The shifting chemical signals of Musth

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Studies of captive elephants have significantly contributed to our understanding of how olfaction influences elephant behaviour throughout their lifetime, with special importance during the reproductive years. These studies allow longitudinal sample collections coupled with observations of individual elephants over time – something that is difficult in field conditions. The infusion of data from captive studies continues to fill in details on the physiology of musth and some of this information has been correlated by observations of wild elephant herds (Rasmussen & Perrin, 1999; Rasmussen *et al.*, 2002; Rasmussen *et al.*, 2005; Greenwood *et al.*, 2005).

Elephants receive chemoesthetic sensations, or smell, via several systems. In the main olfactory system the trunk is a conduit to the extensive olfactory epithelium covering the numerous turbinates. The billions of olfactory neurons are sensitive to low levels of gaseous compounds. Throughout the mucosal lining of the trunk are the free nerve endings of the trigeminal system, detecting compounds present in higher, often toxic concentrations. Furthermore, elephants have a third chemodetection system: the vomeronasal organ. It is the interplay between the main olfactory system and the enormous vomeronasal system that gives elephants one of the most sensitive and precise "smell" detection systems among mammals (Rasmussen, 1999).

We first began our chemical communication research on signals released in urine by female Asian elephants during the periovulatory period. Separation techniques of a postulated pheromone were based on the high frequency of flehmen responses by male elephants assessing isolated fractions. This assessment mechanism presumably involves the vomeronasal organ system as the trunk tip places liquids on the opening to the vomeronasal organ ducts in the roof of the mouth. These studies were begun in facilities in the USA and, in the controlled captive environment, led to the identification of a urine-derived acetate and demonstration of the bioactivity of its synthetic form. This female-to-male signal met the criteria of a pheromone (Rasmussen *et al.*, 1996).

What about male-emitted signals - what were these signals and did any facilitate reproductively oriented behaviors? Early studies showed that cyclohexanone in temporal gland secretions of musth elephants elicited behavioral responses from females with calves (Perrin & Rasmussen, 1994). To better understand the nature and function of any signals, we needed to study the chemosensory influences of male elephants as related to the phenomenon of musth.

A serendipitous observation of a young captive Asian male elephant heightened our male-focused chemosensory studies. The observation by one of us that a young teenage Asian elephant in a first musth smelled like honey triggered detailed studies of these young males and their secretions (Rasmussen *et al.*, 2002). Subsequent chemical analyses revealed that young Asian male temporal gland secretions (TGS) were composed of a bouquet of sweet odors: acetates, an alcohol (3-hexen-2-ol) smelling like leaves, and pleasant smelling ketones (acetophenone and 2-heptanone). This finding provided the real impetus for in depth chemosensory studies comparing young and older males, as well as musth and non-musth males.

Not only do young Asian male elephants experiencing their first musth emit sweet compounds, but their behavior is unpredictable and erratic. These teenage musth episodes are short in duration and have been termed "moda" (Chandrasekharan *et al.*, 1992). Older males are not only much larger but our studies have shown their musth has a distinctive chemical signature; mature Asian male elephants in musth secrete a very different mixture of chemicals than teenage males (Rasmussen *et al.*, 2002). The older males are more socially and sexually adept and, importantly, capable of sustaining long periods of musth, sometimes several months in duration. During these extended periods not only do they release secretions distinctive of adult musth, but compounds characteristic of the specific phase of these long musth periods.

The concurrent chemical and hormonal maturation of musth is an integral part of the long process of male maturesence within elephant society. When teenage males reach their early twenties, sweet smelling acetates are no longer detectable in their secretions. Our captive studies have shown that pleasant-smelling compounds are transitionally replaced by carboxylic acids; such acids reduce the pH of the temporal gland secretions as low as pH 5.5. During this transitional phase, trace amounts of an acrid ketal, frontalin [1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane] a demonstrated pheromone, are occasionally

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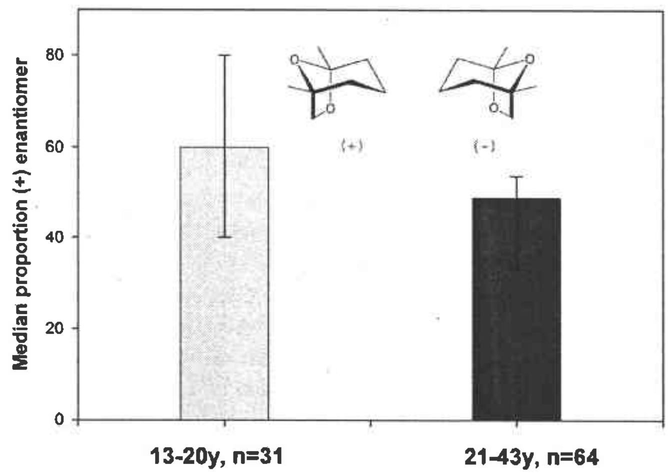

Fig.1 Proportion of enantiomer forms of frontalin during musth

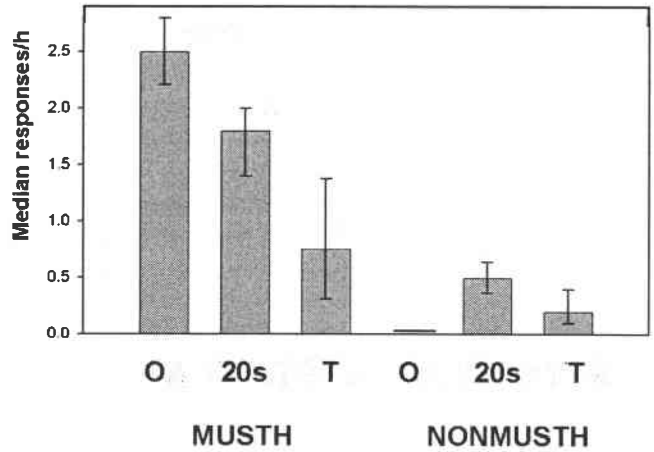

Fig.2 Frequencies of checks to expelled female urine

O = older males (+30 years); 20s = males + 20 years old; T = teenage males detected (Rasmussen & Greenwood, 2003; Rasmussen & Greenwood, in prep.). Significantly, we have recently discovered that these young male Asian elephants in musth release both sterically possible enantiomers, or mirror image forms, of frontalin but the proportion of forms varies widely from day to day, with the (+) form usually the more dominant one (Greenwood *et al.*, 2005). However, as males continue to mature, not only do the pleasant odors make way for malodorous ones, but the proportion of mirror image forms stabilizes, especially at mid-musth, to almost 50% (Greenwood et al., 2005). Eventually and particularly during the mid-point of a musth episode, older males broadcast mixtures of less volatile, more alkaline-based, longer-lasting ketones, increasing amounts of the bicyclic ketal, frontalin as an almost racemic mixture of the enantiomers of frontalin (Fig. 1) (Rasmussen *et al.*, 2002; Greenwood *et al.*, 2005).

Based on this observed gradual change of chemical emissions, especially of frontalin, we conducted behavioral bioassays both with whole collected TGS and the synthetic form of frontalin at three facilities with captive Asian elephants in the USA (Riddle's Elephant and Wildlife Sanctuary, the Ringling Center for Elephant Conservation, the Oregon Zoo) and at the Auckland Zoo in New Zealand. Behaviors observed in response to presentations of synthetic racemic frontalin were consistent with the results observed with whole temporal gland secretion from older males whose mid-musth secretions contained almost racemic mixtures of frontalin. These behavioral responses were related to the sex, developmental stage, and physiological status of the responding individual.

Thus frontalin, as a racemic synthetic compound, elicited behavioral responses from both males and females. Female reactivity varied with hormonal state and male reactivity varied with age and musth status. Frontalin, either released in the natural TGS by older males or presented as a synthetic component, attracted reproductively ready females, but elicited apprehension from pregnant females, whereas luteal phase females were indifferent. Among males, the older adult males were mostly indifferent to frontalin in either presentation medium, whereas sub-adult males were highly reactive, often exhibiting repulsion or avoidance. Such differential responses may facilitate the smooth functioning of elephant society. This facilitation may occur among males by clarifying who is in musth, in what phase of a musth episode, and who is not, and also revealing male maturity. Females' differential responses, and thus the impact of chemical signals on them, may also affect their reproductive strategies. Many of these social influences and controls within and between male and female groups, especially those affecting breeding strategies, are mediated in large part through the dual olfactory systems.

Wild Asian male elephants are found in transitory groups if not in pairs or solitary. A male may dramatically increase its home range during the weeks when he comes into musth (Desai & Johnsingh, 1995; Fernando & Lande, 2000). Older males, especially but not exclusively, during the annual musth period apparently maintain a subtle, somewhat loose control over non-musth and younger males, and such control increases in area concurrent with home range expansion during musth. The presence of older, larger musth males is effectively signaled by their distinctive TGS and urine chemical signatures that include specific ketonic components; statistically significant captive studies have demonstrated retreat by young males to both musth males actively secreting or presented whole TGS or selected secreted compounds, such as higher molecular weight ketones and frontalin (Rasmussen & Greenwood, 2003; Perrin *et al.*, 1996). A recent anecdotal observation noted that wild elephants in Assam (NE India) were deterred from coming close to previously regularly raided paddy fields by the presence of an older (+30 years old) captive male tethered near the fields during his musth (Dr. K. K. Sarma, personal communication). Observations of wild herds have noted instances of retreat by young males in moda musth to TGS left on substrates by older males in musth (Dr. V. Krishnamurthy, personal observation). In the wild, both the presence of such older males (in musth or not) and internal physiological conditions of the moda musth state may be decisive in determining a young male's investigative behaviours toward females. Teenage males in moda musth may or may not retreat in the presence of a dominant male (especially one in musth), whereas non-musth teenage males are certain to retreat (Rasmussen *et al.*, 2002).

Captive Asian elephant studies have implicated that from a female perspective, musth males are preferred over non-musth males. Females near ovulation were observed to show more interest in urine from musth males whose serum testosterone levels are above 10 ng/ml and especially those above 20 ng/ml (Schulte & Rasmussen, 1999a). Recently our studies demonstrated the reciprocal - namely that musth males have the most direct access to females and that chemical signals influencing the outcome of male dominance interactions reinforce this superior access by musth males.

We examined this in greater behavioral depth among a wild population of elephants in Asia (Rasmussen *et al.*, 2005). Wild male elephants were identified as individuals and categorized by age (and thus size) and musth characteristics. The quantitative appraisal of musth in each male was provided using attributes of musth that included physiological and behavioural characteristics. These characteristics were scored and combined with the chemical assessment of the presence, absence, or relative amount of selected urinary ketones. These appraisals gave a measure of the maturity and degree of musth in individual wild males when compared to data available from precisely studied captive males. Our field data have shown that musth males, in comparison to non-musth males, interacted much more frequently with pre-ovulatory females and demonstrated a strikingly higher frequency of chemosensory responses to females and their urine (Rasmussen *et al.*, 2005).

Not only do musth males interact with more females and more frequently, but also specific olfactory-related responses and patterns of response vary with age and are different between musth and non-musth males. Adult males (+20 years old) while in musth demonstrate a higher frequency of responses to females than their non-musth counterparts. These responses include both distant and close sniffs, trunk tip checks to both the urogenital region of females and their expelled urine (Fig. 2), and flehmen responses. Musth males also characteristically monitored multiple females in succession. Apparently they are more skilled at detecting by olfaction the precise ovulatory status of females.

Male age (and thus size) also influenced responsivity and eventually access to females. Larger, older males (+30 years old) in musth performed significantly more distant sniffs and total contact chemosensory responses than their younger counterparts in musth and than older males not in musth. Among all three primary contact responses - urogenital checks, expelled urine checks, and flehmens - older males in musth exhibited higher responsivity than younger males in musth, with non-musth young males not showing any contact interactions with females. This avoidance of contact responses toward females suggests that perhaps either experience has taught these young males to avoid direct contact with females during their non-musth period (as females may not be interested), or male behaviours are more rational during non-musth.

Specific premating behaviours (e.g. mounting) were more frequent among young males in musth than the older group, suggesting that experienced older males could judge proximity of females to ovulation more accurately and thus expended less effort on non-fertile females. Further down the age structure, teenage males show much lower frequency of distant sniffs toward females than older males, again suggesting their inexperience in recognizing the distant odor of reproductively active females. These quantitative differences in varied responses among different categories of males offer clues about the biological roles in breeding tactics and elephant social structure.

Age revealed different frequencies of chemosensory responses and premating behaviours when teenage males (both in moda musth and non-musth) were compared with their older counterparts. Older males (+30 years old) exhibited the highest chemosensory responses, but summed chemosensory responses by musth and non-musth teenage males were slightly higher than in young adult (+20 years old) counterparts. This high responsiveness by the maturing teenage male group may reflect attempts by this male category to gain sexual knowledge about females through increased contact chemosensory responses. Inexperienced teenage males may lack knowledge of the chemical identity of the pheromone or the ability to precisely assess concentrations, either of which could be the result of learning. Teenage males are still in an experience-gaining period and thus are deciphering the attributes of female cycles, fine-tuning how to socialize with females, and decoding which males are dominant or in musth or both.

Breath also contains relevant social signals. In the somewhat redundant chemical signaling system of elephants, many communicative compounds are present in the blood and excreted or secreted into the urine, TGS or breath. The focus of much of our efforts has been on the influence of chemical signals and pheromones from the urine and temporal glands on elephant societal interactions (Rasmussen & Krishnamurthy, 2000). Our research has demonstrated a correlation between blood constituents and TGS components, yet many of these same chemical communicator molecules are excreted to the outside environment through the breath. Frontalin, indicative of musth in older males, is an especially redundant signal present in blood, TGS, urine and breath. Over a 10-year period (1994-2004), using special stainless steel evacuated canisters to collect exhalant breath, we analyzed more than 100 samples from 10 captive male elephants – both in musth and non-musth. As Asian male elephants often reduce their caloric intake during the musth state, they lose body condition (Desai & Johnsingh, 1995) and a weight loss of hundreds of kilograms has been documented in captive elephants during a musth period; such weight loss implies first a depletion of fat reserves and eventually muscle breakdown. Our breath sample analyses and physiological measurements in blood confirmed such patterns. Breath volatiles were different between male elephants in musth and those not in musth, i.e. clear-cut qualitative and quantitative differences distinguished that a spectrum of ketones, as well as several related alcohols are significantly elevated (Rasmussen & Riddle, 2004). However, while at first ketone levels increased subsequently pentane, an indication of muscle breakdown, was elevated. This correlates with documented elevations in serum lipase and triglycerides. However, the lack of changes in serum creatinine phosphokinase during musth suggests that muscle metabolism may be less affected than fat metabolism, and only after a lengthy period of starvation (Schulte & Rasmussen, 1999b; Rasmussen & Perrin, 1999).

What makes breath signals special is that they are more ephemeral than urinary or TGS secretions. They are carried in the air, probably in part on aerosols, and are usually rather volatile. They diffuse rapidly in the air medium and are subject to photo oxidation. Thus their message is immediate, often close range and non-persistent, and may be individual-to-individual communication. Further studies on the meaning of chemical signals released in breath may help explain why captive male Asian elephants in musth will blow at each other across fences or under doors, strongly suggesting a transfer of chemical signals between individuals.

In exploring breeding strategies it is noteworthy that the Asian elephant shares some breeding tactics common to other sexually dimorphic cognitive mammals, such as a roving strategy with similarities to some primates (*e.g.* orangutans) and whales (Singleton & van Schiak, 2002; Whitehead, 1990), while the musth parameter adds a unique feature. Among elephants, highly chemosensory oriented searches aid in successful elephant reproduction. Male elephants are natural roamers and that tendency increases with age and is definitely expanded during musth. Reciprocally, musth influences temporary fusion–fission events as roving males join female groups while tracking preovulatory pheromone concentrations. The pH dependency and the modulation by proteins of the Asian female preovulatory pheromone (Z)-7-dodecenyl acetate) affect its signal lifetime (Rasmussen *et al.*, 2003). Thus, from an olfactory perspective, the two male strategies - roaming and older-controlling-younger - demonstrate a well-balanced, time-dependent system of pheromones. Breeding strategies interwoven into the differential social structure of male and female Asian elephants are apparently influenced by the behavioural and chemical conditions of musth as evidenced by greater chemosensory responsivity and increased mating behaviours by musth males; thus musth plays an important determining factor in reproductive strategies.

How does musth influence reproductive success? This question has been raised in a study of African elephants demonstrating that age, size and musth state interacted to affect a male's reproductive success. Older musth males sired a markedly large number of calves, while non-musth males sired about 10% of the calves in this studied population. Furthermore the genetic data from this study suggests that females mate with more males than previously thought, so male mating behaviour, such as mate guarding, is not a good predictor of paternity as a single musth male does not maintain exclusive access to a female throughout her estrous period (Hollister-Smith, 2005). Paternity studies of Asian elephant populations would be revealing, especially in those with depleted older male populations, to determine reproductive success of younger or non-musth males.

Understanding male elephant societal roles, the functioning of male chemical signals and the subtle shifts in these signals – both in musth and non-musth states - are important for conservation applications.

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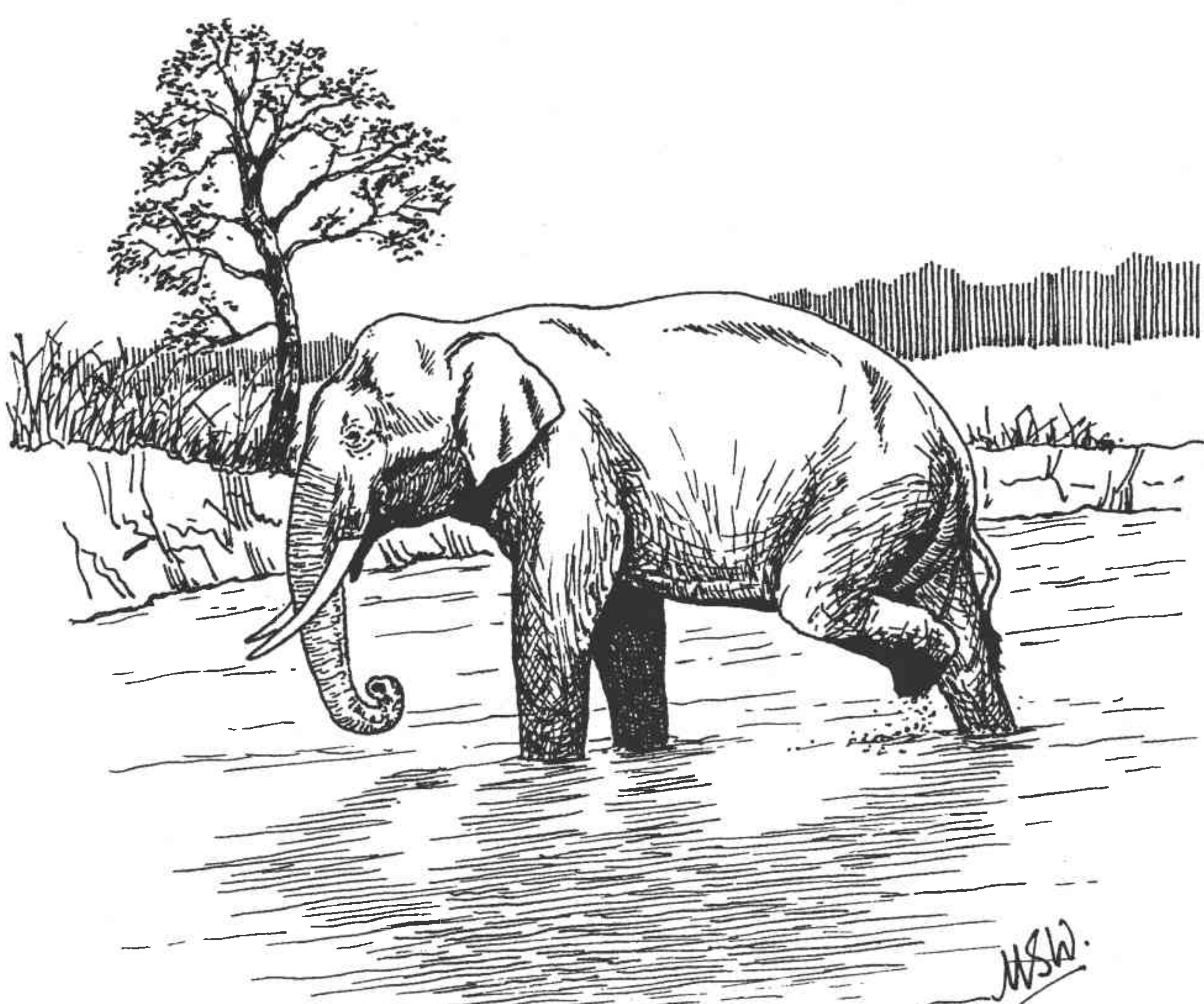

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