the basis of her teat sizes. We ran a GLIMMIX procedure with binomial error distribution, breeding success as the binary numerator response (i.e. assigned or not assigned maternity), one as the response denominator, month as a categorical fixed effect, and both mean teat diameter and mean teat length of each female per trapping event as covariates. The GLMM method (Laplace likelihood approximation) used 242 records from 122 females that had both their teat lengths and diameters recorded (69 females were only measured as breeders, 35 as non-breeders, and 18 as breeders and non-breeders). Badger identity was included as a random factor, to account for repeated measures. We then tested whether mothers that were only assigned genetic maternity of one cub were more likely to be mis-identified as breeders in the previous GLMM than mothers that were assigned maternity of more than one cub. We ran a GLIMMIX procedure with binomial error distribution, GLMM assignment success as the binary numerator response (i.e. correctly or incorrectly assigned as breeders, with 0.80 or 0.95 criteria), one as the response denominator, and a binary fixed effect (assigned maternity of 1 (0) or ≥2 (1) cubs). The GLMM method (Laplace likelihood approximation) was based on the 164 records of 87 breeders. Badger identity was included as a random factor, to account for repeated measures.

Finally, we ran a discriminant analysis (DISCRIM procedure) to classify 122 females (that had both their mean teat length and diameter recorded; two additional females did not have teat length and 12 more females did not have teat diameter recorded) as breeders or non-breeders. We ran two classification procedures: normal and cross-validation. Cross-validation classifies each observation, but removes that observation when calculating the discriminant function, thus providing a better estimate of classification accuracy. We tested for equal variance structure across classifications, using Bartlett's modification of the likelihood-ratio test of the homogeneity of the within-group covariance matrices, and the results were used in the discriminant analysis. One trapping record was selected at random for each female to avoid pseudo-replication, resulting in 47 non-breeders and 75 breeders that had data for both teat measures. Two of these non-breeders were outliers; removal of these resulted in approximately normal data with all groups having a kurtosis of less than two and a skew close to zero. It is possible that these females lost late-stage foetuses or dependent young, but ultrasound data were not available to confirm this. The mean teat sizes of one of these females fell within the 95% quantile-range of breeder teat sizes; the mean teat length of the second female also fell within this range, but her mean teat diameter was in the largest 1% of breeders. The discriminant analyses were run both including, and excluding these two outliers.

Results

Females that were assigned maternity, compared to those that were not, had longer teats (272 records from 135 females: n = 167 records from 89 breeders, mean ± 95% confidence interval = 6.5 ± 0.3 mm; n = 105 records from 65 non-breeders, mean = 3.0 ±

0.4 mm) and wider teats (244 records from 123 females: n = 165 records from 87 breeders, mean = 5.1 ± 0.3 mm; n = 79 records from 54 non-breeders, mean = 2.8 ± 0.3 mm) when analysed in a GLMM that controlled for month and repeated measures (Table 1). The teat length and diameters of mothers increased with their assigned litter size (Table 2). Previous parity did not compromise this: females assigned maternity in one year but not in the following year, had significantly shorter (mean = 6.1 ± 1.2 then 3.5 ± 1.7 mm, $t_9 = 3.43$, $p = 0.0076$) and thinner teats (mean = 4.4 ± 1.7 then 2.5 ± 0.8 mm, $t_9 = 2.88$, $p = 0.0183$) when they were not assigned maternity.

[Table 1 & 2 here]

Mean teat length and diameter were significant positive predictors of whether or not a female was assigned maternity with 95% confidence in a genetic parentage analysis (Fig. 1; Table 3). Month was also a significant effect (Table 3), resulting in the following equation that can be used in the field to quickly assess the probability with which a female should be assigned maternity:

logit probability = -20.0 + 1.8 month + 1.6 mean teat length + 1.0 mean teat diameter

[Figure 1 and Table 3 here]

Teat length had greater significance as a predictor than did teat diameter; however, both terms were significant suggesting that both should be used in the field

(Table 3). Females that were assigned maternity of 1 cub were no more likely to be mis-assigned as a non-breeder in the previous GLMM than were females that were assigned maternity of ≥ 2 cubs (estimate ± S.E. = -8.6 ± 4.9, $F_{1,76}$ = 3.1, p = 0.08 with 0.80 criteria; estimate ± S.E. = -3.4 ± 2.4, $F_{1,76}$ = 2.0, p = 0.016 with 0.95 criteria). Discriminant analyses also suggested that teat length is a better predictor of the probability of maternity assignment than teat diameter; however, the best discrimination was obtained using both terms (Table 4).

[Table 4 here]

The crude classification of breeding status (using cut-off values of: > 5 mm diameter and > 2 mm length) had the highest overall error rate and the lowest sensitivity to the classification of females that were assigned maternity, followed by the discriminant analyses; the best performance was achieved by the GLMM (Table 5). The crude classification had higher specificity of females that were not assigned genetic maternity than the discriminant analyses, but the lowest error rates were again achieved by the GLMM (Table 5). No ultrasound data were available to investigate the reasons for the misclassification of two non-breeders using the GLMM (0.80 criteria; these non-breeders were different females to the two female outliers detected in the discriminant analyses).

[Table 5 here]

Discussion

Molecular genetic techniques provide vital tools enabling validation of conventional ecological methods for assessing breeding success, especially in species such as European badgers where behavioural observations alone are not reliable. Measures of teat sizes are commonly used in a wide variety of mammalian species to assess breeding success (McCravy and Rose, 1992), yet no published study to date has validated this method using genetic techniques. This is particularly important for plurally breeding species where behavioural inferences of breeding status are difficult to make (e.g., nocturnal or subterranean species) or where reproductive failure may occur after behavioural inference of breeding status (e.g. palpation of pregnant females, Cant, 2000). Our results confirm that teat length and diameter, measured May–July, can be applied with confidence to determine whether female badgers have produced young that survived to independence, in that year. This has important application in the field, for example to identify breeding females between May and July so as to avoid leaving dependent cubs to starve if their mother was culled. This technique may also be easily applied to other populations of badgers, or other mammalian species, after validation of the GLMM equation, to account for body size differences between populations and species, and, latitudinal differences that will affect the month parameter.

Our long-term dataset, spanning 1994–2005, enabled us to select data from only those social groups in which all cubs were assigned maternity with 95% confidence. This ensured confidence in the non-breeding status of females that were not assigned maternity. Some females may have experienced the loss of their cubs after birth, potentially due to infanticide (Cresswell et al., 1992) or infantile coccidiosis (Newman et al., 2001) and may therefore have teat data that indicated breeding success, but were not assigned maternity as their cubs were not sampled. Alternatively, false positives may occur if non-breeders pseudo-lactate (Creel et al., 1991). It is not known whether pseudo-lactation occurs in badgers; however, the only published study reporting suckling data from groups in which all mothers were genetically identified, showed that only breeding females were suckled (23 observations of five mothers in Wytham Woods, Dugdale et al., 2010). The GLMM only misclassified 2 non-breeders as breeders using 0.80 criteria (or 1 with the 0.95 criteria; Table 5), but ultrasound data were not available to check if these females were pregnant. Although mothers with larger litter sizes had longer and wider teats, mothers that were only assigned maternity of one cub were no less likely to be mis-assigned in the GLMM than mothers that were assigned maternity of ≥ 2 cubs. The 95% confidence intervals around the mean lengths and diameters of breeding and non-breeding females, however, indicate that such error does not compromise the conclusion that teat length and diameter are generally powerful indicators of breeding success in female badgers, given our large sample size. Furthermore, we demonstrate that teat length and diameter are reduced in females that were assigned maternity in a previous year but not in the current year, and as such will not lead to misclassification due to previous parity.

It has been suggested previously that breeding female badgers have teats of > 5mm diameter and > 2mm length before August (Macdonald and Newman, 2002); however, this had not been validated. We demonstrate that this crude classification has a higher overall error rate than discriminant and GLMM analyses. The GLMM had the lowest misclassification of both breeders and non-breeders, therefore the resulting formula can be confidently and easily applied in the field to assess the probability with which females should be assigned maternity based on both their mean teat length and diameter. This formula will need validating in other populations where body size may vary, or at other latitudes where month may have a different effect.

The use of teat length and diameter data as indicators of breeding success is a cheap alternative to molecular techniques, however, although genetic validation is often necessary, it has not been applied previously to validate such field methods. We demonstrate the feasibility of such validation, and highlight the importance of comparing different analytical methods. The low-cost technique of measuring teat size has broad potential both in terms of the number of mammalian species it can be applied to (although further genetic validation is required), and the fields in which it can be applied from pure science to veterinary practice. For example, it will enable estimation of parameters, important for managing or studying wild populations, such as effective population size (N_e) and litter size (number of young captured within a group divided by the number of breeding females in that group, which produces similar estimates to those using genetic techniques Dugdale *et al.* 2007). Furthermore, the technique could also be applied immediately as a welfare precaution during potential future badger culling operations, instigated to control bovine tuberculosis (but see McDonald et al., 2008).

Acknowledgements

This work was generously supported by the People's Trust for Endangered Species and the Natural Environment Research Council (NERC). The authors are extremely grateful to the Wytham Woods badger team who collected the long-term dataset. Ben Sheldon provided lab space for the hair extractions. Terry Burke, Kristien Erven, Helen Hipperson, Andy Krupa and Lisa Pope (NERC Biomolecular Analysis Facility – Sheffield) provided support during laboratory work. This study was conducted under English Nature Licence 20001537 and Home Office Licence PPL-30/1216.

References

Branch, L.C., Villarreal, D., Fowler, G.S., 1993. Recruitment, dispersal, and group fusion in a declining population of the plains vizcacha (*Lagostomus maximus*, Chinchillidae). J. Mammal. 74, 9–20.

Brooks, R.T., 1997. Nipple dimensions and reproductive status of northeastern Minnesota female black bears (*Ursus americanus*). Am. Midl. Nat. 137, 178–182.

Cant, M.A., 2000. Social control of reproduction in banded mongooses. Anim. Behav. 59, 147–148.

Carpenter, P.J., Pope, L.C., Greig, C., Dawson, D.A., Rogers, L.M., Erven, K., Wilson, G.J., Delahay, R.J., Cheeseman, C.L., Burke, T., 2005. Mating system of the Eurasian badger, *Meles meles*, in a high density population. Mol. Ecol. 14, 273–284.

Cheeseman, C.L., Harris, S., 1982. Methods of marking badgers (*Meles meles*). J. Zool. 197, 289–292.

Creel, S.R., Monfort, S.L., Wildt, D.E., Waser, P.M., 1991. Spontaneous lactation is an adaptive result of pseudopregnancy. Nature 351, 660–662.

Cresswell, W.J., Harris, S., Cheeseman, C.L., Mallinson, P.J., 1992. To breed or not to breed: an analysis of the social and density-dependent constraints on the fecundity of female badgers (*Meles meles*). Philos. Trans. R. Soc. Lond., 338, 393–407.

da Silva, J., Macdonald, D.W., 1989. Limitations to the use of tooth wear as a means of aging Eurasian badgers, *Meles meles*. Revue D Ecologie - La Terre et la Vie 44, 275–278.

Dugdale, H.L., Ellwood, S.A., Macdonald, D.W., 2010. Alloparental behaviour and long-term costs of mothers tolerating other members of the group in a plurally breeding mammal. Anim. Behav. 80, 721–735.

Dugdale, H.L., Macdonald, D.W., Pope, L.C., Burke, T., 2007. Polygynandry, extra-group paternity and multiple-paternity litters in European badger (*Meles meles*) social groups. Mol. Ecol. 16, 5294–5306.

Dugdale, H.L., Macdonald, D.W., Pope, L.C., Johnson, P.J., Burke, T., 2008. Reproductive skew and relatedness in social groups of European badgers, *Meles meles*. Mol. Ecol. 17, 1815–1827.

Frost, H.C., York, E.C., Krohn, W.B., Elowe, K.D., Decker, T.A., Powell, S.M., Fuller, T.K., 1999. An evaluation of parturition indices in fishers. Wildl. Soc. Bull. 27, 221–230.

Garber, P.A., Encarnacion, F., Moya, L., Pruetz, J.D., 1993. Demographic and reproductive patterns in moustached tamarin monkeys (*Saguinus mystax*): implications for reconstructing platyrrhine mating systems. Am. J. Primatol. 29, 235–254.

Hoyle, S.D., Pople, A.R., Toop, G.J., 2001. Mark-recapture may reveal more about ecology than about population trends: Demography of a threatened ghost bat (*Macroderma gigas*) population. Austral. Ecol. 26, 80–92.

Jackson, D.B., 2006. The breeding biology of introduced hedgehogs (*Erinaceus europaeus*) on a Scottish Island: lessons for population control and bird conservation. J. Zool. 268, 303–314.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106.

Kirby, K.J., Thomas, R.C., 2000. Changes in the ground flora in Wytham Woods, southern England from 1974 to 1991 - implications for nature conservation. J. Veg. Sci. 11, 871–880.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS for Mixed Models. SAS Institute Inc., Cary, NC, USA.

Macdonald, D.W., Newman, C., 2002. Population dynamics of badgers (*Meles meles*) in Oxfordshire, UK: numbers, density and cohort life histories, and a possible role of climate change in population growth. J. Zool. 256, 121–138.

McCravy, K.W., Rose, R.K., 1992. An analysis of external features as predictors of reproductive status in small mammals. J. Mammal. 73, 151–159.

McDonald, R.A., Delahay, R.J., Carter, S.P., Smith, G.C., Cheeseman, C.L., 2008. Perturbing implications of wildlife ecology for disease control. Trends Ecol. Evol. 23, 53–56.

McKenzie, J., Page, B., Shaughnessy, P.D., Hindell, M.A., 2007. Age and reproductive maturity of New Zealand fur seals (*Arctocephalus forsteri*) in southern Australia. J. Mammal. 88, 639–648.

McLaren, G.W., Thornton, P.D., Newman, C., Buesching, C.D., Baker, S.E., Mathews, F., Macdonald, D.W., 2005. The use and assessment of ketamine-medetomidine-butorphanol combinations for field anaesthesia in wild European badgers (*Meles meles*). Vet. Anaesth. Analg. 32, 367–372.

Mech, L.D., Meier, T.J., Seal, U.S., 1993. Wolf nipple measurements as indexes of age and breeding status. Am. Midl. Nat. 129, 266–271.

Newman, C., Macdonald, D.W., Anwar, M.A., 2001. Coccidiosis in the European badger, *Meles meles* in Wytham Woods: infection and consequences for growth and survival. Parasitology 123, 133–142.

Oli, M.K., Dobson, F.S., 2003. The relative importance of life-history variables to population growth rate in mammals: Cole's prediction revisited. Am. Nat. 161, 422–440.

Petrides, G.A., 1950. The determination of sex and age ratios in fur animals. Am. Midl. Nat. 43, 355–382.

Queller, D.C., Goodnight, K.F., 1989. Estimating relatedness using genetic-markers. Evolution 43, 258–275.

Roper, T.J., 2010. Badger. Collins, London.

Thompson, E.A., 1976. A paradox of genealogical inference. Adv. Appl. Probab. 8, 648–650.

Thornton, P.D., Newman, C., Johnson, P.J., Buesching, C.D., Baker, S.E., Slater, D., Johnson, D.D.P., Macdonald, D.W., 2005. Preliminary comparison of four anaesthetic regimens in Eurasian badgers (*Meles meles*). Vet. Anaesth. Analg. 32, 40–47.

Vehrencamp, S.L., 1979. The roles of individual, kin, and group selection in the evolution of sociality. In: P. Marler, J.G. Vandenbergh (Eds.), Handbook of Behavioral Neurobiology. Social Behavior and Communication. Plenum, New York, vol. 3, pp. 351–394.

Vernes, K., 2004. Breeding biology and seasonal capture success of northern flying squirrels (*Glaucomys sabrinus*) and red squirrels (*Tamiasciurus hudsonicus*) in southern New Brunswick. Northeast. Nat. 11, 123–136.

Woodroffe, R., Macdonald, D.W., 1995. Female/female competition in European badgers *Meles meles*: effects on breeding success. J. Anim. Ecol. 64, 12–20.

Yamaguchi, N., Dugdale, H.L., Macdonald, D.W., 2006. Female receptivity, embryonic diapause and superfoetation in the European badger (*Meles meles*): implications for the reproductive tactics of males and females. Q. Rev. Biol. 81, 33–48.

Table 1 Estimates and the standard errors (S.E.) of the fixed effects used to model teat length (272 records from 135 females) and diameter (244 records from 123 females). Bred 1 = assigned maternity; Bred 0 = not assigned maternity. Significant (type 3) *P*-values are shown in bold.

		Teat length[1]					Teat diameter[2]				
		Estimate	S.E.	*F*	*df*	*P*	Estimate	S.E.	*F*	*df*	*P*
Intercept		11.53	0.86				7.19	0.86			
Month		-0.86	0.14	38.0	1,135	< **0.0001**	-0.35	0.14	6.3	1,119	**0.0137**
Bred	0	-3.24	0.28	137.0	1,135	< **0.0001**	-2.18	0.28	62.4	1,119	< **0.0001**
	1	0.00	.				0.00	.			

[1]The random effect estimates ± standard errors (S.E.) were: individual variance = 1.6 ± 0.4 (to control for repeated measures on individual females) and residual variance = 2.7 ± 0.3.

[2]The random effect estimates ± S.E. were: individual variance = 2.3 ± 0.5 and residual variance = 2.0 ± 0.3.

Table 2 Estimates and the standard errors (S.E.) of the fixed effects used to model teat length (167 records from 89 mothers) and diameter (165 records from 87 mothers) of females that were genetically assigned as mothers. Significant (type 3) *P*-values are shown in bold.

	Teat length[1]					Teat diameter[2]				
	Estimate	S.E.	*F*	*df*	*P*	Estimate	S.E.	*F*	*df*	*P*
Intercept	11.46	1.10				7.35	1.14			
Month	-1.08	0.17	40.1	1,76	< **0.0001**	-0.50	0.18	8.2	1,76	**0.0053**
Number of cubs	0.94	0.22	18.6	1,76	< **0.0001**	0.54	0.22	5.8	1,76	**0.0182**

[1]The random effect estimates ± standard errors (S.E.) were: individual variance = 1.9 ± 0.6 (to control for repeated measures on individual females) and residual variance = 2.3 ± 0.4.

[2]The random effect estimates ± S.E. were: individual variance = 3.0 ± 0.7 and residual variance = 2.0 ± 0.3.

Table 3 Estimates and the standard errors (S.E.) of the fixed effects used to model the probability that a mother was assigned maternity in a genetic parentage analysis (with 95% confidence), based on 242 records from 122 females (that had data on both their teat lengths and diameters). Significant (type 3) *P*-values are shown in bold.

	Estimate	S.E.	*F*	*df*	*P*
Intercept	-20.01	6.17			
Month	1.75	0.65	7.2	1,117	**0.0084**
Teat length	1.60	0.49	10.7	1,117	**0.0014**
Teat diameter	0.96	0.41	5.5	1,117	**0.0206**

[1]The variance estimate of individual (random effect) ± S.E. was 32.6 ± 26.3.

Table 4 Percentage of females correctly classified in normal and cross-validation discriminant analyses. A restricted dataset was used, containing one entry per female (that had data on both their teat lengths and diameters) for 47 non-breeders and 75 breeders. The analyses were also run excluding two non-breeders that were outliers.

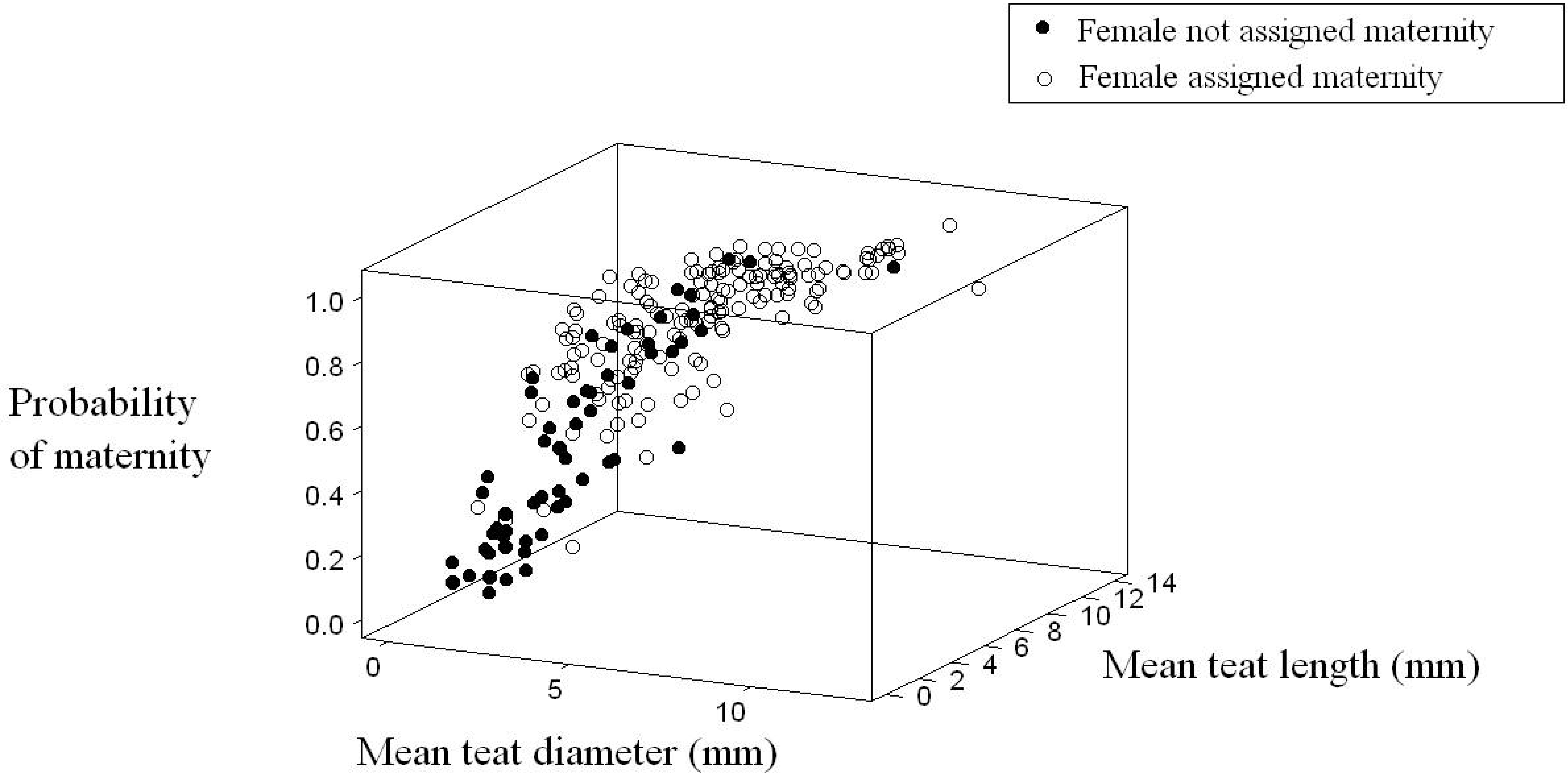
	Percentage correctly classified (excluding outliers)						Percentage correctly classified (including outliers)					
	Normal procedure			Cross-validation procedure			Normal procedure			Cross-validation procedure		
Trait(s)	Overall	Non-breeder	Breeder	Overall	Non-breeder	Breeder	Overall	Non-breeder	Breeder	Overall	Non-breeder	Breeder
Teat length & diameter	83	84	81	81	82	80	80	81	80	79	79	80
Teat length	81	82	79	81	82	79	78	83	74	78	83	74
Teat diameter	75	85	64	75	85	64	74	83	64	74	83	64

Table 5 Rates of misclassification of genetic maternity status from five analyses based on mean teat length and diameter. The predicted probability of maternity from a Generalised Linear Mixed Model (GLMM; Table 2) was either cut-off at 0.80 or 0.95 to classify breeders. The discriminant procedures used a restricted dataset of one record from 45 non-breeders and 75 breeders (excluding two outliers). The GLMM and the cruder analysis (> 5mm diameter and > 2mm length) were based on 242 trapping records (136 females).

	Overall	Breeders	Non-breeders
GLMM, 0.80 criteria	5%	5%	3%
GLMM, 0.95 criteria	12%	15%	1%
Cross-validation discriminant function	19%	20%	18%
Normal discriminant function	17%	19%	16%
Diameter > 5 mm & length > 2 mm	38%	51%	9%

Figure legend

Figure 1 Mean teat diameter (mm; x-axis) and length (mm; y-axis) of each female, per trapping event, against the predicted probability of each female being assigned maternity (z-axis) obtained from a Generalised Linear Mixed Model that controlled for month. Closed symbols represent females that were not assigned maternity and open symbols females that were assigned maternity in a genetic parentage analysis (with 95% confidence).

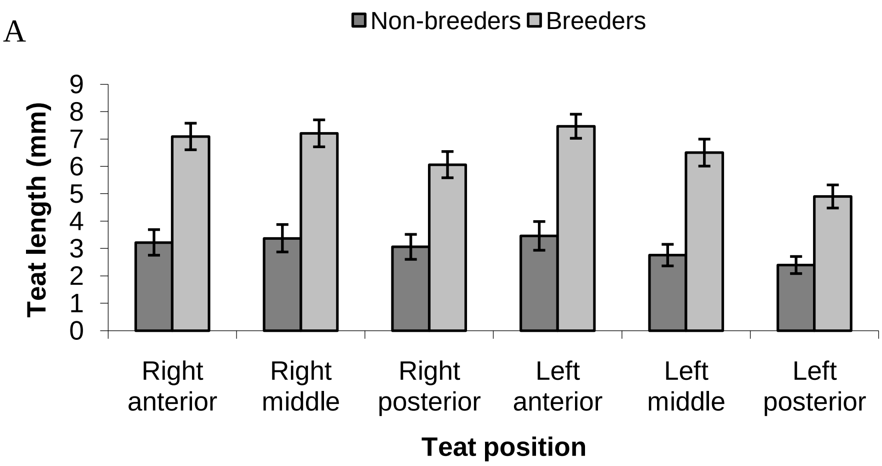

Female not assigned maternity
Female assigned maternity
Probability of maternity
0.0
0.2
0.4
0.6
0.8
1.0
Mean teat diameter (mm)
0
5
10
Mean teat length (mm)
0
2
4
6
8
10
12
14

FEMALE TEAT SIZE IS A RELIABLE INDICATOR OF ANNUAL BREEDING SUCCESS IN EUROPEAN BADGERS: GENETIC VALIDATION

HANNAH L. DUGDALE*[+†], DAN DAVISON[‡], SANDRA E. BAKER[+], STEPHEN A. ELLWOOD[+], CHRIS NEWMAN[+], CHRISTINA D. BUESCHING[+], AND DAVID W. MACDONALD[+]

Supplementary Figure 1: Mean teat (A) lengths [mm] and (B) diameters [mm] of adult females that were assigned maternity with 95% confidence, and that were not assigned maternity and came from a social group in which all cubs were assigned a mother with 95% confidence. Error bars display the 95% confidence intervals around the means.

A



B