

# Behavioural and chemical confirmation of the preovulatory pheromone, (Z)-7-dodecenyl acetate, in wild Asian elephants: its relationship to musth

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## Summary

Mammalian breeding strategies vary depending on particular social contexts and sensory systems emphasized in various species. Among sexually dimorphic non-territorial Asian elephants, *Elephas maximus*, a multiplex olfactory chemical signaling system has been implicated in ensuring effective reproduction. This study explores how, using chemosensory mechanisms, widely roaming, wild male elephants locate periovulatory females in matriarchal-led female family units and precisely assess their ovulatory status. In this species, the dual obstacles of separately living sexes and infrequent oestrus are overcome by lengthy female cycles. During an extended preovulatory period captive females release increasing concentrations of the urinary pheromone (Z)-7-dodecenyl acetate, timed to reach a maximum just before ovulation. The current field studies combined chemical identification and quantification of female urinary (Z)-7-dodecenyl acetate with behavioural observations, monitoring the frequencies of chemosensory responses and pre-mating behaviours by various categories of males. The results suggest the temporal extension of the preovulatory period effectively provides a synchrony between sexes for successful reproduction. Male elephants undergo a two-decade-long maturation process that involves physical, sexual, social, and physiological maturation. Males older than 30 years are generally large, sexually active, socially adept and capable of sustaining long periods of musth, during which they release secretions distinctive of adult musth. These older adult males in musth demonstrated significantly more chemosensory responses and pre-mating behaviours than their younger or non-musth counterparts; they

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apparently are more skilled at detecting the precise ovulatory status of females. Male–male interactions are affected by size, age, and musth; the winners gain greater access to females, as indicated by the high incidence of mate guarding. The Asian elephant shares some breeding tactics common to other mammals including some primates (e.g. orangutans) and whales, while the musth parameter adds a unique feature. Fusion–fission events are influenced by elephant reproductive strategies, as roving males join female groups while tracking preovulatory pheromone concentrations.

*Keywords:* *Elephas maximus*, pre mating behaviours, chemosensory responses, flehmen, urinary volatiles, gas chromatography/mass spectrometry.

## Introduction

Among large mammals, breeding tactics may vary depending on the social structure, the degree of territoriality practiced, and the sensory systems employed in conjunction with mating. Clutton-Brock (1989) and Whitehead (1990) described a roving breeding strategy in species in which sexually dimorphic larger males do not defend territories containing the home ranges of female groups. The Asian elephant, *Elephas maximus*, fits this definition. Usually single males optimize their reproductive success by wandering among female units, seeking out oestrous or preovulatory females (Eisenberg et al., 1971; McKay, 1973). Among many sexually dimorphic species, size is a determining measure of success in male–male competition for female mates. Other species, such as Asian elephants, may exhibit additional determinates of competition that are age-related, experience-related, or physiological condition-related. Among Asian elephants and other large mammalian species including sperm whales, polar bears, killer whales, orangutans and African elephants, the behaviour of younger males in a competitive environment may depend on the controlling behaviour of older males (Poole, 1989a; Whitehead, 1990; Whitehead, 1994; Slotow et al., 2000; Rasmussen et al., 2002). Our current investigation demonstrates how olfactory cues mediate the interweaving of two male strategies — roaming and older-controlling-younger — into the societal fabric of wild Asian elephants.

Social structure sets the outline for these breeding strategies; it defines the ecological relationships between nearby conspecifics and may include cooperation, competition and dominance. Social structure is an important correlate of communicative behaviour, because it sets the scenario within which cognition and communication occur. More cognitively advanced mammals,

such as elephants, cetaceans and primates, usually have complex social structures (Worthy & Hickie, 1986; Tyack, 1999). Such mammals with large brains that develop and mature greatly after birth potentially have the ability to store fluctuating spatial, temporal and specialized olfactory information in long- and short-term memory (Tower, 1954; Koikegami & Ozaki, 1967; Haug, 1987). Such capability not only influences the survival of their immediate associates, but can also affect mate choice decisions.

Early published studies on wild Asian elephants showed that the social organization of Asian elephant populations is apparently similar to that of the African elephant (Poole, 1987; Poole, 1989b; Sukumar, 2003), i.e., that adult males and females live in different social structures, and come together mainly during times of mating (Eisenberg et al., 1971; Eisenberg & Lockhart, 1972; McKay, 1973; Eisenberg, 1980; Sukumar, 1989; Desai, 1991). However, the fine details of the social ways and behavioural strategies of the Asian species in the wild have been elusive. Field studies of Asian elephants have been difficult, as they inhabit different habitats compared with the African savannah species. The low visibility habitats of the Asian elephants include dense rain or deciduous forests, thick, rough shrubland, and tall grasslands, all possessing features that impede behavioural observations.

Recent investigations have begun to reveal substantial differences in the complexity and structure of the social groupings of female Asian elephants in contrast to the African elephant (Fernando & Lande, 2000). In both species, the fundamental unit of association is mother-calf dyads and triads; the basic level of organization is the female group led by a matriarch and several generations of her offspring, forming a closely knit unit with no inter-group transfer of females (Sanderson, 1878; Douglas-Hamilton, 1972; McKay, 1973; Sukumar, 1994). Female Asian and African elephants, along with their daughters and pre-pubertal sons, stay in matriarchal family groups with close bonds among related individuals and between related family groups. The lack of inter-group transfer among females differs from many social mammals such as gibbons (Brokelman et al., 1998), pilot whales (Amos et al., 1993), and lions (Pusey & Packer, 1987; Packer et al., 1991) that do demonstrate inter-group transfer of females. Unlike savannah African elephants (Douglas-Hamilton, 1973; Moss & Poole, 1983), there is no evidence for social groups of higher complexity than family groups among female Asian elephants (Fernando & Lande, 2000). Rather, several studies show that the social structure of the Asian elephant has similarities to that of the

forest African elephants (Marechal et al., 1998), with small groups of related females maintaining their separate group integrity. Studies by McKay (1973) in Sri Lanka demonstrated that the most frequently sighted groupings of females consisted of 3-5 members. In the Gal Oya region in particular, 50% of the sightings were of six or fewer individuals. In Malaysian rain forests, Oliver (1978) observed an average of 5-6 individuals in each matriarch-dominated group with four as the medial group size. Fernando & Lande (2000) in Sri Lanka demonstrated that, unusually, the matriarchal social system of the Asian elephant may consist of perfect matrilineal associating females; even sperm whales which exhibit associating matrilineal groups had more than one haplotype in all sampled female groups (Richards et al., 1996). Radiotracked wild female Asian elephants in Sri Lanka maintained high fidelity to well-defined and comparatively small home ranges (Fernando & Lande, 2000). Although the numbers of groups studied were few, several showed considerable overlaps in home ranges, suggesting tolerance in sharing resources. However, no association between members of two groups was noted (Fernando & Lande, 2000).

The comparison between Asian elephant systems and several primate social systems is relevant, especially with regard to their degree of territoriality and concurrent breeding tactics. Individual female orangutans, *Pongo pygmaeus*, share a home range with a single immature offspring (Rodman, 1984; Galdikas, 1985; Stephens, 1990). Upon maturation, female orangutans may establish a range near the maternal range. Studies of the Sumatra population of orangutans demonstrated a high home range overlap among females (Singleton & van Schaik, 2001) and no territoriality. Similar to maturing young Asian elephants, maturing male orangutans disperse widely, and sexually mature males range more widely than females (McKay, 1973; Rodman, 1984; Galdikas, 1985; Singleton & van Schaik, 2002). Male ranges are larger and overlap female ranges, but the dominant male orangutan has a more limited range, reflecting the availability of sexually attractive females.

A roving breeding strategy has been described in sperm whales (Whitehead, 1994). Sexually dimorphic sperm whales live in groups having similarities to orangutans and to the Asian elephant. Long-term bonds between female sperm whales are fostered by communal babysitting by adult females and immature whales (Best, 1979; Whitehead, 1996). Subadult males disperse from females, first in bachelor herds and later solitary, similar to young Asian male elephants that often disperse as pairs. As adults, male sperm

whales and male elephants search extensively for groups with females in oestrus rather than defend a permanent group waiting for oestrus (Barnes, 1982; Whitehead & Arnbohm, 1987).

The differences between male and female societal structure in Asian elephants becomes important in examining the impact of chemical signals on reproductive strategies. In contrast to female calves, male juveniles receive preferentially longer suckling privileges and thus are larger at similar ages. As youngsters, males venture away from but then rejoin their natal groups. As these males get older, the wandering distance from the natal unit increases and the frequency of return visits decreases until independence is achieved by about 12-15 years of age. Typically, teenage males move solitarily or in temporary all-male groups composed of 2-4 individuals (McKay, 1973). Interwoven in this differential male society are developmental degrees of musth. As teenage elephants get older, gradual physiological changes related to musth begin, including transitory elevations in serum testosterone that affect behaviour (Rasmussen et al., 2002). In other mammals, seasonal increases in testosterone affect behaviour, but evidence suggests a more direct involvement of the central nervous system in driving behaviour among male Asian elephants (Cheeran et al., 2004). Older males, especially but not exclusively during the annual musth period, apparently maintain a subtle, somewhat loose control over nonmusth and younger males (Rasmussen et al., 2002). In McKay's (1973) study, males were solitary in 80% of observations, in pairs — 15%, and in groups of 3-5 — 5%. While adult male home ranges are typically smaller than those of family groups during most of the year, a male may dramatically increase his home range during the weeks when he comes into musth (Joshua & Johnsingh, 1995; Fernando & Lande, 2000). These social influences and controls within and between female groups and males, especially those affecting breeding strategies may be mediated in large part through the dual olfactory systems [main olfaction (MO) and vomeronasal organ (VNO)], although the visual, auditory and tactile systems also play significant roles.

Superimposed on this varying separation of sexes, differential home ranges, and the lack of male defense of territories is the infrequency of oestrus. Studies of captive elephants have demonstrated two pheromones facilitating male dominance interactions, female preference, and male assessment of preovulatory condition. In this study, we explore hypotheses

that olfactory mechanisms allow adult males to locate females and to precisely assess their ovulatory status so that access to mating-ready females is partially dependent on olfactory signals from females to males and between males. It has been long recognized that the sense of smell is paramount among Asian elephants (Krishnan, 1972). We have demonstrated coordination between behaviour and chemistry. Chemical emissions, often specific compounds (i.e., pheromones), allow conspecifics to gain information about each other and facilitate important functional social roles throughout life (Rasmussen et al., 1997, 2003; Schulte & Rasmussen, 1999a; Rasmussen & Krishnamurthy, 2000; Rasmussen & Greenwood, 2003). The well-developed MO and VNO systems play essential transport and reception roles for two pheromones identified in the Asian elephant (Rasmussen, 1999; Lazar, 2001; Lazar et al., 2002, 2004; Rasmussen & Greenwood, 2003). The female-to-male sex pheromone (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac), identified from the preovulatory urine of captive female Asian elephants (Rasmussen et al., 1996), elicited rates of chemosensory response frequencies among captive males that increased proportionally to concentration (Rasmussen, 2001). Among forest camp male elephants in their natural Asian habitat repetitive, multiple flehmen responses to the synthetic pheromone and pre-mating behaviours, including penile erections and mounting demonstrated bioactivity. Response frequencies, however, were affected also by male social contexts, with the presence of dominant older males reducing or eliminating responses to *Z*7-12:Ac by younger males (Rasmussen et al., 1997). The male-emitted pheromone, frontalinal, also affected responses by captive males (Rasmussen & Greenwood, 2003).

In the context of their native habitat and natural social settings, do wild Asian elephants utilize this preovulatory pheromonal system; i.e., do wild Asian female elephants produce and do wild Asian males respond to the female-to-male pheromone clearly delineated in captive Asian elephants, and are the effects of societal breeding strategies apparent? Our objectives were: (1) to demonstrate whether wild male Asian elephants exhibit differential, well-defined, apparently chemosensory-based behaviours and responses toward female elephants, to their expelled urine, and specifically to their naturally emitted *Z*7-12:Ac; and (2) to establish if, among a wild elephant population, the frequencies of various observed behaviours and chemosensory responses by males toward females at the time of mating are affected by age and/or musth condition. Four specific, sequential questions were

posed: (a) Qualitatively and quantitatively, what behaviours and responses do wild male elephants exhibit toward female elephants of varying reproductive states? (b) If high frequencies of male pre-mating behaviours and chemosensory responses to females were observed, could we collect expelled female urine and identify the well-characterized female-released pheromone, Z7-12:Ac? (c) Would studies with wild elephants confirm the significant correlation of male-to-female behaviour with pheromone presence and with concentrations observed during captive studies? (d) Would we observe differential male behaviours reflective of male status, maturity and/or state of musth?

## Methods

### *Overview and study site*

Expanded resolution of olfactory-based wild elephant behaviours is now possible because of new arrays of techniques including global positioning instrumentation, digital photography, laser-based distance measuring devices, and novel field methods of capturing urinary volatiles coupled with high-sensitivity analytical chemistry techniques (Rasmussen & Wittemyer, 2002). The study site along the Kabini River reservoir in Nagarhole National Park in southern India has an unusual feature, namely that during a 3-4-month annual period (February-May) wild Asian elephants can be clearly observed for extended periods and are readily, reliably, and individually identifiable. In this very-short-grass habitat rimmed with sparse bamboo clumps, elephants can be catalogued photographically and notes made of distinguishing features such as sex, ear tears, tusks, tail hair pattern, depigmentation marks, and body lumps. Accordingly, sequences of behaviours of specific males and females can be followed, timed, and correlated with qualitative and quantitative assessment of specific emitted chemical signals.

At our field site, individual males were assessed for age and musth status (Table 1). Overt behavioural series were systematically recorded, focusing on clear-cut behaviours and responses by adult male elephants toward mature females. Concurrently female responses were noted (Table 2). These detailed behavioural observations allowed the division of mature female elephants into two groups: those attracting males and mating-related behaviours, and those not attracting male attention. For the former group, we quantitatively assessed specific pre-mating male behaviours and chemosensory responses,

especially flehmen, allowing some female elephants to be categorized as probably in the preovulatory condition.

### *Field observation techniques*

We observed wild Asian elephants 6-9 h daily for 1 week in 1998, 3 weeks in 2000, and 2 weeks in 2002 at numerous sites along the Kabini River in Nagarhole National Park, Karnataka, India. The sites allowed vistas of up to 2 km. As measured with a laser range finder with an effective range of 1 km and correlated with video camera magnifications up to 72 fold, we were able to clearly delineate elephant trunk and olfactory behaviours up to 300 m.

Focal males were individually identified based on tusk attributes (shape, length, and thickness), dome size, ear characteristics, and other features. Photographs and video camera recordings documented this identity. Age was estimated based on body size, head shape, ear condition, and, if present, musth characteristics (Sukumar, 1989).

In the sexually dimorphic Asian elephant species, four aspects of male maturation were considered: sexual, physical, social and musth condition. Sexual maturation usually begins between 12 and 15 years (Sukumar, 1994). Physical maturation is an ongoing process continuing throughout the male's third and fourth decade and includes increases in total height and head dome size. Social maturity, evidenced by smooth interactions with other males and skillful interactions with females, is achieved only when males reach their early 30s. Lastly, the unique male elephant state of musth has a characteristic maturation. Physiological and chemical signal aspects of musth undergo extended longitudinal and chronological developments (Rasmussen et al., 2002). The first musth episodes begin shortly after young teenage males become sexually mature and are called 'moda' musth. In captivity, these males consistently demonstrate only slight temporal gland (TG) swelling, sparse temporal gland secretion (TGS), no urine dribbling (UD), no mud tusking, only a slightly different walk, and no repetitive trunk twisting. Serum testosterone levels are elevated sporadically up to 15-fold and fluctuate greatly. The infrequent and short-lasting temporal gland secretions correlate with spikes in testosterone concentrations (Rasmussen et al., 2002). The chemistry of these secretions is significantly different from that of fully adult males (Rasmussen & Perrin, 1999; Schulte & Rasmussen, 1999b; Rasmussen et al., 2002). These characteristics and others, including short durations of musth



**Table 1.** Scoring criteria for musth characteristics.\*

Score	TG <sup>1</sup> swelling	TG <sup>1</sup> secretion	Urine dribbling	Self-sniff TG <sup>1</sup>	Tusk mud <sup>2</sup>	MW <sup>3</sup>	TT <sup>2,4</sup>	Ketones <sup>5</sup>
0	no visible swelling	none visible	none	none	0	0	0	0
1	slight increase in outline of gland	visible as small spot	occasional droplet or recent leg staining	distant sniff (1 m)	1 time	0	1 time	C <sub>9</sub> , C <sub>11</sub> detectable
2	moderate increase in outline of gland	streaking to below eye level	thin, steady stream of droplets	close sniff (10-100 cm)	2 times		2 times	C <sub>9</sub> , C <sub>11</sub> some
3	prominent bulging of gland	streaking to corner of mouth	several moderate streams of urine	touch with trunk tip	3 times		3 times	C <sub>9</sub> , C <sub>11</sub> moderate
4	swollen gland intrudes into eye socket	streaking to chin or under	constant single wide stream	suck at with trunk tip	4 times	+	4 times	C <sub>9</sub> , C <sub>11</sub> lots

<sup>1</sup> Temporal gland.

<sup>2</sup> Tusking mud and trunk twist were scored per number of times behavior observed in one session.

<sup>3</sup> MW = Musth walk; scored as all (+) or none (0).

<sup>4</sup> TT = trunk twist; a characteristic repetitive twisting of trunk around the head, reaching from eyes to mouth, only observed during musth.

<sup>5</sup> Ketones were quantified in urinary volatiles.

\* Nonmusth (NM), if TG swelling, TG secretion (TGS), urine dribbling (UD), and ketones were 0; musth (M), if two or more of the three criteria of TG swelling, TGS or UD scored between 1 and 4; premusth (TG swelling = 3, TGS = 0, 2-butanone (C<sub>4</sub>) elevated); light musth (TG secretion = 2, urine dribbling = 0); heavy musth (TG swelling, TG secretion, and urine dribbling all score 3-4), 2-nonanone (C<sub>9</sub>) and/or 2-undecanone (C<sub>11</sub>) elevated.

**Table 2.** Chemosensory-based responses and behaviors.

Responses	Definition
<i>Main olfactory (MO)</i>	
Sniff	Olfactory input toward the primary olfactory turbinals
distant	Trunk elevated, with tip as directional periscope, trunk compression may be seen
close	Tip may hover over sample or female, compression may be seen, inhalant air maybe heard
<i>Either MO or VNO</i>	
Check	Placement of dorsal trunk tip finger on urine, urogenital region or other body areas (primary olfactory input to turbinals and probably additional input to vomeronasal organ)
regular place	A variation on check response in which entire trunk tip surrounds a sample (again involvement of both main and vomeronasal organ olfaction)
suck	A variation on place response in which the whole trunk tip surrounds the sample and trunk musculature is seen to contract, suggesting the air flow is forcibly increased over the turbinals (subsequent flehmen responses usually occur)
<i>Vomeronasal (VNO) system (presumed)</i>	
Flehmen	Trunk curled and tip containing drops of urine (or other substances) is placed onto the openings of the vomeronasal organ ducts
<i>Trunk-to-</i>	
Mammary glands	Check to nipple region of mammary gland
Palatal pit area	Check to numerous, small blind-ended crypts located bilaterally dorsal, anterior mouth cavity
MALE ACTIONS	
Penis peek	Only tip of penis visible
Erection	Penis fully extended and rigid
Trunk on F back	
Mounting	Male's forelimbs and trunk on female's back
Avoidance	Fails to initiate or ceases forward motion toward female or sample
Circling	Moves laterally around sample area or female
FEMALE ACTIONS	
Tail flick	End hairs of tail tip are dragged across the clitorius and then waved behind like a flag
Tail out	Tail out horizontally
Urinate	Urine expressed
Check male TG	Trunk tip touches male temporal gland opening

periods, masturbation episodes, erratic, sporadic movements, and distinctive honey-scented secretions, define 'moda' musth (Chandrasekharan et al., 1992; Rasmussen et al., 2002).

The attributes of Table 1 were scored to establish the extent of musth in each individual male elephant. For two categories of adult males [males older than 30 years (OA) and males between 20 and 30 years (Y20sA)], an individual male was deemed in musth if two or more of the following three criteria were evident and scored between 1 and 4: temporal gland swelling, temporal gland secretion, and urine dribbling (Table 1). Pre, light and heavy musth and nonmusth are defined in greater detail in Table 1. Thus, according to the characteristics in Table 1, young adult teenage males could only score numerically above 0 in the TG swelling and TG secretion attributes, not in urine dribbling as they did not exhibit this feature.

Second, we measured distances to males and females using a Bushnell 1000 laser range finder. This equipment had an effective range of 1 km and at 500 m was accurate to less than 1 m. By triangulation, we calculated the male-to-female distance. Distance measurements were conducted simultaneously with video recordings of responses including distant sniffs. Wind direction was recorded and wind conditions qualitatively scored as calm, slight, moderate, or very breezy.

Third, video recordings were made for the duration of all visible adult male-to-adult female elephant interactions including purviews, proximities, and contacts. Field notes recorded the time duration of discrete interactive sessions and were confirmed by timed video recordings of the entire session. Purviews were defined as elephants being within visual sight of each other, and thus within limits of olfactory detection if wind direction and conditions are favorable (between 50 and 100 m). Proximity was defined as a male and a female being within 1-5 trunk lengths (1-5 m). Contacts were defined as the trunk touching any part of another elephant's body. Interactions between a focal male and females were our major emphasis, but relevant overt female behaviours and responses and behaviours of other males present were also recorded.

We recorded the focal male's olfactory chemosensory-based behaviours toward females. Olfactory interest by males toward females was scored as definite interest (+) or no interest (-). Such interest at purview, proximity, or contact distances was clearly documented by, respectively, directional

distant sniffs, close sniffs, and other behaviours and responses listed in Table 2. If interest was followed by overt attraction toward a female, change of direction and speed of movement toward females were recorded. Close-up videotaping recorded the sequence of responses suggestive of either the MO or VNO sensory systems (Table 2). The following chemosensory-based responses and behaviours were scored from playbacks of the video recordings: (a) distant sniffs, close sniffs, blows, and trunk shakes involving MO; (b) checks and several variations including places, sucks, and scrubs involving either MO or the VNO; (c) flehmens presumably involving the VNO. All these responses were clearly seen and readily countable (Rasmussen, 1999). Additional male-to-female 'trunk-to' behaviours recorded included trunk-to-nipples and trunk-to-palatal pits (located dorsally, bilaterally in anterior oral cavity) (Table 2). We recorded all episodes of erections, masturbations, full trunk on back, and mountings as were female behaviours of tail out, urination, tail flicking (tail hairs are dragged over clitoral region and then tail is waved backwards and upwards in quick flicking motion), solicitation, avoidance, and trunk to male temporal gland (Table 2).

Our previous research demonstrated that the presence of males or their forceful blowing of air toward the female urogenital area elicited female urination. Less than 10 ml of urine, often only a few drops, were required to stimulate male flehmen responses if the proper concentrations of specific chemical compounds are present (Rasmussen et al., 1986). We thus watched for such male-toward-female behaviours among wild elephants. When we observed urinations followed by multiple flehmens by males, we waited until we could safely approach the sample site and then collected samples of urinary volatiles. Likewise, if males performed multiple urogenital checks followed by flehmens on a particular female, we discretely tracked this female until she urinated, sometimes several hours later. Samples of urinary volatiles were collected immediately after urination (2-5 min) after both male and female elephants moved away to a safe distance. For the control samples, a similar collection procedure was followed.

Many females were characterized individually so that sequential testing by different males could be substantiated. We observed several females that were checked on sequential days by the same male or by multiple males on the same day. In addition to male responses toward females and their urine, characteristic female-to-female behaviours and chemosensory responses confirmed their oestrous status (either preovulatory or non-

reproductively active). Subsequent chemical analyses confirmed these field observations of oestrus.

### *Field collection procedures*

#### Evacuated canister capture (ECC) of urinary volatiles

If male elephants display multiple flehmens to specific females and these females concurrently or subsequently urinated, we should detect Z7-12:Ac in this urine. Hence, we analyzed field-collected samples of male-attracted-to female urine and controls for the presence and concentration of Z7-12:Ac. The volatile compounds (i.e., low molecular weight, gaseous compounds) from urine were captured as headspace samples in evacuated (to ~30 inches Hg vacuum), specially designed, stainless steel bottles (0.85-l or 6-l capacity) that allowed long-term storage at ambient temperature until gas chromatographic/mass spectrometric (GC/MS) analyses were conducted (Perrin et al., 1996; Rasmussen & Perrin, 1999). All samples were taken in duplicate or triplicate to ensure sufficient concentrations for detailed qualitative analyses of multiple compounds and for accurate quantitative analyses of Z7-12:Ac. The canister collection of elephant urine headspace volatiles followed one of the following two procedures. Both yielded similar relative levels of compounds, but concentrations were higher using the second procedure (Rasmussen & Wittemyer, 2002):

Procedure 1. If sufficient urine remained above ground, a stainless steel measuring cup was used to scoop carefully 25-100 ml of urine into a clean 250-ml glass jar fitted with a special lid that contained two Swagelok fittings. One fitting was connected to the jar via ultra-clean Nupro SS-4H4 bellows-stem valves to the evacuated stainless steel receiving bottle, and the other fitting controlled entry of clean replacement air. The urine samples were heated to 35-37°C for 30 min allowing volatilization at a controlled temperature. This equilibration time was considerably longer than that of funnel-gathered samples (see below). Subsequently, at 15-min intervals for 2 h, the stainless steel evacuated receiving bottle was briefly opened to allow the entry of the compounds developed in the headspace until the receiving bottle reached ambient pressure.

Procedure 2. If it was not possible to obtain liquid samples, an inverted funnel attached to the evacuated canister was placed in situ firmly over the recently voided urine sample on the ground. After equilibration, we drew

headspace volatiles into the evacuated canister in a slow, steady manner over a period of less than 5 min to capture the compounds volatilizing from the fallen urine (Rasmussen & Wittemyer, 2002). Control background air samples were obtained over vegetation or ground substrate adjacent to the urine sample. Both procedures 1 and 2 captured urinary volatiles into the stainless steel bottles for subsequent GC/MS analyses (Rasmussen & Perrin, 1999).

### Solid phase microextraction procedure

We used a second field technique to capture a different but overlapping array of urinary compounds. Subsequent analysis allowed additional qualitative confirmation of *Z7-12:Ac* in the urine of the wild females. A field sampler solid-phase microextraction (SPME) fiber, 100- $\mu\text{m}$  polydimethylsiloxane, was immersed for 2-5 min in droplets of female urine identified as either bioactive (or not) by previously observed responses by male elephants. All of these SPME samples were duplicates of the canister samples. The fibers were kept between  $-20$  and  $-25^\circ\text{C}$  prior to analysis in the laboratory.

### GC/MS analyses

#### ECC samples

We conducted GC/MS analyses on equivalent aliquots of samples by the methods described in detail in Perrin et al. (1996) and Rasmussen & Perrin (1999), for qualitative and quantitative assessment of urinary volatiles. The mass spectrometer was programmed for mass scans between 33 and 300 mass units, which allowed for identification of compounds from  $\text{C}_3$  through  $\text{C}_{14}$ . The conditions allowed quantitation as low as 0.10 ppbv (parts per billion volume). Compounds were identified using an NBS 75 K Hewlett-Packard MS ChemStation library search and were rechecked manually with the NIST/EPA/NIH Mass Spectral Data Base Version 4.01, the Wiley library, and our own library of elephant-specific compounds. Internal standards of authentic synthetic compounds of measured concentration for compounds of specific interest (including *Z7-12:Ac*) as well as several simple and cyclic ketones provided positive identification and quantitation.

## SPME samples

These analyses by GC/MS followed the procedures developed for analysis of Z7-12:Ac (Rasmussen, 2001). The mass spectrometer was programmed at 0.83 scans/s for a mass scan of 33-550, which produced mass spectra facilitating identifications of compounds from C<sub>3</sub> through C<sub>18</sub>. Most compounds were identified using an NBS 75 K Hewlett-Packard Mass Spectrometer ChemStation library search and were rechecked manually with the NIST/EPA/NIH Mass Spectral Data Base Version 4.01 + 1.6d and the Wiley Library Version 6-275. For Z7-12:Ac and (Z)-7-dodecenol, authentic synthetic standards were analyzed on our particular equipment, allowing confirmation of two parameters of identification: retention time by GC separation, and mass ion and dominant ion patterns by mass spectrometry. Internal authentic standards compared to real samples confirmed the presence of Z7-12:Ac.

Previously, studies with urine from captive female Asian elephants established the identification of more than 100 compounds by ECC-GC/MS and an additional 25 by SPME-GC/MS; many of these compounds were confirmed by simultaneous retention time analysis of authentic synthetic chemicals. Most compounds were present in the urine in the same proportions and total concentration throughout the oestrous cycle (Rasmussen et al., 1982, 1986, 1993, 1997). The notable exception was the preovulatory elevation of Z7-12:Ac (Rasmussen, 2001).

## *Data analyses and statistics*

### Behavioural data

Our first criterion was to identify all focal males and most females. The second criterion was to determine the extent of musth.

Because of its exploratory and field nature, we employed statistics with caution due to low sample size and varying conditions. We used the broad terms 'encounters' or 'sessions' of chemosensory interest rather than attempting at this stage to separate these into smaller units. Each session or encounter period was timed from the initial male-female interactions until the departure of one or both participants. From real-time video records and field notes, we calculated frequencies of male-female interactions per unit of time (rates/hour). In addition, we focused on sequences of behaviours. Within four male age categories, we compared musth and nonmusth male

chemosensory behaviours. We scored the data as purviews, proximities, and contacts between males and females and tabulated the data as total number of male-to-female interactions per unit of time. Male chemosensory responses toward females were scored as discrete response types and later expressed as total chemosensory responses per hour. Male-to-female premating behaviours were also scored separately and subsequently summed as premating behaviours and expressed per hour. Female actions toward males that were relevant to this study were also noted.

Several statistical tests were employed. Age differences in responses and behaviours were tested between old adult, young adult, and teenage males in musth by Kruskal-Wallis one way analysis of variance (ANOVA) (Siegel & Castellan, 1988). Teenage males and young males in nonmusth were also compared using Kruskal-Wallis ANOVA followed by pairwise multiple comparisons (Dunn's method) or, when only two groups were involved, Mann Whitney rank sum (Sokal & Rohlf, 1995). Fisher's exact test was used on occasion to test whether the one nonmusth older male differed from the six musth males by evaluating whether his data fit inside or outside the 95% confidence intervals of the six musth males. In all figures, data are expressed as responses or behaviours per hour.

### Chemistry data

Field collected samples of female urine diagnosed as preovulatory by male response behavior were limited because of logistics involved in the collection and transfer of the samples to the laboratory. We were conservative and constrained on the use of statistics (Sokal & Rohlf, 1995). Duplicate samples were collected for chemical analysis. We had no repeated samples, thus no pseudoreplication, but we present semi-quantitative results on concentration differences for Z7-12:Ac and qualitative results for apparent concentration differences in other compounds by comparing follicular/preovulatory samples with luteal samples. The statistical significance of the differences could not be assessed due to sample size limitations.



## Results

### *Groupings and general observations*

#### General information

Thirty-eight interactive sessions of 29 males with 105 females were observed and recorded. Of a total of 1440 min recorded, ~400 min of definitive male-to-female encounters were documented, including sessions lasting from 1-35 min with an average of 11 min. The entire sequence of each of 38 interactive sessions was video recorded.

#### Males and musth

From field notes, photos, and videotape analyses, males were categorized into four age groups and their male groupings described (Table 3) and then each male was labeled as musth or nonmusth per their musth characteristics scores (Table 1).

The three young subadult males (YSA) were predominantly an integral part of the family unit and were usually in close proximity or contact with females but occasionally located in the close periphery (Table 3). No overt male-male interactions by this group were observed; older males ignored the subadult youngsters. The eleven young teenage adult (YTA) males (~ages 13-19 y) were usually solitary or in pairs. Nine young adult males in their 20s (Y20sA) were not as large physically as the older adult males; they were either alone or in pairs. Six older adult males age 30 y or more (OA) were observed; they were almost solitary-not in close proximity to any other males (Table 3).

As musth is an important component of being a male elephant, males in each age group were characterized as in musth or not (nonmusth) and, when possible, described further as being in pre, light, or heavy musth. These behavioural assessments were substantiated by chemical analyses of the male urine (Table 1). Of the eleven YTA males, four were in moda musth (MoM) (Table 3). Three of these males were solitary or not near another male. Some male-male interactions between these YTA and older males were apparent at purview distances (greater than 5 trunk lengths). Interactive behaviours between YTA and OA in musth varied with musth status of the YTA. One solitary YTA male in moda musth maintained a constant, 100-m distance from an older, larger musth male but nevertheless interacted opportunistically with females. In contrast, a nonmusth YTA male in the presence of

**Table 3.** Total male-to-female interactions per male groupings at three spacing distances.

Male categories	Definition	Symbol	Musth	N <sup>2</sup>	Number of females <sup>1</sup> interacted via			Total females	Average # females/male	Total interactions	Average interactions/male
					Purviews	Proximity	Contact				
Adult males (30 y or more)	OA	M <sup>2,3</sup>	NM <sup>2</sup>	5	4.0	7.0	10.4 <sup>3</sup>	70	12	109	22
				1	0	3.0	7.0	4	7	10	10
Adult males (20-30 y)	Y20sA	M	NM	3	2.3	2.3	3.0 <sup>3</sup>	9	3	25	8
				6	0.1	1.0	0 <sup>4</sup>	6	1	7	1
Young teenage males (YTA) (~13-19 y)	YTA	MoM <sup>2</sup>	NM	4	0.5	0.3	2.0	10	2	11	2
				7	0.4	0.7	1.0	12	1	15	2
Young subadult males (<12 y)	YSA	NM	NM	3	0	1.0	1.0 <sup>6</sup>	3	1	6	2

<sup>1</sup> 105 females were interacted with by males. Females may be in a distance classification more than once. Twelve females were contacted more than once by same male on different days.

<sup>2</sup> N = number; A = average; M = musth; NM = nonmusth; MoM = moda musth; see text for specific stages of musth.

<sup>3</sup> Two females, despite their nearness to ovulation as demonstrated by pheromone release eliciting high flehmen frequency, were apprehensive of one musth male.

<sup>4</sup> All responses were to previously expelled urine.

<sup>5</sup> Responses were close sniffs to the urogenital region of the mother.

<sup>6</sup> Responses were either to expelled urine or to the urogenital region of the female.

a nearby older musth male was apprehensive and apparently deterred from female interactions, performing only distant sniffs.

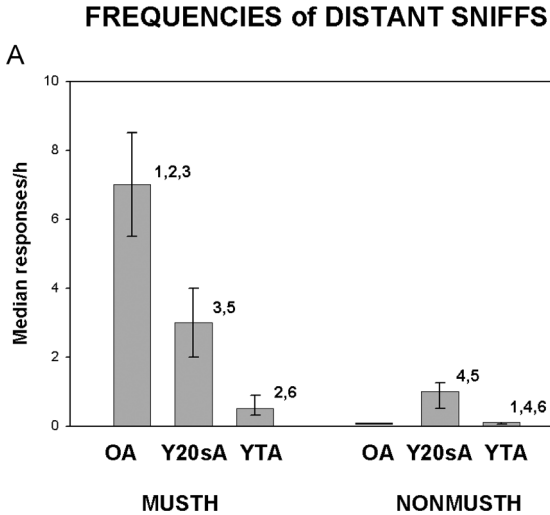
Among the nine Y20sA males, six were in the nonmusth state (Y20sA-NM) (Table 3), two were in light musth with a TGS score of 2 and not evidencing urine dribbling (Table 1), and a third was in premusth as indicated by swollen temporal glands, a TG score of 3, but no visible secretions (see Table 1).

Among the six OA males, only one in this age category was in nonmusth. Two musth males, observed for several successive days, were the largest and oldest males observed; they were in heavy musth with swollen temporal glands, a TG score of 4, TGS varying daily with a score between 2 and 4, and urine dribbling varying with a score of 0 to 1. The other three males in musth in this group scored between 3 and 4 on many of the musth characteristics, also indicating heavy musth (Table 1).

### Females and interactions

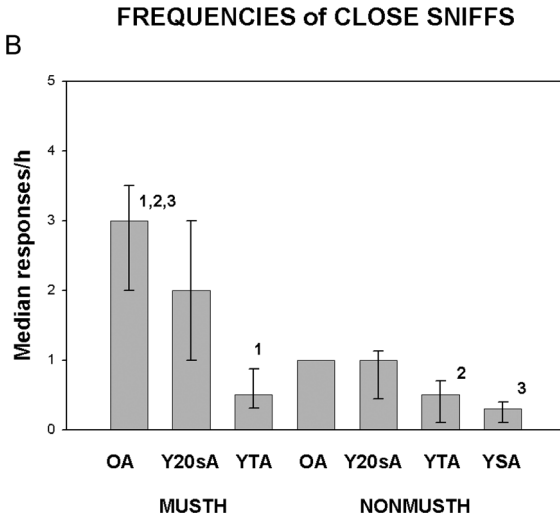
The 29 males (in four age groupings, in musth or nonmusth, constituting seven categories) interacted with 105 females. The number of females with whom males interacted are presented per male category and further subdivided into three categories of distances in Table 3. On average, musth males in all age categories interacted with more females than nonmusth males (Table 3). The frequencies of male-to-female responses and behaviours at these three inter-animal distance categories — purview, proximity and contact — are shown in Figures 1, 2, and 3, respectively.

For male-to-female purview interactions, the frequency of distant sniffing is shown for each male category in Figure 1a, and the total and average females interacted with per group are listed in Table 3. OA males interacted with more total females and more females/male (Table 3) and exhibited significantly higher median frequency of long distance sniffs toward females than the males in all other categories, including musth (M) and non-musth (NM) males in their 20s (Figure 1a). Statistically significant differences (SSD) are indicated by matching numbers in bold (1-1, 2-2, 3-3, 4-4, 5-5, 6-6) as seen in Figure 1a. Kruskal-Wallis ANOVA revealed SSD between the 3 musth groups ( $H = 9.73$ ,  $df = 2$ ,  $p \leq 0.001$ ) and pairwise multiple comparisons Mann-Whitney rank sum test and Dunn's method additionally indicated SSD between groups. OA-M males performed significantly higher frequency of distant sniffs than YTA-MoM males (2-2, Figure 1a) [ANOVA,



**Figure 1.** (A) Purview interactions: frequencies of distant sniffs. Abbreviations: M = musth; NM = nonmusth; for male categories and number of males per category: OA = older adult (M,  $N = 5$ ; NM,  $N = 1$ ); Y20sA = young twenties adult (M,  $N = 3$ ; NM,  $N = 6$ ); YTA = young teenage adult (M [MoM],  $N = 4$ ; NM,  $N = 7$ ). No purview interactions were observed for the single OA-NM as indicated by solid bar above baseline. Data depicted graphically as medians with two-way error bars of 75%-25%. Statistically significant differences (SSD) are indicated by matching numbers in bold (1-1, 2-2, 3-3, 4-4, 5-5, 6-6). (B) Proximity interactions; frequencies of close sniffs. Additional abbreviation: YSA-NM = nonmusth young subadults. Data depicted graphically as medians with two-way error bars of 75%-25%. Kruskal-Wallis ANOVA on ranks revealed one (1-1) SSD between 3 musth groups and two (2-2, 3-3) SSDs between musth and nonmusth groups. Note that the frequency of close sniffs by the one nonmusth OA male was not within the confidence limit of the OA-M males.

pairwise multiple comparisons, Diff of Ranks (DR) = 7.50,  $Q = 3.10$ ,  $p \leq 0.05$  and pairwise Mann Whitney rank sum test  $T = 10.0$ ,  $p = 0.016$ ]. Distant sniffs by OA-M males were also significantly elevated over those of Y20sA-M males (3-3, Figure 1a), although the difference was less [pairwise Mann Whitney rank sum test  $T = 6.0$ ,  $p \leq 0.036$ ]. Comparison of the musth and nonmusth categories showed a significantly higher responsivity of OA-M males as compared to YTA-NM males (1-1, Figure 1a) [ANOVA  $H = 22.21$ ,  $p \leq 0.001$  and pairwise multiple comparisons Diff of Ranks 19.00,  $Q = 4.41$ ,  $p \leq 0.05$ ]. The Y20sA-M males on average interacted with more females (Table 3) and responded with significantly more distant sniffs than nonmusth males (5-5, Figure 1a) [ANOVA  $T = 20.5$ ,  $p = 0.05$  and Mann Whitney rank sum  $T = 20.50$ ,  $p = 0.036$ ]. Among the YTA

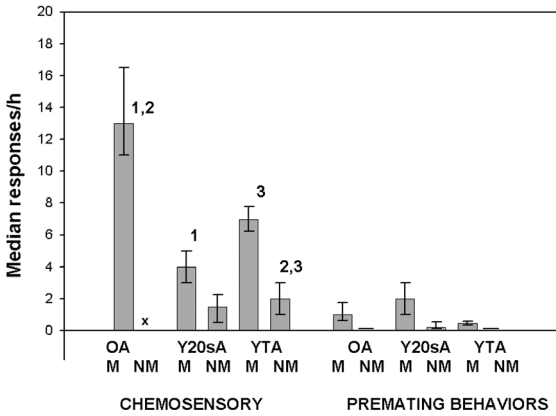


**Figure 1.** (Continued).

category, both moda and nonmusth males interacted on average with similar numbers of females, but males in moda musth exhibited a 2-fold higher frequency of distant sniffs compared with nonmusth counterparts (6-6, Figure 1a) [Mann Whitney rank sum  $T = 38.00$ ,  $p = 0.006$ ]. The one nonmusth OA male showed no distant sniffs. Within only nonmusth groupings, Y20sA-NM demonstrated significantly higher distant sniffs than YTA-NM (4-4, Figure 1a) [pairwise Mann Whitney rank sum test,  $T = 63.00$ ,  $p \leq 0.001$ ].

For proximity interactions OA-M males interacted with more than twice the number of females than did Y20sA-M males and with more than four times the proximity interactions of males in all other categories (Table 3). In Figure 1b significant differences in frequencies are indicated by matching numbers in bold (1-1, 2-2, 3-3). The OA-M males exhibited a significantly higher frequency of close sniffs than all other groups of males except Y20sA-M males (Figure 1b). ANOVA revealed one (1-1, Figure 1b) SSD between 3 musth groups [ $H = 7.79$ ,  $df = 2$ ,  $p \leq 0.007$ ]; and two (2-2, 3-3, Figure 1b) SSDs between musth and nonmusth groups [ $H = 20.06$ ,  $df = 6$ ,  $p \leq 0.003$ ]. OA-M males performed significantly higher frequencies of close sniffs than YTA-MoM males (1-1, Figure 1b) [Pairwise multiple comparisons (Dunn's method)  $DR = 6.58$ ,  $Q = 2.72$ ,  $p \leq 0.05$ ] or than YTA-NM (2-2, Figure 1b) [ $DR = 20.53$ ,  $Q = 3.30$ ,  $p \leq 0.05$ ], or YSA who had never experienced musth (3-3, Figure 1b) [ $DR = 17.41$ ,  $Q = 3.49$ ,

## Frequencies of summed contact and preming behaviors



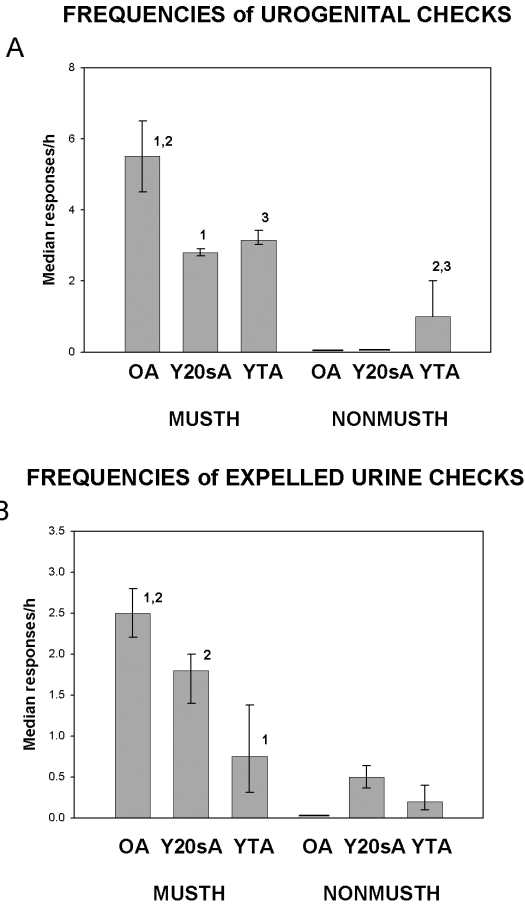
**Figure 2.** Summed male-to-female contact interactions and preming behaviours. Similar abbreviations as Figure 1a and b. Data from single OA-NM were not depicted; he contacted seven females and exhibited 10 summed contacts (Table 3). His chemosensory responses were all urogenital checks (x), and he exhibited no preming behaviours as indicated by solid bar above baseline. The frequency of either his summed contact or preming behaviour was not within the 95% confidence limit of the OA musth males. Data depicted graphically as medians with two-way error bars of 75%-25%. Kruskal-Wallis ANOVA on ranks revealed two (1-1, 2-2) SSDs in chemosensory responses between all groups. YTA-NM also showed no preming behaviours as indicated by solid bar.

$p \leq 0.05$ ]. The frequency of close sniffs by the one nonmusth OA male was not within the confidence limit of the OA-M males. Y20sA-M males also were more interactive with females than nonmusth males of the same age; the musth males demonstrated a 2-fold higher median frequency of close sniffs than the nonmusth group (Figure 1b), although the difference was not statistically significant. Only one YTA in moda musth interacted proximally with a single female, exhibiting a single close sniff; this male maintained a controlled distance from a large musth male mentioned earlier, whereas YTA-NM males, with no adult males present, exhibited a similar response frequency as seen with YSA males (who have never experienced musth).

OA-M males contacted the greatest number of females, 1.3-fold more than the single OA-NM and 3-5-fold more than Y20sA-M and YTA males, both in moda musth and nonmusth (Table 3). The contact responses by Y20sA-NM males were not to females, but only to their expelled urine. YTA-MoM males contacted twice as many females as their nonmusth equivalents (Table 3).

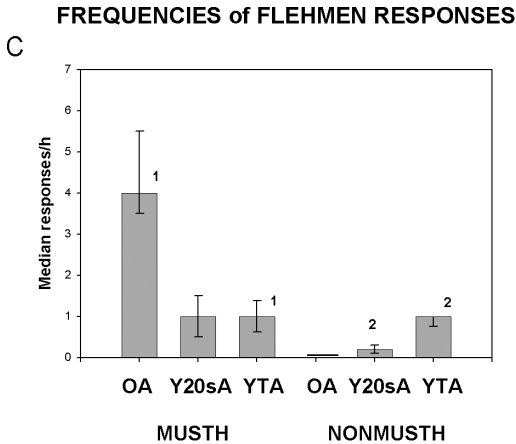
Frequencies of total contact chemosensory interactions from males toward females (Figure 2) were higher for musth males in all categories compared with their nonmusth counterparts (Figure 2). ANOVA revealed two (1-1, 2-2, Figure 2) SSDs in chemosensory responses between all groups [ $H = 34.48$ ,  $df = 8$ ,  $p \leq 0.001$ ]. Among musth male groups, the OA-M demonstrated the highest frequency of contact chemosensory responses, and this frequency was significantly higher than those of Y20sA-M (1-1, Figure 2) [Pairwise multiple comparisons (Dunn's method)  $DR = 34.50$ ,  $Q = 4.64$ ,  $p \leq 0.05$ ]. Y20sA-M and YTA-MoM exhibited more of these chemosensory responses than their nonmusth counterparts (Figure 2), with the difference significant between the YTA-MoM and YTA-NM (3-3, Figure 2) [Mann Whitney rank sum,  $T = 38.00$ ,  $p = 0.006$ ]. A wide and significant difference was seen between OA-M males and YTA not in musth (2-2, Figure 2) [ $DR = 31.50$ ,  $Q = 3.83$ ,  $p \leq 0.05$ ]. The YTA-NM males showed no premating behaviors as indicated by the solid bar (Figure 2). Among YTA males, most of the male-female interactions by nonmusth males occurred only in the proximity of females. In conjunction with the high degree of contact interaction by musth males, the higher chemosensory responsivity of YTA-MoM males was evident, being 2-3-times higher across all scored responses than YTA-NM males (3-3, Figure 2).

When individual types of responses and behaviours were examined, OA-M males exhibited higher frequencies of urogenital checks, urine checks, and flehmens than all other categories of males (Figures 3a, b, c); these higher frequencies were statistically significant for most groups. For urogenital checks ANOVA revealed one (1-1, Figure 3a) SSD among musth groups, [ $H = 9.69$ ,  $df = 2$ ,  $p \leq 0.001$ ] and two (2-2, 3-3, Figure 3a) SSDs between musth and nonmusth groups [ $H = 15.81$ ,  $df = 3$ ,  $p \leq 0.001$ ]. OA-M males exhibited significantly higher frequencies than Y20sA-M males (1-1, Figure 3a) [pairwise multiple comparisons (Dunn's method)  $DR = 8.00$ ,  $Q = 3.04$ ,  $p \leq 0.05$ ]; expelled urine checks and flehmens were also higher between these two groups. The median frequency of urogenital checks was significantly higher in OA-M males than YTA-NM males (2-2, Figure 3a). [ $DR = 12.50$ ,  $Q = 3.70$ ,  $p \leq 0.05$ ]. Among YTA-males the YTA-MoM males showed significantly higher responses than YTA-NM males (3-3, Figure 3a) [ $DR = 7.85$ ,  $Q = 2.77$ ,  $p \leq 0.05$ ]. Among YTA-MoM and Y20sA-M males, the observed frequencies of urogenital checks (Figure 3a) and flehmens (Figure 3c) were similar whereas Y20sA-M males exhibited a



**Figure 3.** (A) Frequencies of urogenital checks to the female urogenital or vulva region. Data depicted graphically as medians with two-way error bars of 75-25%. Kruskal-Wallis ANOVA on ranks revealed one (1-1) SSD in urogenital checks between musth groups and Kruskal-Wallis ANOVA on ranks revealed two (2-2, 3-3) SSD in urogenital checks between musth and nonmusth groups (2-2). Solid bars above baseline indicate no responses observed. (B) Frequencies of expelled urine checks; no contact with body of female. Data depicted graphically as medians with two-way error bars of 75-25%. Kruskal-Wallis ANOVA on ranks revealed (1-1, 2-2) SSD in expelled urine checks between musth groups. Solid bars above baseline indicate no responses observed. (C) Frequencies of flehmen responses. Data depicted graphically as medians with two-way error bars of 75-25%. Kruskal-Wallis ANOVA on ranks revealed one (1-1) SSD in flehmen responses among musth groups and by Mann Whitney rank sum test one (2-2) SSD in flehmen responses among nonmusth groups. Solid bar above OA (nonmusth) indicates no responses observed.





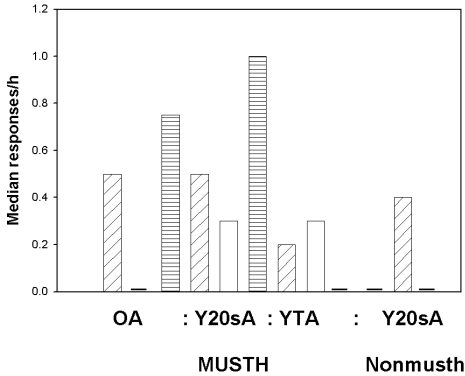
**Figure 3.** (Continued).

higher frequency of expelled urine checks than YTA-MoM males, although the difference was not significant (Figure 3b).

However, responses to expelled urine differed significantly between OA-M and the other two categories-Y20sA-M and YTA-MoM. ANOVA revealed two (1-1, 2-2, Figure 3b) SSDs in expelled urine checks between musth groups [ $H = 9.29$ ,  $df = 2$ ,  $p \leq 0.001$ ]. Pairwise multiple comparisons (Bonferroni test) indicated SSD between the musth groups OA-M and YTA-MoM, with the responses by the OA-M significantly higher than those by YTA-MoM (1-1, Figure 3b) [Mann Whitney rank sum  $T = 10.0$ ,  $p = 0.016$ ; Diff of means 0.99,  $t = 3.1$ ,  $p \leq 0.036$ ]. In addition, OA-M males responses to expelled urine were also significantly greater than those by Y20sA-M males (2-2, Figure 3b) [Diff of means 0.99,  $t = 3.1$ ,  $p \leq 0.036$ ]. In the two groups, Y20sA and YTA, the median frequency of the expelled urine checks was higher but non significant in the musth males than the nonmusth males (Figure 3b).

Flehmen responses showed one significant difference among musth groups by ANOVA (1-1, Figure 3c) [ $H = 8.28$ ,  $df = 2$ ,  $p \leq 0.001$ ] and among non-musth groups by Mann Whitney rank sum test (2-2, Figure 3c). [ $T = 21$ ,  $p = 0.001$ ]. Among musth categories, OA-M males demonstrated higher responses than Y20sA-M and YTA-MoM, but the difference was only significant for the YTA-MoM group (1-1, Figure 3c) [pairwise multiple comparison (Dunn's method),  $DR = 6.00$ ,  $Q = 2.48$ ,  $p \leq 0.05$ ]. No significant flehmen frequencies were observed between musth and nonmusth male cate-

## FREQUENCIES of Premating Behaviors



**Figure 4.** Frequencies of individual types of premating behaviours are depicted as median responses/h. Paucity of numbers precludes statistics. In legend, slanted lines = erections, clear bars = mounts, horizontal bars = mate guard. Solid bars above baseline indicate no behaviours observed.

gories, but YTA-NM performed significantly higher flehmen responses than their Y20sA-NM counterparts (2-2, Figure 3c) [ $T = 21$ ,  $p = 0.001$ ].

Among the Y20sA, nonmusth males exhibited no contact interactions, such as urogenital checks, toward females (Table 3, Figure 3b). Rather they only performed checks and flehmen responses to previously expelled urine spots (Figure 3b). In this age category, both flehmen responses and erections were 2 times lower in nonmusth males than musth males, although paucity of observations per behaviors precludes statistics (Figures 3c and 4).

Olfactory-based behavioural sequences involving YSA (<12 y old) males toward female elephants were infrequent and involved responses to female urine or to the urogenital region of females (Table 3). Three older females permitted urogenital checks by sub-teenager males; other young females thwarted similar checking attempts. In one session, olfactory checks were performed on expelled female urine by a sub-teenager male, but the matriarch and older females immediately chased him away.

### Premating behaviours

Erections occurred in both musth and nonmusth groups and were equally frequent in OA-M and Y20sA-M groups. A trend toward higher frequency of erections occurred in Y20sA-NM males, showing little difference between

the musth and nonmusth groups (Figure 4). Mate guarding was only observed by OA-M and Y20sA-M (Figure 4), whereas mounting occurred in YTA-MoM and Y20sA-M (Figure 4).

Two musth OA males wandered at least several kms per day, whereas the nonmusth OA male exhibited more restrictive movements. Chemosensory interactive responses followed this general pattern. One musth male checked more than 12 females within a 5-min period; in contrast the nonmusth male interacted with only five females even though the same females were available for a longer period (Table 3). The nonmusth OA male was large and exhibited other dominance attributes including displacement of other elephants from grass foraging spots. Another OA-M male interacted with 39 females during three successive days, checking three females daily. A third OA musth male interacted with 21 females, including two females on successive days. Twelve females were either rechecked on sequential days or checked by multiple OA males within a short interval, supporting the postulate that males monitor pheromone concentrations during the preovulatory period.

Ten cases of male responses to palatal pits on the palatal ridge in the dorsal buccal cavity of females were observed, with three OA males in musth performing 90% of these responses. All palatal pit responses occurred prior to urogenital or urine checks. The palatal pit responses were predominantly to female-calf dyads. Some examples include a male checking the palatal pits of a calf and then mate guarding the mother. In another episode, after a palatal pit response to a mother, the male sniffed her calf. Although an OA-M male exhibited palatal pit responses to a tail-flicking female, no urine or urogenital checks followed. None of the males in any category reacted to tail-flicking females, even when passing within one trunk length of the rear of such females. In contrast, matriarchs often checked and performed flehmen to tail flicking females.

Examination of total pre mating behaviours (Figure 2) and discrete pre mating behaviours — erections, mounts and mate guards (Figure 4) — demonstrated that Y20sA males in musth engaged equally or more often in such behaviours as OA males in musth. Mounting attempts in the Y20sA age category were observed only among musth males (Figure 4). All three musth Y20sA males were observed to mate guard at least one female (Figure 4). With one exception, all males conducting mate guarding were in musth, although no teenage males in moda musth were observed to mate guard. Mate

**Table 4.** Five characteristic sequential chemosensory responses and pre-mating behaviours toward females by YTA males in moda musth.

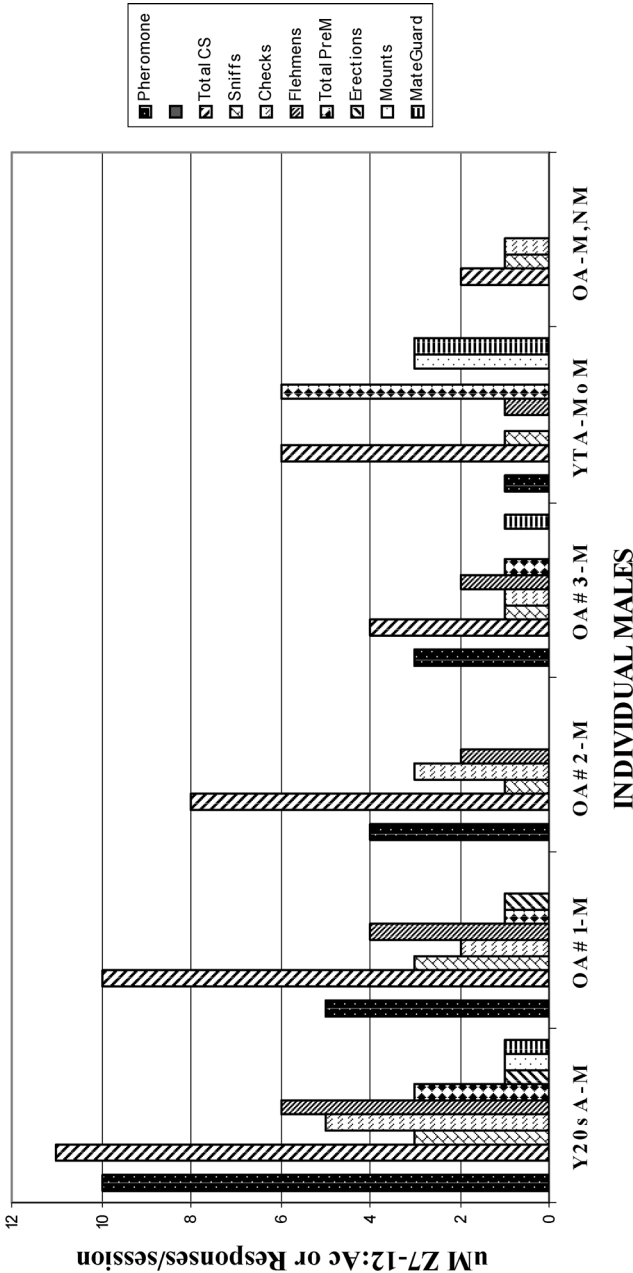
Male #	Discrete behavioural sequences
1	Checks urogenital region of female with calf.
2	Successively interacts with 5 females, checking palatal pits of two, and checking nipples of one.
3	Performs 2 flehmens toward a solicitous female; then mounts an adjacent log.
4	Rapidly performs urogenital checks and flehmen to young female, but retreats as older musth bull approaches.
5	Initially mounts female (prior to any urine checking), subsequently performs urogenital checks and induces urination by exhaling into vulva area of female.

guarding was conducted by all Y20sA-M, by four out of five OA-M, and by the single nonmusth OA (Figure 4). Seven males had erections, including three OA-M, one OA-NM, one Y20sA-M, and two Y20sA-NM.

Behavioural interactions between YTA-MoM males exhibited some unusual sequential characteristics (Table 4). An example of this variability included a YTA-MoM male physically ignoring a solicitous female who backed up to him; after performing two flehmens to her expelled urine, he instead mounted a nearby log (Table 4).

### *Preovulatory urine samples*

We obtained samples of 'presumably preovulatory' urine after six different males pursued five individual females. Indicators from five females that suggested they could be in the follicular phase included: (1) no observed mammary gland enlargement, suggesting that they were not in mid- to late pregnancy; (2) no observed nursing episodes in ~2-3-years-old calves accompanying these females; (3) no noted tail flicking. Subsequent sequential male-to-female interactive behaviours strongly suggested these five females were in the preovulatory phase of oestrus. This supposition was confirmed by later chemical analyses quantifying Z7-12:Ac (Figure 5). Males at purview distances [50-100 m] exhibited olfactory detection of these females, i.e. elevated trunks and tips pointed in the direction of female groups. Subsequently, often maintaining elevated trunk positions, males approached the females, where some were sniffed from an intermediate distance (1-5 trunk lengths, 1-5 m), and others were ignored. Next, selected females were approached closely. During two of the five encounters that resulted in sample collections, males



**Figure 5.** Summed chemosensory (CS) responses, then three discrete responses (sniff, check, flehmen) and total preming (PreM) behaviours, then three discrete preming behaviours (erectons, mounts, mate guarding) are compared to urinary pheromone (Z7-12:Ac) concentrations in individual female elephants. Y-axis represents either micromolar concentration ( $\mu\text{m}$ )  $\times 10$  of Z7-12:Ac or responses/session. The age and musth condition of the individual males is indicated on the X-axis.

gently touched the urogenital area, resulting in female urinations. During the other three encounters, females urinated as males approached. In all five encounters, the male placed his trunk tip into the urine and subsequently performed between two and four flehmen responses (Table 2; Figure 5). Other pre-mating behaviours also occurred (Figure 5). Headspace urine samples were gathered after the elephants moved off.

For five control episodes, the headspace urine samples were obtained at a similar time interval after female urination. During these episodes, the male, if present, did not approach the females and the females were (1) with nursing calves, (2) pregnant (as suggested by enlarged mammary glands), or (3) presumably not in the follicular phase of oestrus. Chemical analyses of the urine volatiles confirmed the non-preovulatory status.

The urinary volatile samples collected in the wild from non-preovulatory Asian female elephants (pregnant, young, or females in which males exhibited no olfactory interest) contained a complement of well-characterized gaseous compounds, including many ketones dominated by a constant acetone concentration (Rasmussen et al., 1997; Rasmussen, 2001; Rasmussen & Krishnamurthy, 2001). Aldehydes, alcohols, furans, acids, and sulfur-containing compounds were also present in measurable concentrations.

In contrast, the volatile samples from the urine of wild female elephants defined as preovulatory, not only contained the regular complement of compounds but also some distinguishing compounds. The most striking compound was *Z*-7-dodecenyl acetate (*Z*7-12:Ac), present in micro-to-millimolar concentrations (Figure 5). Four related compounds — two alcohols (2-dodecanol and (*Z*)-7-dodecen-1-ol), the aldehyde (dodecanal), and the ketone (2-dodecanone) — were detected at concentrations similar to levels measured in captive preovulatory elephants (Table 5).

In these preovulatory urine samples from wild female elephants, the levels of seven ketones were elevated. Five of these ketones had previously been demonstrated to be elevated in preovulatory captive female Asian elephants (Table 5, footnote 4). Three other ketones were also elevated in three of the five wild preovulatory females (Table 5, footnote 3). Similar to results from captive elephants, two bicyclic compounds were elevated in the urine of wild preovulatory females (Table 5).

Acids detected in the preovulatory urine of captive elephants were found in greater variety and concentration in wild elephants. Propanoic, butanoic, 3-methylbutanoic, and heptanoic acids predominated in non-preovulatory

**Table 5.** Compounds elevated or prominent in preovulatory (compared to non-preovulatory) urine of wild female Asian elephants.<sup>1</sup>

Class of chemical	Compound
Esters/acetates	3-Methylbutanoic acid methyl ester
	2-Butenoic acid, ethyl ester
	Butyl acetate
	1,1-Dodecanediol diacetate
	11-Dodecenoic acid methyl ester
	(Z)-7-dodecenyl acetate <sup>2</sup>
Aldehyde	Dodecanal
Alcohols	2-Dodecanol
	(Z)-7-dodecen-1-ol
Acids	Benzoic acid <sup>3</sup>
Ketones	4-Methyl-3-penten-2-one <sup>4,5</sup>
	2-Methylcyclopentan-1-one <sup>3</sup>
	2,3,4-Trimethylcyclopent-2-en-1-one <sup>3</sup>
	2-Cyclohexen-1-one <sup>3</sup>
	3-Octen-2-one <sup>4,5</sup>
	2-Dodecanone <sup>4,5</sup>
	2-Buten-1-one, 4-(2,6,6-trimethylcyclohexen-1-yl) <sup>4,5</sup>
	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl) <sup>4,5</sup>
Heterocyclics	2-Oxabicyclo[4.4.0]dec-7-ene <sup>4,6</sup>
	2,6,10,10-Tetramethyl-1-oxa-spiro[4,5]dec-6-ene <sup>4</sup>
Diphenyl pentenes	2,4-Diphenyl-4-methyl-1-pentene <sup>5</sup>
	(E)-2,4-Diphenyl-4-methyl-2-pentene

<sup>1</sup> Trapped by ECC or SPME, detected, identified, and measured by GC/MS.

<sup>2</sup> Concentrations varied from micro- to millimolar.

<sup>3</sup> Elevated in preovulatory urine from 3 of 5 wild female elephants.

<sup>4</sup> Present in urine of captive preovulatory females also.

<sup>5</sup> Elevated concentrations.

<sup>6</sup> Detected only or primarily by SPME.

wild females, whereas benzoic acid dominated in preovulatory urine (Table 5, footnote 3). In addition to Z7-12:Ac, five esters were detected in preovulatory urine, with the ester of 3-methylbutanoic acid elevated. These esters were not detected or were considerably lower in urine from pregnant, nursing, or non-attractive females (Table 5). As seen in studies of captive female Asian elephants, the major difference between preovulatory and non-preovulatory urine in the wild females was the strikingly higher concentrations of Z7-12:Ac (10-100  $\mu\text{m}$ ) (Table 5). As Z7-12:Ac was not detected in

urine samples from control female elephants, the difference between the two groups was presumed significant.

Chemosensory responses and behaviours by males were generally correlated with the measured concentrations of Z7-12:Ac (Figure 5). When individual response types or specific pre-mating behaviours were examined, the effect was less clear cut (Figure 5), perhaps reflective of the individual nature of male responses.

## Discussion

### *Why a female preovulatory pheromone in wild Asian elephants?*

Wild Asian elephants appear to utilize a complex olfactory chemical signaling system to ensure efficient and effective reproduction. Olfactory sensory mechanisms in Asian elephants allow adult males to locate females and to assess their ovulatory status precisely (Rasmussen et al., 1997). Access to mating-ready females is partially dependent on olfactory signals from males-to-females (Schulte & Rasmussen, 1999a). Signals between males, dependent on age, size, and an unusual male physiology, may be a decisive factor in determining which particular male has access to females to assess their reproductive phases. In the wild, the female-to-male pheromonal system was confirmed by chemical corroboration of the presence of the female urinary pheromone Z7-12:Ac, in correlation with observed high frequencies of chemosensory responses and pre-mating behaviours by wild male elephants. The synchronization of urinary pheromone concentrations, chemosensory responses, and behavioural acts facilitates breeding strategies of Asian elephants. Breeding tactics of this large, sexually dimorphic mammal occur within the context of its social structure, particularly female home ranges, small but multigenerational female family units, and roaming males. Chemical senses have an essential unifying role. The behavioural responses of older wild males in musth (three OA-M and one Y20sA-M) correlated with measured female urinary concentrations of Z7-12:Ac (Figure 5). This correlation corroborates two features initially delineated in female captive Asian elephants (Rasmussen et al., 1997; Rasmussen, 2001). First, captive female elephants demonstrated a 1000-fold increase in urinary Z7-12:Ac concentration, from nondetectable amounts during the luteal phase to millimolar levels just prior to ovulation. In urinary samples from wild females,



Z7-12:Ac was not detected in control samples from nonreproductively active females. However, all five urine samples from wild females to whom males were chemosensorily responsive revealed millimolar concentrations of the pheromone (Figure 5). Second, captive males showed robust bioresponses to urine, its extracts, and synthetic pheromone, which increased linearly with the concentration of Z7-12:Ac (Rasmussen et al., 1997; Rasmussen, 2001). Similarly, wild males demonstrated more chemosensory responses and pre-mating behaviours as the concentration of detected Z7-12:Ac increased (Figure 5). The detection of other variably elevated preovulatory compounds related to Z7-12:Ac (Table 5), previously described in captive elephants (Rasmussen, 2001; Rasmussen & Krishnamurthy, 2001), suggested further information could be encoded in such chemical repertoires.

Because the chemical identity of the ovulatory signal is known in Asian elephants, captive studies not only demonstrated that male elephants detected the preovulatory pheromone as an indication of approaching ovulation by females, but also that males could pinpoint this impending event precisely by assessing increasing pheromone concentrations (Rasmussen, 2001). Wild male elephants in native range states appear to do the same assessment of female reproductive status (Table 5, Figure 5). The daily rechecking of some females and the multiple checking of a particular female by a number of males within several hours supports the hypothesis that males monitor pheromone concentrations during the preovulatory period. The unusual elongation of the follicular phase of the oestrous cycle may provide an adaptive advantage for elephant society where sexes live physically separated. A temporal extension of the follicular phase could facilitate reproductive synchrony between sexes. From the male perspective, the long period of increasing pheromone concentration effectively extends 'receptivity' of females. From the female perspective this extended time to encounter more widely wandering males increases the probability of attracting an older, larger male. This lengthy period may allow adequate time for female-to-male primer pheromones to be functionally effective.

### *Influences of musth*

Female captive Asian elephants in follicular phase prefer the urine of musth males over that of nonmusth males (Schulte & Rasmussen, 1999a). We demonstrate a reciprocal effect — that musth males have the most direct

access to females and that chemical signals influencing the outcomes of male dominance interactions reinforce this priority of access by musth males. We categorized males by age (and thus size) and musth characteristics. The quantitative appraisal of musth in each male was provided by using eight attributes of musth combined with the semi-quantitative chemical assessment of the presence, absence, or relative amount of key urinary ketones (C<sub>8</sub>-C<sub>11</sub>). This appraisal gave a measure of the maturity and degree of musth in individual wild males when compared to data available from precisely studied captive males (Rasmussen et al., 2002; Rasmussen & Greenwood, 2003). Musth males, in comparison to nonmusth males, interacted significantly more frequently with preovulatory females and demonstrated a strikingly higher frequency of chemosensory responses to females and their urine. Thus musth must be an important determining factor in reproductive strategies.

Not only do musth males interact with more females and more frequently, but specific responses and patterns of response varied with age and differed between musth and nonmusth males. OA and Y20sA males in musth demonstrated a higher frequency of distant sniffs, close sniffs, urogenital checks, expelled urine checks, and flehmen responses than their nonmusth counterparts; most of these differences were significant. With the exception of erections, pre mating behaviours including mate guarding and mounting were also observed more frequently in musth than nonmusth males, occurring in all but one of the eight musth OA and Y20sA males, but only one non-musth male, a large dominant male (Figure 4). Predominantly musth males attempted to actually mate. Musth males also monitored multiple females in succession. Which factor — size or musth — tips the decisive balance in determining response and behaviours remains an open question.

### *Male age effects*

Age of males and, therefore, size also influenced responsivity and access to females. In the Y20sA males, the amount of spatial contact during interactions with females differed between musth and nonmusth males. The larger OA-M males performed significantly more distant sniffs and total contact chemosensory responses than the younger, smaller Y20sA-M males (Figure 2). When the three types of contact responses — urogenital checks, expelled urine checks, and flehmens — were examined separately among musth male groupings, the OA-M category maintained higher responsivity

for each type of response (Figures 3a, b, c). The Y20sA-NM males exhibited no contact interactions, such as urogenital checks, toward females (Table 3); rather they performed checks and flehmen responses only to previously expelled urine spots (Figures 3a, b). The robust avoidance of contact responses with females suggests that perhaps either experience has taught these Y20sA males to avoid direct contact with females during their nonmusth period (as females may not be interested) or male sexual behaviours are reduced during nonmusth.

Premating behaviours also differed with Y20sA-M males demonstrating a greater frequency of mounting episodes than their OA-M counterparts (Figure 5). The more experienced OA males may be more able to judge the proximity of females to ovulation more accurately and thus wasted less effort on non-fertile females. These quantitative differences in various responses and among different categories of males offer clues about the biological roles in breeding tactics and elephant social structure. Among OA and Y20sA males, distant sniffs toward females are performed at a higher frequency than among YTA males (Figure 1a), whereas an age-dependent response was less evident with close sniffs and contact responses (Figures 1b and 2). Again, older males may be more experienced in recognizing the distant odor of reproductively active females than the inexperienced teenagers. We suspected that response to the female pheromone Z7-12:Ac (and presumably the adult male dominance pheromone, frontalin) could be the result of learning by a particular male as he interacts socially with females of different reproductive status and sorts out dominance relationships with other males.

Different frequencies of chemosensory responses and premating behaviours were seen when YTA (both in moda musth and nonmusth) were compared with older males. OA males exhibited the highest chemosensory responses (Figure 5), but the summed chemosensory responses by both musth and nonmusth YTA males were slightly higher than their Y20sA counterparts. In particular urogenital checks were significantly greater in YTA-MoM males than Y20sA-M males. This high responsiveness by the maturing male group, YTA, may reflect attempts by this male category to gain sexual knowledge about females through increased contact chemosensory responses.

The frequency of flehmen responses were also slightly higher in YTA-MoM compared to Y20sA-M, contributing to the higher totals of contact interactions among the YTA-MoM (Figures 2, 3a, c). Reasons for the elevations of these two types of responses, flehmens and urogenital, by YTA-MoM may involve both maturation processes and peculiarities of teenage

moda musth. Males in moda musth only have moderately elevated serum testosterone and dihydrotestosterone (DHT) concentrations during sporadic short-duration musth episodes. These testosterone and DHT fluctuations constantly challenge homeostasis (Rasmussen et al., 2002). In comparison to OA-M and Y20sA-M males, YTA-MoM males showed an incomplete, often inappropriate behavioural repertoire of premating acts, confirming observations in captivity (Chandrasekharan et al., 1992; Rasmussen et al., 2002). Mountings and erections were frequent, but no mate guarding was observed, similar to young male tamarins (Ginther et al., 2001). We do not know to what degree the moda state affects the sexual impulses of YTA males or whether the physiological disturbances associated with the moda state simply make young males exhibit more sporadic behaviors. Nonmusth YTA males did not often exhibit sexual behaviours, but they were also apprehensive of nearby musth males and often demonstrated distant sniffs and avoided interactions with females. In addition, response patterns toward females or their urine did not show the clear differences between musth and nonmusth states as seen among the two older male groups (OA and Y20sA). One YTA-MoM male showed a strong chemosensory response to female urine later demonstrated to contain Z7-12:Ac (Figure 5). This inexperienced male may have lacked knowledge of the chemical identity of the pheromone or the ability to precisely assess concentrations, either of which could be the result of learning. Alternatively experienced older wild males may possess greater olfactory sensitivity under the androgen influence of musth, and may conduct female monitoring from far distances as demonstrated by the high frequency of distant and close sniffs by two OA-M males.

### *Controlling effect of older males on younger males*

A basic question of Asian elephant male society is whether older males, especially OA-M, control young males, especially those in moda musth. Studies of African elephants demonstrated that young males lost physical signs of musth shortly after an aggressive encounter with a higher-ranking musth male (Slotow et al., 2000). Asian elephants show a difference between emissions such as TGS and urine from moda males and older adult males. Older males in musth (larger and more dominant) release compounds that elicit behavioural retreat by young males, demonstrating definitive behavioural influences on YTA, especially those in moda musth (Rasmussen et al., 2002).

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. Moda males may or may not retreat in the presence of a dominant male (especially one in musth), whereas YTA-NM males retreat.

In some primates such as orangutans, the social environment can affect reproductive development as androgen levels and the expression of secondary sexual characteristics may be depressed in the presence of dominant males (Maggioncaida & Sapolsky, 1999). In some cooperatively breeding primates a similar effect is noted, but in other cooperatively breeding primates such as the cotton-top tamarin reproductive maturity is achieved within the natal group, although the time required for the completion of puberty may vary with the presence or absence of a dominant, breeding male (Ginther et al., 2002). Behaviourally adult offspring tamarins engaged in homosexual behaviour and were as sexually active as reproducing males, presenting some analogues to YTA-MoM male elephants (Ginther et al., 2001). More detailed studies of the sexual, social, and endocrinological maturation of the young teenage Asian elephant should reveal the roles of primer and alerter pheromonal systems on these processes.

### *Relevance of Asian elephant chemical signal-integrated society to other large mammal societies*

In mostly solitary, non territorial mammals, the nature of the male-male competition and female mating preferences may determine both the mating system and social structure (Singleton & van Schaik, 2001, 2002). Females may be responsible for the social and reproductive decisions that generate the diversity of social structures. In Asian elephants, females exhibit a range of social tactics toward other females that range from affiliative to agonistic to aggressive (Slade et al., 2003). Females may also control relationships with males, as they can detect musth and degrees of musth (Schulte & Rasmussen, 1999a). Females may be able to control where and when they release pheromones and may prime the males with increasing concentrations of pheromones as ovulation approaches.

Elephants are a polygynous species with pronounced sexual dimorphism. Such species are expected to have intense male-male competition (Clutton-Brock et al., 1977; Clutton-Brock & Harvey, 1983). As physical competition may be energetically costly, the use of chemical signals may be more

efficient and effective than fighting. In Asian elephants dominance rank is obtained by several factors and may have advantages over other mammalian ranking systems. In long-tailed macaques, *Macaca fascicularis*, both dominant and subordinate males show strongest interest in females during and around the fertile phase of the ovarian cycle, and males differ in their ability to obtain access to females according to rank (Engelhardt et al., 2004). Asian male elephants show such age and size ranking, but the musth status is the determining factor of female access. Male Asian elephants seem to avoid the majority of possible overt aggressive encounters by broadcasting differential, distinctive musth/nonmusth signals that influence appropriate behaviours in other males. Social status, age, and musth correlate positively with each other. Males can declare their state and social status with olfactory signals. For example, strong-smelling, medium-duration, higher molecular weight ketones released by older males can inform younger males of the presence of larger, older musth males. The chemical signaling repertoire of female Asian elephants is so finely tuned that changing Z7-12:Ac concentrations enables males to pinpoint the day of ovulation. Coupled with the close temporal relationship between the occurrence of elevated urinary Z7-12:Ac and ovulation is the superior ability of experienced older dominant musth males to assess differential pheromone concentrations. Uneven responses to increasing concentrations of the pheromone by younger subordinate males, both captive and wild, suggest that they are less accurate in determining ovulation. The influence of the musth condition on bioresponse levels merits further studies, as other species demonstrate increased olfactory sensitivity during elevated hormonal periods (Sisneros & Tricas, 2000) and olfactory stimulation can lead to rapid hormonal change such as seen in male common marmosets (Ziegler et al., 2005). The evidence is not conclusive in either primates or proboscideans whether the skewed successful mating ratio in favor of the dominant males is a product of female reproductive choice or reflects the energetically costly ability of males to monopolize or mate guard females during their fertile period (de Ruiter et al., 1994). For the Asian elephant, several factors may be balanced. In captivity, females are positively excited by and are attracted to urine and temporal gland secretions of musth males; wild musth males preferentially mate guard and mount females. Musth males can indicate their quality (fitness), health, and dominance position by their signals. This is similar to broadcasts by roving sperm whales of their extent of nonpigmented, intraspecific scarring as an indication of superiority in aggressive interactions (MacLeod, 1998).

*Fission-fusion in Asian elephant society*

Social organization refers to the spatiotemporal distribution of individuals in a population and is based on the nature and quality of interactions between these individuals (Hinde, 1976; Whitehead, 1997). The examination of the degree of fission-fusion among elephant society in comparison with other large, highly cognitive mammals is revealing in the context of known chemical signaling systems. Many primate and marine mammalian societies demonstrate a variety of degrees of fission-fusion. Asian elephants share a similar tropical forest habitat and female social structure as the nonterritorial primate, the orangutan (*P. pygmaeus*). Females of the *Elephas* species are generally in small, multi-generational family units, and females of the *Pongo* species are often in mother-calf dyads. Among orangutan society adolescent females associate often with their mothers. Adolescent males return less and less to the natal unit. Subadult males are highly mobile and search for females over large areas. Both species may form travel associations. Female orangutans form travel parties that may include sexual consortships and multiple females with offspring (thus an individual-based fission-fusion system). Female elephants travel with multiple family units, accompanied by sexually interested males (McKay, 1973). One outcome of long-distance breeding forays by both species is that genes may be more widely dispersed than in the actual physical, semi-permanent location of individuals such as seen in kangaroo rats (Winters & Waser, 2003). Widely roaming musth elephants and widely searching male orangutans have similar objectives of finding and mating with fertile females, and they achieve a wider gene dispersal. Range use by male orangutans is dictated by the presence of receptive females, but, like male elephants, different classes of males have different mating strategies. In elephants, different ages (and thus experience), sizes, and musth states affect ranking, and subsequently behaviour toward females, resulting in differing degrees of access. Among orangutans, females living in adjacent home ranges show convergence in mate choice on the same dominant male in the area (Singleton & van Schaik, 2002). The question is relevant as our knowledge of chemical signals in wild Asian elephants widens to determine if female elephants tend to agree on a particular musth male as a desirable mate.

Subtle differences also exist between orangutans and Asian elephants. Asian elephant society is exemplified by a dual spectrum: the fused multi-generational stability of female family units and fission among males. With

a six-decade lifespan, only the infrequent death of a matriarch results in the occasional fission of a family group. Unlike nonterritorial female Sumatran orangutans that demonstrate a high home range overlap with neighboring, possibly related adult females preferentially associating with each other (Singleton & van Schaik, 2001), Asian female elephants do not exhibit such fusion. Also unlike the orangutans, they apparently do not exhibit fission-fusion behaviour by forming travel association parties that include both sexual consortships and multiple females with offspring (van Schaik, 1999; Singleton & van Schaik, 2002).

In a broader sense, these two species — orangutans and Asian elephants — have similarities in a fission-fusion modular concept. Such models can take various emphases, with the degree of social cohesion somewhat dependent on the synchrony of activities of a social group (Conradt & Roper, 2000). The frequent occurrence of intersexual social segregation in sexually dimorphic species such as primates, cetaceans, and proboscidea may result from sex differences in activity rhythms (Conradt, 1998a, b). The social segregation of the Asian elephant fits this concept: females are spatially coherent, live in small social units, and synchronize their foraging activities; such activities are slower and more methodical than those of the more adventurous males (Sukumar & Gadgil, 1988). In both orangutans and elephants, males seeking high-protein, more diverse diets wander farther in search of varied foods. Their groupings are less homogeneous than female units with the males of both species tending to be solitary, having a lower activity synchrony, and thus a looser social cohesion. However, only 35% of the observed degree of intersexual segregation could be explained by differences in activity synchronization. In red deer (Condrat, 1999; Condrat & Roper, 2000), other causes of sex segregation were suggested, namely competitive interference between males and females or differences in environmental requirements (Clutton-Brock et al., 1987). The fission-fusion model of Asian elephant society is likely to be equally important with such general factors as societal aspects unique to elephants, especially the prolonged female oestrus.

#### *Integrated breeding strategies: pheromones and identity mechanisms*

The central theme of this paper is that an integrated system of chemical signals provides intra- and intersexual information important both in ontogeny and also carrying out of breeding strategies. Elephants and other mammals



need to know the intra- and intersexual reproductive states of conspecifics. Highly cognitive mammals, especially those with segregated sexes such as many of the larger sexually dimorphic mammals, need to establish genetic relationships, i.e., know who are kin and recognize individual identity. Pheromones are external chemical messages that elicit specific responses or behaviours; pheromones and vocalizations have not yet been demonstrated to reveal individual identity in elephants. Urinary and other odor emissions can be remote in time and place and may reveal sex, sex state, health, and even kinship information, but how is individual identification accomplished?

The sequences of behaviours conducted by males during initial reconnoiters of females offer a hint as a possible mechanism for individual recognition. Wild male elephants upon encountering 'new' females, i.e., females possibly never encountered before or not seen for a long time, inserted their trunk tip into the dorsal lateral region of the mouth of the female, specifically onto the palatal pits located on the palatal ridge. Males performed these palatal pit checks before checking the urogenital regions. Urogenital and urine odors provide sex and reproductive state information, but males may be obtaining different information from these palatal pits. We hypothesize that males may be getting kin information from unfamiliar females via palatal pit responses. Histologically, palatal pits are composed of lymphoidal tissue, capable of producing proteins of the major histocompatibility complex variety that have been demonstrated to function in individual and kin recognition in other species (Yamazaki et al., 2000; Brennan, 2004), as have other polymorphic proteins (Beynon & Hurst, 2003). These initial trunk-to-mouth investigations are probably relevant to breeding strategies since almost all palatal pit responses by males were performed by males in musth.

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## References

- Amos, B., Schlotter, C. & Tautz, D. (1993). Social structure of pilot whales revealed by analytical DNA profiling. — *Science* 260: 670-672.
- Barnes, R.F.W. (1982). Mate searching behaviour of elephant bulls in a semi-arid environment. — *Anim. Behav.* 30: 1217-1223.
- Best, P.B.A. (1979). Social organization in sperm whales, *Physeter macrocephalus*. — In: Behaviour of marine mammals, Vol. 3. Cetacea (Winn, H.E. & Olla, B.L., eds). Plenum Press, New York, p. 227-290.
- Beynon, R.J. & Hurst, J. (2003). Multiple roles of major urinary proteins in the house mouse, *Mus domesticus*. — *Biochem. Soc. Trans.* 31: 142-146.
- Brennan, P.A. (2004). The nose knows who's who; chemosensory individuality and mate recognition in mice. — *Horm. Behav.* 46: 231-240.
- Brokelman, W.Y., Reichard, U., Treesucon, U. & Raemakers, J.J. (1998). Dispersal, pair formation and social structure in gibbons (*Hylobates lar*). — *Behav. Ecol. Sociobiol.* 42: 329-339.
- Cheeran, J.V., Easwaran, E.K. & Nagara, B.N. (2004). Behavioural modifications in an elephant using a neuroleptic agent. — Oct. 15, 2004 Elephant Managers Association Conference, Tulsa, OK.
- Chandrasekharan, K., Radhakrishnan, K., Cheeran, J., Nair, K.M. & Prabhakaran, T. (1992). Some observations on musth in captive elephants in Kerala (India) — In: The Asian elephant: Ecology, biology, disease, conservation and management (Silas, E., Nair, M. & Nirmalan, G., eds). Kerala Agric. Univ. India, p. 71-74.
- Clutton-Brock, T.H. (1989). Mammalian mating systems. — *Proc. R. Soc. Lond. B* 236: 227-290.
- Clutton-Brock, T.H. & Harvey, P.H. (1983). The functional significance of variation in body size among mammals. — *Spec. Publ. Am. Soc. Mammal.* 7: 632-663.
- Clutton-Brock, T.H., Harvey, P.H. & Rudder, B. (1977). Sexual dimorphism, socioeconomic sex ratio and body weights in primates. — *Nature* 269: 797-800.
- Clutton-Brock, T.H., Major, M., Albon, S.D. & Guinness, F.E. (1987). Early development and population dynamics in red deer. 1. Demographic consequences of density dependent changes in birth weight and date. — *J. Anim. Ecol.* 56: 53-67.
- Conradt, L. (1998a). Could asynchrony in activity between sexes cause inter-sexual social segregation in ruminants? — *Proc. R. Soc. Lond. B* 265: 1359-1363.
- Conradt, L. (1998b). Measuring the degree of sexual segregation in group living animals. — *J. Anim. Ecol.* 67: 217-226.
- Conradt, L. (1999). Social segregation is not a consequence of habitat segregation in red deer and feral soay sheep. — *Anim. Behav.* 57: 1151-1157.
- Conradt, L. & Roper, T.J. (2000). Activity synchrony and social cohesion: a fission-fusion model. — *Proc. R. Soc. Lond. B* 267: 2213-2218.
- Desai, A. (1991). The home range of elephants and its implications for management of the Mudumalai Wildlife Sanctuary. — *J. Bombay Nat. Hist. Soc.* 88: 145-156.
- Douglas-Hamilton, I. (1972). On the ecology and behaviour of the African elephants. — PhD thesis, Oxford University.
- Douglas-Hamilton, I. (1973). On the ecology and behaviour of the Lake Manyara elephants. — *E. Afr. Wildl. J.* 11: 401-403.

- Eisenberg, J.F. (1980). Recent research on the biology of the Asiatic elephant (*Elephas m. maximus*) on Sri Lanka. — *Spolia Zeylanica* 35: 213-218.
- Eisenberg, J.F. & Lockhart, M. (1972). An ecological reconnaissance of Wilpattu National Park, Ceylon. — *Smithson. Contrib. Zool.* 101: 1-30.
- Eisenberg, J.F., McKay, G.M. & Jainudeen, M.R. (1971). Reproductive behaviour of the Asiatic elephant (*Elephas maximus*). — *Behaviour* 38: 193-225.
- Engelhardt, A., Pfeifer, J.B., Heistermann, M., Niemitz, C., van Hooff, J.A.R.A.M. & Hodges, J.K. (2004). Assessment of female reproductive status by male longtailed macaques, *Macaca fascicularis*, under natural condition. — *Anim. Behav.* 67: 915-924.
- Fernando, P. & Lande, R. (2000). Molecular genetic and behavioural analysis of social organization in the Asian elephant (*Elephas maximus*). — *Behav. Ecol. Sociobiol.* 48: 84-91.
- Galdikas, B.M.F. (1985). Orangutan sociality at Tanjung Putting. — *Am. J. Primatol.* 9: 101-119.
- Ginther, A.J., Carlson, A.A., Ziegler, T.E. & Snowdon, C.T. (2002). Neonatal and pubertal development of males of a cooperatively breeding primate, the cotton-top tamarin (*Saguinus oedipus oedipus*). — *Biol. Reprod.* 66: 282-290.
- Ginther, A.J., Ziegler, T.E. & Snowdon, C.T. (2001). Reproductive biology of captive male cotton-top tamarin monkeys as a function of social environment. — *Anim. Behav.* 61: 65-78.
- Haug, H. (1987). Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: a stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores and one elephant). — *Am. J. Anat.* 180: 126-142.
- Hinde, R.A. (1976). Interactions, relationships and social structure. — *Man* 11: 1-17.
- Joshua, J. & Johnsingh, A.J.T. (1995). Ranging patterns of elephants in Rajaji National Park: Implications for reserve design. — In: A week with elephants (Daniel, J.C. & Datye, H.S., eds). Bombay Natural History Society, Bombay and Oxford University Press, New Delhi, p. 256-260.
- Koikegami, H. & Ozaki, N. (1967). Studies on the paralimbic brain structures. 3. On the nucleus accumbens and on the olfacto-tegmental tract in the Indian elephant. — *Acta Med. Biol.* 15: 131-40.
- Krishnan, M. (1972). An ecological survey of the large mammals of peninsular India: the Indian elephant. — *J. Bombay Nat. Hist. Soc.* 69: 298-315.
- Lazar, J. (2001). Elephant sex pheromone transport and recognition. — PhD thesis, University of Utah, Salt Lake City, Utah, USA.
- Lazar, J., Greenwood, D., Rasmussen, L.E.L. & Prestwich, G.D. (2002). Molecular and functional characterization of the odorant binding protein of the Asian elephant, *Elephas maximus*: Implications for the role of lipocalins in mammalian olfaction — *Biochemistry* 41: 11786-11794.
- Lazar, J., Rasmussen, L.E.L., Greenwood, D.R., In-Soek, B. & Prestwich, G.D. (2004). Elephant albumin: a multi-purpose shuttle. — *Chem. Biol.* 11: 1093-1100.
- MacLeod, C.D. (1998). Intraspecific scarring in odontocete cetaceans: an indicator of male 'quality' in aggressive social interactions? — *J. Zool. London.* 244: 71-77.
- Maggioncalda, A.N. & Sapolsky, R.M. (1999). Reproductive hormone profiles in captive male orangutans; implication for understanding developmental arrest. — *Am. J. Phys. Anthropol.* 109: 19-32.

- Marechal, C., Maurois, C. & Chamberlan, C. (1998). Size (and structure) of forest elephants groups (*Loxodonta africana cyclotis*, Matschie, 1900). — *Mammalia* 62: 297-300.
- McKay, G.M. (1973). Behaviour and ecology of the Asiatic elephant in southwestern Ceylon. — Smithsonian Inst. Press, Washington, DC.
- Moss, C.J. & Poole, J. (1983). Relationships and social structure of African elephants. — In: Primate social relationships (Hinde, R.A., ed.). Blackwell, Boston, p. 315-325.
- Oliver, R.C.D. (1978). On the ecology of the Asian elephant, *Elephas maximus* Linn., with particular reference Malaya and Sri Lanka. — PhD Dissertation, University of Cambridge.
- Packer, C., Gilbert, D.A., Pusey, A.E. & O'Brien, S.J. (1991). A molecular genetic analysis of kinship and cooperation among African lions. — *Nature* 351: 562-565.
- Perrin, T.E., Rasmussen, L.E.L., Gunawardena, R. & Rasmussen, R.A. (1996). A method for collection, long-term storage, and bioassay of labile volatile chemosignals. — *J. Chem. Ecol.* 21: 207-221.
- Poole, J.H. (1987). Rutting behaviour in African elephants: the phenomenon of musth. — *Behaviour* 102: 283-316.
- Poole, J.H. (1989a). Announcing intent: the aggressive state of musth in African elephants. — *Anim. Behav.* 37: 140-152.
- Poole, J.H. (1989b). Mate guarding, reproductive success and female choice in African elephants. — *Anim. Behav.* 37: 842-849.
- Pusey, A.E. & Packer, C. (1987). The evolution of sex-biased dispersal in lions. — *Behaviour* 101: 275-310.
- Rasmussen, L.E.L. (1999). Elephant olfaction. — *ChemoSenses* 2: 4-5.
- Rasmussen, L.E.L. (2001). Source and cyclic release pattern of (Z)-7-dodecenyl acetate, the preovulatory pheromone of the female Asian elephant. — *Chem. Senses* 26: 611-624.
- Rasmussen, L.E.L. & Greenwood, D.R. (2003). Frontalin: a chemical message of musth in Asian elephants. — *Chem. Senses* 28: 433-446.
- Rasmussen, L.E.L. & Krishnamurthy, V. (2000). How chemical signals integrate Asian elephant society: the known and the unknown. — *Zoo Biol.* 19: 405-423.
- Rasmussen, L.E.L. & Krishnamurthy, V. (2001). Urinary, temporal gland and breath odors from Asian elephants of Mudumalai National Park. — *Gajah* 20: 1-7.
- Rasmussen, L.E.L., Lazar, J. & Greenwood, D.R. (2003). The olfactory adventures of elephantine pheromones. — *Trans. J. Biochem.* 31: 137-141.
- Rasmussen, L.E.L., Lee, T.D., Daves, G.D., Jr. & Schmidt, M.J. (1993). Female-to-male pheromones of low volatility in the Asian elephant, *Elephas maximus*. — *J. Chem. Ecol.* 19: 2115-2128.
- Rasmussen, L.E.L., Lee, T.D., Roelofs, W.L., Zhang, A. & Daves, G.D., Jr. (1996). Insect pheromone in elephants. — *Nature* 379: 684.
- Rasmussen, L.E.L., Lee, T.D., Zhang, A., Roelofs, W.L. & Daves, G.D., Jr. (1997). Purification, identification, concentration and bioactivity of (Z)-7-dodecen-1-yl acetate: sex pheromone of the female Asian elephant, *Elephas maximus*. — *Chem. Senses* 22: 417-438.
- Rasmussen, L.E.L. & Perrin, T.E. (1999). Physiological correlates of musth: lipid metabolites and chemosignal composition. — *Physiol. Behav.* 67: 539-549.
- Rasmussen, L.E.L., Riddle, H.S. & Krishnamurthy, V. (2002). Mellifluous matures to malodorous in musth. — *Nature* 415: 975-976.

- Rasmussen, L.E., Schmidt, M.J., Henneous, R., Groves, D & Daves, G.D., Jr. (1982). Asian bull elephants: flehmen-like responses to extractable components in female elephant oestrous urine. — Science 217: 159-162.
- Rasmussen, L.E., Schmidt, M.J. & Daves, G.D. (1986). Chemical communication among Asian elephants. — In: Chemical signals in vertebrates: evolutionary, ecological, and comparative aspects (Duvall, D., Silverstein, M. & Muller-Schwarze, D., eds). Plenum Press, New York, p. 627-646.
- Rasmussen, L.E.L. & Wittemyer, G. (2002). Chemosignaling of musth by individual wild African elephants (*Loxodonta africana*): implications for conservation and management. — Proc. R. Soc. London 269: 853-860.
- Richards, K.R., Dillion, M.C., Whitehead, H. & Wright, J.M. (1996). Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses. — Proc. Natl. Acad. Sci. U.S.A. 93: 8792-8795.
- Rodman, P.S. (1984). Foraging and social systems of orangutans and chimpanzees. — In: Adaptations for foraging in nonhuman primates (Rodman, P.S. & Cant, J.G.H., eds). Columbia University Press, New York, p. 6-16.
- de Ruiter, J.R., van Hooff, J.A.R. & Scheffrahn, W. (1994). Social and genetic aspects of paternity in wild long-tailed macaques (*Macaca fascicularis*). — Behaviour 129: 203-224.
- Sanderson, G.P. (1898). Thirteen years among the wild beasts of India. — W.H. Allen, London.
- van Schaik, C.P. (1999). The socioecology of fission-fusion sociality in orangutans. — Primates 40: 73-90.
- Schulte, B.A. & Rasmussen, L.E.L. (1999a). Signal-receiver interplay in the communication of male condition by Asian elephants. — Anim. Behav. 57: 1265-1274.
- Schulte, B.A. & Rasmussen, L.E.L. (1999b). Musth, sexual selection, testosterone and metabolites. — In: Advances in chemical communication in vertebrates 8 (Johnston, R.E., Muller-Schwarze, D. & Sorenson, P.W., eds). Kluwer Academic/Plenum Press, New York, p. 383-397.
- Siegel, S. & Castellan, N.J. (1988). Nonparametric statistics. — McGraw-Hill, New York.
- Singleton, I. & van Schaik, C.P. (2001). Orangutan home range size and its determinants in a Sumatran swamp forest. — Int. J. Primatol. 22: 877-911.
- Singleton, I. & van Schaik, C.P. (2002). The social organization of a population of Sumatran orang-utans. — Folia Primatol. 73: 1-20.
- Sisneros, J.A. & Tricas, T.C. (2000). Androgen-induced changes in the response dynamics of ampullary electrosensory primary afferent neurons. — J. Neurosci. 20: 8586-8595.
- Slade, B.E. Schulte, B.A. & Rasmussen, L.E.L. (2003). Oestrous state dynamics in chemical communication by captive female Asian elephants. — Anim. Behav. 65: 813-819.
- Slotow, R., van Dyke, G., Poole, J., Page, B. & Klocke, A. (2000). Older bull elephants control young males. — Nature 408: 425-426.
- Sokal, R.R. & Rohlf, J.F. (1995). Biometry, 3rd Ed. — W.H. Freeman, New York.
- Stephens, M.E. (1990). Longevity factor in hominoid social organization. — Am. J. Phys. Anthropol. 81: 357-361.
- Sukumar, R. (1989). The Asian elephant: ecology and management. — Cambridge University Press, Cambridge, U.K.
- Sukumar, R. (1994). Elephant days and nights. — University Press, Delhi, Oxford.

- Sukumar, R. (2003). The living elephants: evolutionary ecology, behaviour and conservation. — Oxford University Press, New York.
- Sukumar, R. & Gadgil, M. (1988). Male-female differences in foraging on crops by Asian elephants. — *Anim. Behav.* 36: 1233-1235.
- Tower, D. (1954). Structural and functional organization of mammalian cerebral cortex: the correlation of neurone density with brain size. — *J. Comp. Neurol.* 101: 19-51.
- Tyack, P.L. (1999). Communication and cognition in biology of marine mammals (Reynolds, J.E. & Rommel, S.A., eds). Smithsonian Institution Press, Washington, DC, p. 287-324.
- Whitehead, H. (1990). Rules for roving males. — *J. theor. Biol.* 145: 355-368.
- Whitehead, H. (1994). Delayed competitive breeding in roving males. — *J. theor. Biol.* 166: 127-133.
- Whitehead, H. (1996). Babysitting, dive synchrony, and indications of alloparental care on sperm whales. — *Behav. Ecol. Sociobiol.* 38: 237-244.
- Whitehead, H. (1997). Analysing animal social structure. — *Anim. Behav.* 53: 1053-1067.
- Whitehead, H. & Arnborn, T. (1987). Social organization of sperm whales off the Galapagos Islands, February-April 1985. — *Can. J. Zool.* 65: 913-919.
- Winters, J.B. & Waser, P.M. (2003). Gene dispersal and outbreeding in a philopatric mammal. — *Molec. Ecol.* 12: 2251-2259.
- Worthy, G.A.J. & Hickle, J.P. (1986). Relative brain sizes in marine mammals. — *Am. Nat.* 128: 445-459.
- Yamazaki, K., Beauchamp, G.K., Curran, M., Bard, J. & Boyse, E.A. (2000). Parent-progeny recognition as a function of MHC odortype identity. — *Proc. Natl. Acad. Sci. U.S.A.* 97: 10500-10502.
- Zeigler, T.E., Schultz-Darken, N.J., Scott, J.J., Snowdon, C.T. & Ferris, C.F. (2005). Neuroendocrine response to female ovulatory odors depends upon social condition in male common marmosets, *Callithrix jacchus.* — *Horm. Behav.* 47: 56-64.
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