# A microsatellite perspective on the reproductive success of subordinate male honey badgers, *Mellivora capensis*

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**The dominance hierarchy system of male honey badgers, *Mellivora capensis,* does not appear to determine reproductive success in the species as subordinate males are frequently seen to gain brief access to receptive females. To establish whether these interactions lead to fertilization, five microsatellite loci were used to infer paternity in a honey badger population from the Kgalagadi Transfrontier National Park. In total, 26 individuals comprising 10 cubs, eight known mothers and eight potential fathers were analysed. Exclusion analysis and a likelihood-based approach identified the dominant male in the study population as the most likely father of 50% of the cubs (≥80% likelihood confidence). The remaining five cubs were most likely fathered by four different subordinate males (≥80% likelihood confidence). The results suggest that the brief mating opportunities afforded to subordinate males lead to fertilization, and hence that the dominance hierarchy system of the honey badger is not strong enough to exclude sneak fertilizations.**

**Key words:** **Mustelidae, ratel, paternity, dominance hierarchy.**

The social organization and reproductive biology of the honey badger, *Mellivora capensis* (family Mustelidae) is not well documented and, owing to the elusive nature of this carnivore, most reports are based on rare sightings. To address these uncertainties a behavioural study on the honey badger was recently conducted by Begg (2001). The mating behaviour of 15 adult male honey badgers was monitored within an 844 km$^2$ area in the central dune region of the Kgalagadi Transfrontier National Park (KTNP) over a period of 42 months. It was found that in this region the species is solitary, with marked sexual dimorphism and a polygynous or promiscuous mating system. In other words, older males (identified by a prominent scar in the middle of their backs, believed to be the result of repeated bites in the same area over time) were always dominant over younger, non-scarback males. Within scarback males, dominant individuals tended to be heavier (75% of interactions) and have larger testes (100%) than appeasing males, but otherwise no pattern in shoulder height or body length was evident. In addition it was suggested that adult males do not follow the typical mustelid pattern of intra-sexual territoriality (Powell 1979), but instead have large, overlapping home ranges (mean = 548 km$^2$) that incorporate the smaller home ranges (mean = 138 km$^2$) of up to 13 females (Begg 2001). Asynchronous breeding and a long inter-birth interval (>12 months) also result in a skewed operational sex ratio (OSR; Emlen & Oring 1977): at any time there are more males than females looking for mating opportunities (Begg 2001). Male honey badgers thus compete directly for access to receptive females and typically adopt intimidation or appeasement postures upon contact with each other. Because these behavioural patterns are retained towards each other on subsequent encounters, Begg (2001) suggested the existence of a non-linear dominance hierarchy among male honey badgers.

The hierarchy system is, however, no guarantee that only the dominant male will reproduce. Subordinate males frequently, albeit briefly, gain access to receptive females (Begg 2001). Whether these 'sneak' visits result in fertilization cannot be inferred by simple observation. The situation is exacerbated by the fact that mating is concealed within a burrow and a single female is usually exposed to several males during her receptive period. Genetic markers, and more specifically microsatellite analysis, have proven to be an effective way to evaluate reproductive success in wildlife populations (Gullberg *et al.* 1997; Hughes 1998; Coltman *et al.* 1999). These markers combine high variability with nuclear co-dominant inheritance and can be typed following non-invasive sampling. In addition, primers developed in one species can

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**Table 1**. Sample collection. Social status of each adult male and cub(s) born to each adult female are indicated. Names and social status as given by Begg (2001).

| Adult male | Social status | Adult female | Cub(s) |
|---|---|---|---|
| HB-12 | Dominant scarback | HB-16 | HB-27 |
| HB-18 | Subordinate scarback | HB-20 | HB-21 |
| HB-23 | Subordinate scarback | HB-25 | HB-44 & HB-26* |
| HB-24 | Subordinate non-scarback | HB-30 | HB-29 |
| HB-36 | Subordinate non-scarback | HB-31 | HB-40 & HB-32* |
| HB-43 | Subordinate scarback | HB-34 | HB-33 |
| HB-46 | Subordinate non-scarback | HB-38 | HB-39 |
| HB-47 | Subordinate scarback | HB-41 | HB-42 |

*Born from separate litters.

often be used in closely related taxa (Burland *et al.* 2001; Kayang *et al.* 2002; Williamson *et al.* 2002).

To assess whether the brief mating opportunities afforded to subordinate males contribute to the genetic diversity of the population, five microsatellite markers were used to infer paternity in a honey badger population from the KTNP. Hair samples were obtained from the dominant male (HB-12) and from seven subordinate males (Table 1). HB-12 was awarded dominant status based on 41 visual observations of his interactions with seven males where he was observed to be the aggressor in each event (Begg 2001). The eight adult females with whom the eight males interacted and the 10 cubs born consequently were also sampled (Table 1). Honey badgers in the KTNP appear to only raise a single cub at a time (Begg 2001).

In the present study there could thus only be one father per reproductive cycle, a scenario in contrast to most other mustelids that rear more than one cub (Johnson *et al.* 2000).

DNA was extracted from the hair samples through exposure of the roots to standard phenol/chloroform extraction procedures (Sambrook *et al.* 1989). At present no species-specific primers are available for *M. capensis,* and 17 mustelid microsatellite loci, isolated from the wolverine, (*Gulo gulo;* Gg prefix), Eurasian otter (*Lutra lutra*; Ll), fisher (*Martes americana*; Ma) and American badger (*Taxidea taxus*; Tt); were thus screened for cross-species amplification: Gg7, Ma1, Ma10, Ma14, Tt1, Tt3, Tt4 (Davis & Strobeck 1998), Ll615 (Dallas & Piertney 1998) and Gg10, Gg14, Gg25, Gg42, Gg50, Gg443, Gg452, Gg454, Gg465 (Walker *et al.* 2001). Of these, five loci were sufficiently polymorphic for paternity analyses. One primer of each primer pair was labelled with a fluorescent dye to allow for simultaneous analyses on an automated sequencer (ABI 3100). The primers were labelled according to product size (Table 2): Gg10, Gg454 and Ma1 with FAM, Tt1 with VIC, Tt4 with PET.

Amplifications were carried out on a GeneAmp PCR System 2700 (AB) in 10 $\mu$l reaction volume containing 1 × PCR buffer, 5–10 mM $MgCl_2$ (Table 2), 20 mM of each primer, 1 mM of each dNTP, 1 unit of Supertherm DNA polymerase (Southern-Cross Biotechnology) and approximately 25 ng DNA. Microsatellites were scored and visualized using GENESCAN and GENOTYPER software (ABI).

Paternity was assessed using two methods.

**Table 2**. Number of alleles, expected heterozygosities ($H_E$), estimated frequency of null alleles and estimates of paternity exclusion probabilities of microsatellite loci used in paternity analyses.

| Locus | No. of alleles | Fragment size | $MgCl_2$mM | $H_E$* | Null alleles† | Exclusion probability |
|---|---|---|---|---|---|---|
| Gg10 | 10 | 164–184 | 10 | 0.884 | –0.0285 | 0.73 |
| Gg454 | 8 | 128–150 | 10 | 0.837 | +0.0472 | 0.64 |
| Ma1 | 5 | 198–204 | 5 | 0.735 | –0.0734 | 0.48 |
| Tt1 | 6 | 148–160 | 5 | 0.580 | –0.0272 | 0.36 |
| Tt4 | 6 | 164–184 | 5 | 0.726 | –0.1368 | 0.49 |

*Expected heterozygosity under Hardy-Weinberg equilibrium.
†Null allele frequencies were estimated using CERVUS, according to Summers & Amos (1997).

**Table 3**. Number of paternity exclusions at each informative locus for all sampled males. Total number of paternity exclusions and percentage of calves excluded (out of the 10 mother/cub pairs analysed) are indicated for each male.

| Male | Locus | | | | | Total no. exclusions | % Cubs excluded |
|---|---|---|---|---|---|---|---|
| | Gg10 | Gg454 | Tt1 | Tt4 | Ma1 | | |
| HB-12* | 5 | 1 | 0 | 0 | 0 | 5 | 50 |
| HB-18† | 0 | 1 | 0 | 0 | 0 | 1 | 10 |
| HB-23† | 7 | 5 | 0 | 1 | 2 | 9 | 90 |
| HB-24° | 7 | 8 | 0 | 1 | 3 | 9 | 90 |
| HB-36° | 7 | 6 | 0 | 0 | 3 | 10 | 100 |
| HB-43† | 8 | 6 | 0 | 0 | 2 | 9 | 90 |
| HB-46° | 9 | 7 | 0 | 0 | 0 | 10 | 100 |
| HB-47† | 7 | 0 | 0 | 1 | 6 | 9 | 90 |

*Dominant scarback.
†Subordinate scarback.
°Subordinate non-scarback.

Exclusion analysis was performed through exclusion of all males possessing at least one microsatellite allele incompatible with the genotype of the cub in question (Queller *et al.* 1993). Such an approach was possible since each cub could also be matched to the known maternal profile. Cognizant of the fact that exclusion analysis might be biased by the possible paternity of males not sampled, a likelihood-based approach was also employed to assign paternity. The paternity simulation program CERVUS was used to predict critical log-likelihood (ΔLOD) scores to assign paternity at confidence levels of 80% and 95% (Marshall *et al.* 1998). It is important to realize that paternities assigned with 80% confidence are more accurate than can be achieved by a purely exclusionary approach, especially where confidence in paternity of non-excluded males is generally unknown (Marshall *et al.* 1998). Based on field observations (Begg 2001), the number of candidate males (i.e. per mating) was set at three and the 'Proportion of males sampled' was set at 53%. Other parameters set for CERVUS were 0.88 for the proportion of loci typed and 0.01 for rate of typing error (see also Coltman *et al.* 1999; Taylor *et al.* 2000; Whitehouse & Harley 2002).

The five loci showed relatively high levels of polymorphism, with an average of seven alleles per locus, and an average expected heterozygosity of 0.75 (Table 2). Exclusion probabilities ranged from 0.36 to 0.73 (Table 2) with a high combined average exclusion probability of 0.98.

Although locus Gg454 had a relatively high estimated null allele frequency (0.0472; Table 2) the locus was not excluded from parentage analyses (Marshall *et al.* 1998). No pattern of repeated homozygote-homozygote mismatches between mother/cub pair was detected by CERVUS, suggesting that homozygote excess at locus Gg454 was not due to a null allele.

Using exclusion analysis, HB-12, the dominant male, was rejected as the father of 50% ($n = 5$) of the cubs (Table 3). Subordinate males were excluded from fathering between 10% (HB-18) and 100% (HB-36 & HB-47) of the cubs (Table 3). Most of these exclusions were based on single loci (for HB-12, four of five exclusions were based on a single locus, one was based on two loci). For six of the cubs (HB-27, HB-32, HB-39, HB-40, HB-42, HB-44) multiple males remained non-excluded.

CERVUS was able to assign paternity with 80% confidence to all 10 cubs and with 95% to two of the cubs (Table 4). HB-12 was identified as the most likely father of 50% ($n = 5$) of the cubs. Three

**Table 4**. Delta criterion scores, predicted and observed number of paternities assigned at varying levels of confidence. Values were derived from 10 000 simulations using CERVUS.

| Confidence level | Delta criterion | Predicted paternities | Paternities allocated |
|---|---|---|---|
| 95% | 1.56 | 4 (43%) | 2 (20%) |
| 80% | 0.00 | 6 (64%) | 10 (100%) |

of these paternities were assigned with 80% confidence and two with 95%. Similar to the results obtained using an exclusion principle, the remaining five cubs were assigned to subordinate males with 80% confidence (HB-18 probably fathered two cubs while HB-23, HB-24 and HB-46 probably fathered a single cub each). The percentages of cubs for which paternity could be assigned varied from those predicted by the program's simulation test and the delta criterion value for the 80% confidence interval was lower than the value obtained for the 95% interval (also see Hatchwell *et al.* 2002). The differences between the predicted and expected number of paternity assignments could be attributed to several factors, including the inaccurate assessment of the number of potential males in the study area (Marshall *et al.* 1998).

While sample sizes were small, the congruence among the two methods used by us provide evidence that at least 50% of the cubs in the study population were probably not fathered by the dominant male (≥80% likelihood confidence). It is also encouraging to report that in support of the concept of a hierarchy system, the dominant male was selected as the most likely father in more instances than any one subordinate male (≥80% likelihood confidence). It thus seems reasonable to conclude that although the dominant male in the study area contributed most to the gene pool, subordinate males also play a role in the reproductive success of the population.

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