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 (27:12: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.

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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 Wittermeyer, 2012). During sexually active periods, especially when in musth (Poole, 1987; Fernando et al., 2008), males wander widely in search of females for
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 Wittenmyer, 2002; Rasmussen et al., 2005). For instance, female Asian elephants secrete a urinary pheromone, (Z,7)-7-dodecen-1-yl acetate (Z7:12:Ac) (Rasmussen et al., 1996) during their follicular/ovulatory phase as a female-to-male signal. The concentration of Z7:12: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 Z7:12: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 specifics 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

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

* All animals included in the study are those conditioned in the camp 10 years prior to the initiation of the experiment.

15 years, thus negating any stress-related influences in the animals' physiology and behavior from recent capture.

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, P4) 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 immunooassay, 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 P4 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 P4 were low, remaining below 0.3 μg·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 P4 above 0.3 μg·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
other 9 males received luteal–luteal (L–L) phase pairing across the 3 trials for a particular male; thus by chance no male received a follicular–follicular (F–F) pairing. Out of the 9 males receiving L–L combination, the behavioral response of only 8 males, each for 3 trials, was considered. One of the males (Santosh, Table 1) behaved as an outlier with respect to the frequency of response toward the luteal-phase samples. The response of this animal was excluded from subsequent analyses (but we discuss below the possible significance of its behavior).

2.4. Design of experiment

Dung samples collected from three of the females at BNP were used to test the males of the MWLS forest camps (Table 1). To the best of our knowledge all the Bandipur females whose dung was presented were strangers to the Mudumalai males and the researcher was not aware of the estrous phase of the females prior to the bio-assays. Thus the behavioral experiments were performed using a double-blind approach.

In total 16 males (Table 1) were tested over a period of 4 months. None of these males was in musth during the period of testing. Each male was tested separately in three individual trials (at an interval of 1–3 days between each trial), against the same female combination. Before starting the trial the ground was cleaned and washed with water to remove any urine or fecal contamination. Three dung samples (two from two different females, and one control that was a male calf) were randomly placed on ground-level concrete or soil, about 1 m apart prior to the entry of the test elephant. The dung samples were discarded after each trial. None of the samples was reused for a particular male or across males at any time in the study. Elephants were observed for 30 min from a distance (~5–10 m) adequate to see their behavior clearly but without distracting the animal. The males were exposed to the aliquots of the same samples for a particular female combination, during subsequent trials. During trials on subsequent days with a particular male, the position of the three dung samples (from two adult females and one control) was shuffled. Behavioral observations were carried out on a focal animal using a pre-established ethogram adapted from Bagley et al. (2006) on African elephants.

All the bioassay trails were recorded on video (Sony Handy-cam, HDR-SR10E) and the observed behaviors of the males toward the presented dung samples incorporated into an ethogram. The behaviors were categorized into two main types: (a) those directly related to chemosensory response such as sniff (distant and close), check, and place and (b) accessory behaviors such as blowing air through the trunk while switching from one sample to another, feeding, belly hit, penis hang, penis droop, urination, defecation, trunk fold. However, we scored only the former category, i.e., frequency of the four predominant chemosensory behaviors, for determining the preference of males for any putative chemical signals present in the dung as they reflect the specific sensory responses associated with the vomeronasal organ (Rasmussen, 1998). The accessory behaviors did not unambiguously represent the chemosensory response of the males and were therefore not included in further analyses. The sum of frequencies of the predominant behaviors were calculated from the play-backs of video recordings and expressed as the total chemosensory response of a male toward a presented sample for a 30 min trial session.

2.5. Statistical analyses

Statistical analyses were performed using R statistics package (R 2.8.1) and Graph Pad Prism 5 software to see if the males showed any behavioral preference toward the dung samples of the females belonging to different reproductive phases. As a particular male was tested against the same female combination for three consecutive trials, the issue of pseudo-replication had to be addressed in the analysis (Crawley, 2007). We thus used the Mixed Effect Model and incorporated male identity as a random effect. The mixed effect model addresses the problem of pseudo-replication and is more appropriate for this study than the application of a linear model (Wittemyer et al., 2006; Rasmussen et al., 2008; Bolker et al., 2009). Data followed a normal distribution, as shown by the quantile–quantile plot and the plot of the fitted against the residual values. Apart from this, in the post hoc analyses we also used unpaired t-tests to compare between the behavioral frequency toward the follicular and luteal phase samples and repeated measures ANOVA to assess the pattern of behavioral response toward the follicular phase samples over time (Bagley et al., 2006). The significance level for all the tests was set at \( \alpha < 0.01 \) and any values equal to or below that were considered to be significant.

3. Results

3.1. Ethogram of male behavioral response

Male elephants tested against the dung samples of female elephants showed the four predominant chemosensory responses, namely, distant sniff, close sniff, check and place behaviors toward the presented samples (see Table 2 for definitions of these responses). A total of 45 behavioral trials were considered for further analyses since the trials for the male Santosh were analyzed separately. The most commonly exhibited response across trials (each trial of 30 min; \( n = 45 \) trials in all cases) was ‘close sniff’ (frequency/30 min: \( 20.4 \pm 13.05 \) (mean \( \pm SD \))) followed by ‘distant sniff’ (12.02 \( \pm 7.12 \)), ‘check’ (11.14 \( \pm 9.8 \)) and ‘place’ behavior (2.09 \( \pm 3.3 \)) (Table 2).

3.2. Relationship between the behavioral response of the male and the reproductive phase of the female

In linear mixed effect model analyses (lme4 package, R 2.8.1) we incorporated male identity as a ‘random effect’, and behavioral response as a function of phase and trial as the ‘fixed effect’. We also included an interaction between phase and trial as one of the fixed effects (Model 1). Using likelihood tests, we compared this complex model with its simpler version where we excluded the interaction term between phase and trial (Model 2) and found that the removal of the interaction term caused a considerable difference in the model fit with an associated significant P-value (\( \chi^2 = 19.09, \ P < 0.001 \), Table 3). Thus, we retained the phase–trial interaction as assigned in the first model (Model 1). In this analysis, the unsigned absolute difference in the frequency of response between the two given phases (either F–L or L–L) was calculated for a male during a particular trial and then incorporated in the model. The variable interval length (1–3 days) between the trials for a particular male had no effect on the observed behavior of the males.

Mixed effect analyses showed that the mean F–L response for males cannot be compared to the mean L–L response across all trials, as each male behaves uniquely depending on the phase of the sample presented in combination with the trial number. Thus, we have represented the complete spread of the data set, separately for each of the trials in the box and whisker plot (Fig. 1). The plot indicates that the median F–L response lies quite apart from the median L–L response along the y-axis, across all the males for every trial performed and the upper quartile range for the former is comparatively broader than for the latter in each of the trials (Fig. 1). We also performed three separate unpaired t-tests (two-tailed) to check whether or not there is any significant difference between the mean responses of the F–L and L–L categories for each trial. Results of the t-test indicated that the mean F–L response across all
Table 2
List and description of chemosensory behaviors observed during the bioassays with male Asian elephants. Each trial was of 30 min duration.

<table>
<thead>
<tr>
<th>Chemosensory behaviors</th>
<th>Definition</th>
<th>Behavioral frequency/trial (mean ± SD; n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distant sniff</td>
<td>Trunk lifted and the tip pointed in the direction of the presented sample line; always from a distance in the air; with or without the “closing–opening” of the trunk tip; no contact of the trunk tip with the sample; never accompanied with mucus secretion from the trunk</td>
<td>12.02 ± 7.12</td>
</tr>
<tr>
<td>Close sniff</td>
<td>Trunk tip hovers or moves around a particular presented sample; with or without the “closing–opening” of the trunk tip; no contact of the trunk tip with the sample; occasionally may or may not be accompanied with mucus secretion from the trunk</td>
<td>20.40 ± 13.05</td>
</tr>
<tr>
<td>Check</td>
<td>Male touches a particular sample with the fingers of the trunk tip; as if the trunk finger is trying to pick up a small amount of the presented sample; may or may not be accompanied with mucus secretion from the trunk</td>
<td>11.14 ± 9.8</td>
</tr>
<tr>
<td>Place</td>
<td>Flatten the trunk tip over a particular presented sample; almost blocking the trunk opening with the sample; not accompanied by prominent mucus secretion</td>
<td>2.09 ± 3.3</td>
</tr>
</tbody>
</table>

Table 3
Comparison of models to understand the nature of association between the phase of the dung presented and the day of trial during the bioassays of male Asian elephants.

<table>
<thead>
<tr>
<th></th>
<th>AIC</th>
<th>BIC</th>
<th>logLik</th>
<th>Chisq chi</th>
<th>Pr (&gt;Chisq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mod2</td>
<td>328.65</td>
<td>339.49</td>
<td>ˆ−158.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>313.56</td>
<td>328.02</td>
<td>ˆ−148.78</td>
<td>19.090</td>
<td>7.15e−05</td>
</tr>
</tbody>
</table>

* Mod2 < lmer (response~phase*trial+(1|Male ID.)).
* Mod1 < lmer (response~phase+trial+(1|Male ID.)).
* Represents P < 0.001.

4. Discussion

Our observations indicate that male Asian elephants can behaviorally discriminate the dung samples of females belonging to different reproductive phases, based on the same set of behavioral responses such as sniff (distant and close), check and place that have been observed in other studies on captive African elephants (Bagley et al., 2006) and in Asian elephants (Rasmussen et al., 1996; Schulte and Rasmussen, 1999) using only urine samples. A recent study on free-ranging African elephants also observed that males inspected dung near water-holes at a higher frequency than did female elephants, presumably to obtain inter- or intra-specific chemical signals (Merte et al., 2010). The reproductive state of the animals whose dung was examined was, however, not determined in that study.

Flehmen, a prominent male olfactory response recorded toward presented urine samples for both captive Asian and African elephants (Rasmussen et al., 1982; Bagley et al., 2006), was not observed in our study. In the current study, adult males showed greater interest in sniffing behavior as compared to check and place behaviors while examining the dung samples of female Asian elephants, similar to observations carried out on the male African elephants (Merte et al., 2010) and in other mammalian species (Brashares and Arcese, 1999; Wronski et al., 2006; Palphramand and White, 2007) for fecal pellets/dung samples.

The behavioral response of male elephants toward each sample was associated with not only the phase of the dung sample (reproductive status) presented but also with the sequence of the bioassay trial (temporal scale) (Fig. 1). The males and the females involved in the bioassays were strangers to each other and, thus, it can be deduced that this behavioral response is not based on pre-existing affiliation among the individuals, but seems to be driven by the inherent behavior of male elephants to prefer females close to the pre-ovulatory period (follicular phase) over those in the post-ovulatory period (luteal phase) (Rasmussen, 2001). Thus, our study indicates the probable existence of a potential pheromone candidate in the dung of the female elephants that allows male to evaluate the reproductive status of the females.

Based on similar sequential bioassay trials on musth/urine samples carried out in captive Asian and African elephants in zoos, it was proposed that a novel substance response (Rasmussen et al., 1996, 2003; Rasmussen and Schulte, 1998) or a “novel stimulus hypothesis” (Bagley et al., 2006) could explain the response of
individual elephants over repeated trials. In these studies, individuals examined any sample type presented to them on the first day of the trial with a relatively high frequency without discrimination. During following trials there was a gradual decrease in overall frequency of chemosensory response toward the samples, but the response toward the follicular-phase sample was still significantly higher than toward the luteal-phase and the control samples. However, in our study, the tested males were able to discriminate between the presented samples on the first day of the trial itself by showing higher preference toward the dung of the female close to ovulation (Figs. 1 and 2). In this respect, our observations of forest camp elephants differ from the behavioral pattern reported for zoo elephants, as in the latter the elephants were behaviorally indiscriminate toward the presented samples on the first day of the trial (Rasmussen et al., 1996; Bagley et al., 2006). This difference in the behavioral response can perhaps be attributed to the social conditions of the elephants employed in the studies—the current study on forest-camp elephants with unrestricted mixing of both the sexes (Sukumar, 2003) versus zoo elephants with restricted access to the opposite sex only for the purpose of breeding (Rasmussen et al., 1996) or males having chemical exposure to females (Bagley et al., 2006).

Although the male elephants in our study showed behavioral discrimination toward the follicular-phase samples even on the first day of the trial, there was a significant reduction in the frequency of response across progressive trials (Fig. 2). Such reduction in overall frequency over time is similar to that described in the “novel substance response” in zoo elephants (Rasmussen et al., 1996; Rasmussen and Schulte, 1998) and as “habituation effect” in other species (May and Hoy, 1991; Yadon and Wilson, 2005; Dacler et al., 2006; Temple et al., 2006).
In bioassay studies on elephants carried out on muth or urine samples, the control is usually a non-biological sample such as water or vanillin solution (Rasmussen et al., 1996; Rasmussen, 2001). In these studies the response of an individual toward the control sample has been observed to be negligible or close to baseline. However, in our study, the magnitude of response toward the control samples is almost comparable to that of the response recorded for the luteal-phase samples (Fig. 2). The reason may lie in our use of a male calf's dung as the control, a biological sample without the relevant chemical signal as in the case of luteal-phase female dung. Thus, it can be hypothesized that the degree of sensory response toward a biological sample will be higher than that toward a non-biological sample.

The unusually high response of the 'outlier' male elephant Santosh toward the female Diana's luteal-phase sample needs an explanation. Serum and dung analyses indicated that Diana (see Ghosal et al., 2012) entered the follicular phase of the estrous cycle five days after the trial sample was collected. Studies by Rasmussen (2001) showed that the urinary pheromone (Z7-12: Ac) was not detectable during the luteal phase of the estrous cycle. With the decline of progesterone concentration toward the end of the luteal phase and the initial stage of the follicular phase, a low concentration of Z7-12:Ac was detected that then showed a linear increase through the follicular period (Rasmussen, 2001). Thus, the pheromone concentration was negatively correlated with the progesterone concentration (Rasmussen, 2001). A similar pattern can perhaps be expected for the potential pheromonal compound (or compounds) in the dung of female Asian elephants, with the male being able to perceive the impending ovulation a few days in advance. However, since only one male showed such an unusual behavior, no firm conclusion can be drawn without more sampling. It so happened in our study that all the other males receiving L-L combination were presented with dung samples that belonged to the luteal phase of the females on an average 2–3 weeks prior to entering the follicular phase of the cycle.

In conclusion, this is the first report of bioassay trials using dung samples and investigating its role in inter-sexual chemical signaling in Asian elephants. It can thus be postulated that the elephants use simultaneous broadcast channels (such as urine, breath, dung, temporal gland secretions) to advertise their physiological state. However, the function of the multiple pheromone-sources in the case of elephants requires further investigation in the context of the social organization (e.g., solitary male versus hierarchical female herd) and the mating strategies (relative short estrous window) adopted by the species. Further, chemical analyses are necessary to isolate and characterize the compound (or compounds) in dung responsible for signaling reproductive status, and to check for possible structural similarity to the urinary pheromone (Z7-12:Ac) present in female Asian elephants.

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