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# Pregnancy is detected via odour in a wild cooperative breeder

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Among mammals, scent has long been known to encode oestrus; however, in many species, detecting pregnancy may also be important in terms of both competition and mate-choice. Here, we show, through odour presentation experiments, that pregnancy is discernible via scent by both sexes in the cooperatively breeding banded mongoose, *Mungos mungo*. Males spent more time investigating and were more likely to scent mark the odours of non-pregnant females, compared to pregnant females. Females showed increased levels of scent marking when odours were of the same reproductive state as themselves. These results present the first direct demonstration that pregnancy is detectable via scent in wild cooperative breeders. Detecting pregnancy may be particularly important in cooperative breeders as, in addition to the competition between males for receptive mates, there is also intense competition between females for access to alloparental care. Consequently, dominant females benefit from targeting reproductive suppression towards subordinates that represent direct threats, such as pregnant females.

## 1. Introduction

Scent cues are heavily used among mammals and are known to encode information on female reproductive state, with many studies demonstrating that males can detect oestrus [1–3]. However, relatively little is known about whether scent can communicate pregnancy status [4,5]. Detecting pregnancies may help males to avoid courting pregnant females, while it may help females assess their competitive landscape [6]. This may be particularly beneficial among cooperative breeders, where competition over access to breeding positions, and hence access to alloparental care, is intense in both sexes [7]. Here, *per capita* breeding success generally declines when multiple females breed, and dominant females may respond by suppressing subordinates that may be a particular threat, for example, those that are pregnant or are likely to become pregnant [7–9]. In addition, dominant males often invest highly in guarding females during fertile periods [7]. Thus, in cooperative breeders, the communication of pregnancy may benefit both mate-choice and intra-sexual competition.

Studies investigating olfactory cues to pregnancy in mammals have so far focused on investigating the chemical profiles of female scents before and during pregnancy, e.g. [4,10,11]. While these studies have discovered chemical differences between pregnant and non-pregnant females, they do not demonstrate whether these changes are detected or acted upon by conspecifics. It is therefore possible that differences in chemical profiles are simply a by-product of hormonal changes that occur during mammalian gestation [4] and are not used to detect pregnancy.

Here, we investigate behavioural responses to scents of pregnant and non-pregnant female banded mongooses *Mungos mungo*. This species lives in mixed sex groups (mean group size = 29) where a ‘core’ of one to five dominant breeders of each sex breed up to four times per year, and younger subordinates breed occasionally [12]. Reproduction is synchronized within groups, with all adult females entering oestrus within the same week and giving birth together, often on the same night [12]. The resulting litter is raised communally by both breeders and non-breeders [12]. Dominant females benefit from increased reproductive success when breeding alongside other females, probably due to reduced levels of infanticide [8]. However, once the number of breeding females exceeds seven, *per capita* reproductive success declines due to increased pup mortality [8]. Dominant females in large groups respond by evicting subordinate females, particularly targeting those that are pregnant [8]. Thus, the detection of pregnancies could provide a mechanism for assessing the competitive landscape of the group. In addition, synchronous oestrus constrains the number of females a male can guard, so dominant males invest highly in one or two mates per reproductive bout [12]. Pregnancy detection could therefore help males to avoid guarding already-mated females.

We predict that (i) if males use scent signals within mate-choice, they should show heightened responses to non-pregnant females and (ii) if females use scent signals within reproductive competition, they should show heightened responses to the odours of females representing direct reproductive threats, in particular pregnant females should show a greater response to odours from other pregnant females.

## 2. Material and methods

This study was conducted in Queen Elizabeth National Park, Uganda (0°8′2″ S, 29°51′42″ E), where a population of wild but habituated banded mongooses has been studied continuously since 1995. Groups are visited by trained observers approximately every 2 days to collect life-history and behavioural data. Detailed descriptions of the population, habitat and climate are provided by Cant *et al*. [12].

### (a) Odour collection

Banded mongooses are prolific scent markers, engaging in conspicuous anal marking, urination and defecation at latrine sites [13]. Previous work has found that anal marking plays a key role in within-group communication and intra-sexual competition [13], so for this study, we focused on anal gland secretions (AGS).

AGS were collected from females in four social groups between April and July 2015 following [14]. We obtained 111 samples (63 pregnant and 48 non-pregnant but non-oestrus) from 54 individual females that were each sampled one to three times. In brief, animals were trapped in baited Tomahawk traps and anaesthetized using isoflurane [13]. Pregnancy status was determined by an ultrasound scan 7–14 days after behavioural oestrus and AGS were collected in a clean 2 ml snap-cap glass vial and were transferred immediately to liquid nitrogen. Further details are provided in the electronic supplementary material.

### (b) Odour presentations

A total of 142 odour presentations were conducted from July to August 2015 on 32 males and 28 females from two well-habituated social groups. Recipients were presented with freshly defrosted AGS samples from pregnant or non-pregnant females. AGS samples were spread upon a clean ceramic tile using an autoclaved cotton swab, and presented directly to the recipient individual following [14]. Presentations were conducted when the recipient was foraging at least 1 m away from other mongooses. Responses were filmed using a handheld camera and scored after the field session. Three measures of response to odour presentations were considered: (i) the time before returning to foraging behaviour; (ii) the time spent inspecting the odour (within 30 cm); and (iii) the number of scent marks deposited on or around the odour. Previous research on banded mongooses and other species suggests that direct over-marking can obliterate the original scent and is therefore likely to function in competition [13,15,16]. For presentations to female recipients, who may use scent cues for intra-sexual competition, we recorded the number of marks deposited directly on top of an odour. For presentations to male recipients, we recorded the number of marks deposited within 30 cm of the odour as vicinity marking is thought to function within mate-acquisition, rather than competitive interactions [15]. The three measures of scent marking behaviour are not fully independent of one another as both scent marking and time spent inspecting an odour correlate with the time taken to return to foraging in male and female datasets. For full details of this correlation, see electronic supplementary material, table S4. Donors and recipients were sexually mature adults (aged over 12 months for females and over 24 months for males [12]). Presentations were made to recipient females within 7 days of an ultrasound scan confirming their reproductive state. Where multiple presentations were made to an individual, a minimum of 48 h lapsed between presentations to prevent habituation to the protocol. Recipients were presented with odours from non-neighbouring groups to avoid confounding results with previous information on the reproductive state of odour donors.

General linear mixed effect models (GLMMs) were constructed in R (v. 3.0.2) using the lme4 package [17] to test the effect of odour donor pregnancy status on the response of male and female recipients. Where significant interactions were detected, the Multcomp package [18] was used to perform Tukey *post hoc* comparison tests compare response measures. All models were fit with Gaussian assumptions as response variables were normally distributed. For full model details and outputs, see electronic supplementary material, tables S1–S3.

## 3. Results and discussion

Pregnancy appears discernible by scent in the banded mongoose, with both sexes responding differently to odours from pregnant and non-pregnant females. In line with our first prediction, males spent longer investigating non-pregnant odours (GLMM: $t = -2.282$, $p = 0.029$, figure 1*a*) and took longer to return to foraging (GLMM: $t = -2.454$, $p = 0.019$, figure 1*b*), suggesting that odours encode information relevant to mate-choice. Detecting pregnancy via scent is likely to be beneficial to males, as it could prevent them from wasting time and energy mate-guarding pregnant females.

Male banded mongooses also deposited more scent marks around the odours of non-pregnant females (GLMM: $t = -3.275$, $p = 0.002$, figure 1*c*). Increased scent marking by males may function in intra-sexual competition, whereby males that invest highly in scent marking are more effective mate-guards [19]. Alternatively, scent marking may be involved in female-choice, as has been demonstrated in other mammals [20]. Despite being mate-guarded while in oestrus, banded mongoose females often refuse the mating attempts of their guards and 68% of pups are fathered by a male not observed to guard the female [21]. Scent marking in the vicinity of receptive females may therefore serve to advertise males to potential mates.

Female banded mongooses responded differently to pregnant and non-pregnant odours in their over-marking response depending on their own pregnancy status (GLMM:

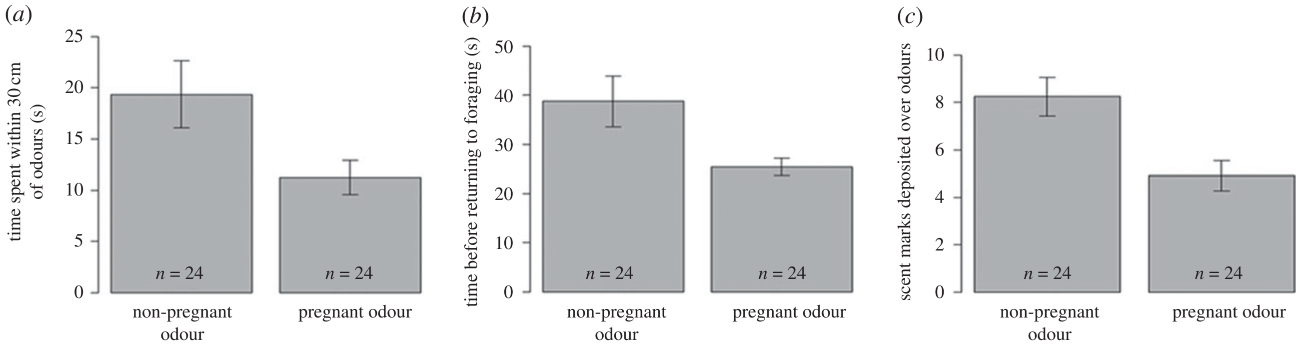

**Figure 1.** Differences in the response of males to the odours of pregnant and non-pregnant females in relation to (*a*) the length of time spent within 30 cm of the odour, (*b*) the length of time before returning to foraging and (*c*) the number of scent marks deposited within 30 cm of the odour. Error bars show standard error.

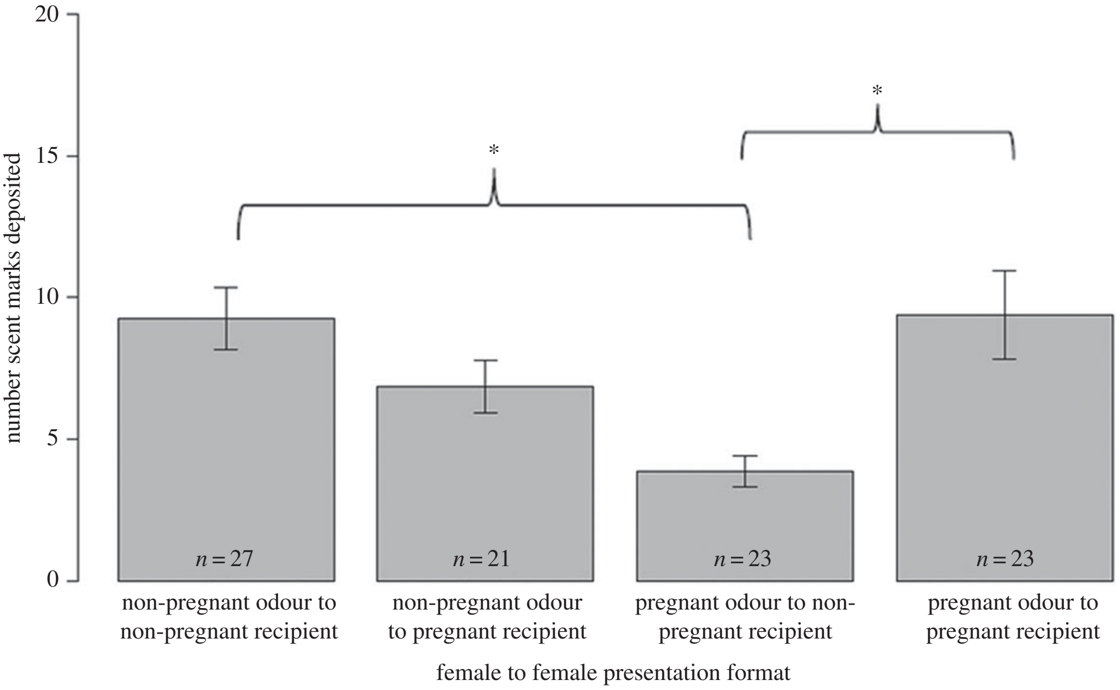

**Figure 2.** The number of scent marks deposited by pregnant and non-pregnant recipients on scents from pregnant and non-pregnant donors. Brackets and asterisks illustrate significant differences between conditions at either end of the bracket. Error bars show standard error.

$t = 3.231$, $p = 0.0017$, figure 2), suggesting that they can detect pregnancy in other females. In line with our prediction, pregnant odours received more over-marks from pregnant recipients than from non-pregnant recipients (Tukey: $z = 3.338$, $p = 0.004$). Similarly, non-pregnant recipients marked the odours of non-pregnant females significantly more than they did the odours of pregnant females (Tukey; $t = -2.811$, $p = 0.025$). The finding that females show heightened over-marking when odours were from females in the same reproductive state suggests that scent marking may be related to intra-sexual competition, whereby the scents of potential competitors are over-marked in order to obliterate their scent [13,15].

The finding that pregnant females inspected scents for longer (GLMM: $t = 2.686$, $p = 0.009$) and took longer to return to foraging (GLMM: $t = 2.245$, $p = 0.027$) than non-pregnant females suggests that detecting the reproductive state of others could be particularly important when pregnant. Indeed, evictions are most common when dominant females are pregnant [8]. We also found that younger females spent longer inspecting odours (GLMM: $t = -3.143$, $p = 0.002$) and deposited more scent marks around odours (GLMM: $t = -2.313$, $p = 0.023$) than older females, possibly as younger subordinate individuals are more likely to be targeted for eviction and their litters are more vulnerable to infanticide than those of dominants [22]. Furthermore, abortion and reabsorption of pregnancies are known to occur in the banded mongoose [8] and, as in other mammals [9], these may be adaptive strategies for mothers who find themselves out-competed or out of synchrony with other breeders. Detecting pregnancies may therefore help females to avoid or respond to reproductive competition.

In many territorial species, reproductive threats come not just from within the social group, but also from competing social groups [9]. In the banded mongoose, neighbouring groups engage in frequent aggressive encounters over territory, often resulting in severe injury and deaths [23]. As we presented odours from individuals that recipients are unlikely to be familiar with, it is possible that scents may be used to assess the competitive landscape between social groups. For example, knowing the reproductive status of females in other groups could allow individuals to time aggressive encounters to periods of vulnerability, such as when pups are present (young pups are most likely to be present when females are non-pregnant and non-oestrus). In addition, mating between groups sometimes occurs during aggressive encounters [23]. Through inspecting scent marks, males may be able to assess potential inter-group mating opportunities. Future work investigating the timing of inter-group interactions will shed light on these possibilities.

Ethics. Research was approved by the Uganda National Council for Science and Technology and Uganda Wildlife Authority (EDO/35/01), and procedures were approved by the University of Exeter's Ethical Review Committee.

Data accessibility. Data are available in Dryad (http://dx.doi.org/10.5061/dryad.0ss0k) [24].

Authors' contributions. J.M. conceived the study, collected data and conducted analyses. H.J.N. and J.M. wrote the paper with comments from M.A.C. H.J.N. supervised data collection and analyses. M.A.C. coordinated the field project. All authors gave final approval for publication and agree to be accountable for the all aspects of the work.

Competing interests. We declare we have no competing interests.

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