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Dung as a potential medium for inter-sexual chemical signaling in Asian elephants (*Elephas maximus*)

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ABSTRACT

Chemical signaling is a prominent mode of male–female communication among elephants, especially during their sexually active periods. Studies on the Asian elephant in zoos have shown the significance of a urinary pheromone (*Z*7-12:Ac) in conveying the reproductive status of a female toward the opposite sex. We investigated the additional possibility of an inter-sexual chemical signal being conveyed through dung. Sixteen semi-captive adult male elephants were presented with dung samples of three female elephants in different reproductive phases. Each male was tested in 3 separate trials, within an interval of 1–3 days. The trials followed a double-blind pattern as the male and female elephants used in the trials were strangers, and the observer was not aware of the reproductive status of females during the period of bioassays. Males responded preferentially ($P < 0.005$), in terms of higher frequency of sniff, check and place behavior toward the dung of females close to pre-ovulatory period (follicular-phase) as compared to those in post-ovulatory period (luteal-phase). The response toward the follicular phase samples declined over repeated trials though was still significantly higher than the corresponding response toward the non-ovulatory phase in each of the trials performed. This is the first study to show that male Asian elephants were able to distinguish the reproductive phase of the female by possibly detecting a pre-ovulatory pheromone released in dung.

1. Introduction

Chemical signals are reliable indicators of an individual's reproductive condition because they are closely linked to physiological changes associated with reproduction. They are probably honest signals that convey to the receiver the reproductive state of the sender (Gittleman, 1989). Most chemical signals are highly species-specific for, e.g., copulin from the vagina of the rhesus monkey (*Macaca mulatta*: Michael, 1973), valeric acid or a mixture of fatty acids produced by oestrous felids (Bland, 1979) and frontalin in Asian elephants (*Elephas maximus*: Rasmussen and Greenwood, 2003). These signals trigger a whole set of behavioral responses. Several studies have documented the behavioral responses of individuals toward the chemical signals generated by members of the opposite sex as, for example, African elephant (*Loxodonta africana*: Bagley et al., 2006), black-tailed deer (*Odocoileus hemionus*: Henderson et al., 1980), sable antelope (*Hippotragus niger*: Thompson, 1995) and several bovine species (Vandenbergh and Izard, 1983). Thus, the importance of chemical signals has increasingly been recognized in the field of reproductive biology to understand the reproductive state and performance of individuals through analysis of species-specific behavioral cues (Berger, 1992).

Being highly developed social animals, elephants rely mostly on chemical signals to extract information from their surroundings (Rasmussen and Schulte, 1998; Rasmussen and Krishnamurthy, 2000). The importance of chemical senses to elephants was recognized in early Sanskrit writing (for example, *The Matanga-Lila of Nilakantha*; see translation by Edgerton, 1931). This millennium-old Sanskrit text states: "Upon smelling their own dung and urine, let them always be producing a tickling of the palate (an attraction for it)!" [Chapter 1, Stanza 38], and ". . .those who are born in the spring are scent elephants. And from the smell of their sweat, dung, urine and *must*-fluid [i.e., musth fluid] other elephants instantly are excited." [Chapter 1, Stanza 40]. The extraordinary sense of smell of elephants and their use of such ability to communicate important social and physiological information is quite evident (Krishnan, 1972; Rasmussen and Krishnamurthy, 2000).

Among elephants, adult males and females experience different social environments, where males are usually solitary while the females are part of a highly structured, hierarchical society (Moss, 1983; Sukumar, 2003; de Silva and Wittemeyer, 2012). During sexually active periods, especially when in musth (Poole, 1987; Fernando et al., 2008), males wander widely in search of females for

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the purpose of mating. Female elephants have an estrous cycle comprising a shorter follicular phase of 4–6 weeks and a longer luteal phase of 8–10 weeks (Brown, 2000), with a window of behavioral receptivity lasting approximately only 2–8 days (Jainudeen et al., 1971). Studies have thus shown that, under this condition, individuals of both sexes rely mostly on chemical signals for the purpose of mating (Rasmussen and Wittemyer, 2002; Rasmussen et al., 2005). For instance, female Asian elephants secrete a urinary pheromone, (*Z*)-7-dodecen-1-yl acetate (*Z*7-12:Ac) (Rasmussen et al., 1996) during their follicular/ovulatory phase as a female-to-male signal. The concentration of *Z*7-12:Ac in urine gradually increases from the beginning of the follicular phase, reaching its peak about a day prior to ovulation, and then declines to levels below detection (Rasmussen, 2001). Males show typical chemosensory response (for example, sniff, check, place, flehmen; Rasmussen et al., 1997) toward *Z*7-12:Ac (both natural and synthetic), where the response is positively related to the urinary concentration of the compound, indicating that they are obtaining cues from this pheromone on the reproductive phase of the female.

The role of dung in indicating the reproductive state, dominance status and home range boundary of an individual toward its conspecifics has been investigated in many species, e.g., male oribi (*Ourebia ourebi*: Brashares and Arcese, 1999), bushbuck (*Tragelaphus scriptus*: Wronski et al., 2006) and badger (*Meles meles*: Palphramand and White, 2007). However, until now, the role of dung in signaling the reproductive state of an individual has not been studied in Asian elephants. Several studies have shown that both male and female Asian elephants usually broadcast the chemical signals through different media such as urine, temporal gland secretion, vaginal mucus, and inter-digital glands, perhaps to increase the probability of an encounter with signal receivers (Lamps et al., 2001; Rasmussen, 1998, 1999; Rasmussen et al., 2003). Thus, it can be hypothesized that dung might advertise a similar or an unique compound as compared to other media. This study makes the first attempt to understand the role of dung in indicating the reproductive status of female Asian elephants, using behavioral bioassays on male elephants kept under semi-captive condition. The objectives of the study are two-fold: (i) to examine the type of behavioral response by male elephants toward the dung sample of non-familiar female elephants. (ii) To analyze the frequency of response of male elephants toward dung samples from different reproductive phases of a female elephant.

2. Materials and methods

2.1. Study area and animals

The trials were carried out on sixteen male Asian elephants kept at Mudumalai Wildlife Sanctuary (MWLS), Tamil Nadu (Table 1), using dung samples collected from three female Asian elephants (Ghosal et al., 2012) and a male calf at Bandipur National Park (BNP), Karnataka, India. Elephants at these camps have been either captured from the wild by the Forest Department or born in captivity (Sukumar et al., 1997; Wemmer et al., 2006). At both camps the elephants are maintained as mixed groups of adult and sub-adult females, calves, and males of different age groups. Unlike elephants kept in zoos and temples, these elephants in a forest camp experience living conditions as close to natural as possible for this species under captivity. The elephants are brought to the camps typically once or twice a day, early in the morning and/or late in the afternoon for supplementary feeding, while they are released (with restraining chains/ropes) in the forest for foraging at other times. Females in the forest camps have access to mating with wild and captive males. The elephants used in this study were captured from the wild and maintained under semi-captive conditions for a minimum period of 15 years, thus negating any stress-related influences in the animals' physiology and behavior from recent capture.

Table 1
Male Asian elephants in the Mudumalai forest camps and their experimental details.

Animal I.D.[a]	Age (year)[b]	Experiment duration period
Indhar	57	09/06/08, 10/06/08, 11/06/08
Subramani	56	02/06/08, 03/06/08, 04/06/08
Moorthy	49	05/04/08, 07/04/08, 08/04/08
Mudumalai	46	02/06/08, 03/06/08, 05/06/08
Sanker	46	04/04/08, 07/04/08, 09/04/08
Ganesh	39	05/05/08, 08/05/08, 12/05/08
Sujay	37	17/04/08, 21/04/08, 22/04/08
Vijay	37	14/05/08, 15/05/08, 16/05/08
Santosh	37	19/05/08, 20/05/08, 21/05/08
Wasim	30	05/05/08, 07/05/08, 08/05/08
Cheeran	22	20/03/2008, 24/03/08, 26/04/08
Jumbu	22	14/05/08, 15/05/08, 16/05/08
Wilson	20	13/05/08, 14/05/08, 15/05/08
John	17	17/04/08, 21/04/08, 23/04/08
Uddayan	9.5	17/04/08, 21/04/08, 23/04/08
Bomman	9	19/05/08, 20/05/08, 21/05/08

[a] All animals included in the study are those conditioned in the camp 10 years prior to the initiation of the experiment.
[b] Approximate age in years (in August 2008) based on height measurements at time of capture or records of birth in captivity.

2.2. Sample collection

Fresh dung samples were collected in the morning from three female elephants at the BNP forest camp. Samples were collected from each female in different aliquots in separate bags. On an average, one bolus was collected in every aliquot from a particular female on a specified date. Sometimes elephants urinate and defecate simultaneously; however dung samples were collected only when there was no urination in order to avoid any possible contamination. All samples were frozen at −20 °C within 2–3 h of collection at the field site and presented within a maximum of 2 days for a particular male. When necessary, samples were thawed overnight before presenting them to the males.

2.3. Determination of estrous cycle

Estrous cycle was determined by generating the profiles of both circulating (in serum) reproductive hormone (progesterone, P_4) and its metabolite (Allopregnanolone, 5α-P-3OH) in dung. Hormonal measurements of both serum and dung samples were carried out as a part of our ongoing work on the assessment of reproductive state of female Asian elephants, through the development of non-invasive assays (Ghosal et al., 2012). Blood and fecal samples were collected at weekly intervals from all the three females kept at the BNP forest camp. The concentration of serum progesterone and that of fecal progesterone metabolite levels were determined through radioimmunoassay and enzyme-linked immunoassay, respectively. The samples were analyzed according to a protocol described elsewhere (Ghosal et al., 2010). Analyses of both the serum and fecal samples were carried out after the completion of experimental trials with all the males.

Based on the levels of serum P_4 and fecal 5α-P-3OH, the follicular phase of the estrous cycle was assigned to a female when the values of both fecal 5α-P-3OH and serum P_4 were low, remaining below 0.3 $\mu g\,g^{-1}$ and 0.3 $ng\,mL^{-1}$, respectively, for a time period >3 week. The luteal phase was characterized by a rise in levels of fecal 5α-P-3OH and serum P_4 above 0.3 $\mu g\,g^{-1}$ and 0.3 $ng\,mL^{-1}$, respectively, over a period >5 week (Ghosal et al., 2012). The post-trial analyses of the both the serum progesterone and the fecal progesterone metabolite showed that, out of the 16 males tested, 7 males received a combination of follicular–luteal phases (F–L) while the