Review

Evolution of chemical signals in the Asian elephant, *Elephas maximus*: behavioural and ecological influences

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In antiquity, the Asian elephant, *Elephas maximus*, gradually spread southward and eastward to become a successfully surviving, ecologically dominant megaherbivore in the tropical environment of south-east Asia. The changing physical environment forced dynamic fluxes in its social structure and altered its metabolism. Such events shaped the production and ultimately the stability of certain chemicals released by body effluvia. Some of these chemicals took on significance as chemical signals and/or pheromones. This article demonstrates by experimental and observational evidence, and hypothesizes based on speculative reasoning, how and why specific chemical signals evolved in the modern Asian elephant. Evidence, including the functional criteria required by elephant social structure and ecology, is presented for the hypothesis that the recently identified female-emitted, male-received sex pheromone, (Z)-7-dodecenyl acetate evolved first as a chemical signal. Subsequently, the cohesiveness and harmony of small, matriarchally-led female groups were strengthened by a female-to-female chemical signal, recently defined behaviourally. The looser societal structure of freer, roaming males also became bounded by chemical signals; for the males, breath and temporal gland emissions, as well as urinary ones function in chemical signalling. Basic knowledge about elephant chemical signals is now linking chemical information to behaviour and beginning to demonstrate how these signals affect elephant social structure and enable the species to cope with environmental changes.

1. Introduction

In Asian elephants, *Elephas maximus*, the functionality of some emitted metabolites as chemical signals presumably developed gradually over millennia, influenced by ecological and climatic events and the structure of elephant society. Such development occurred concurrently with anatomical changes and amid biochemical restrictions. In present day *E. maximus*, five categories of chemical signals have been demonstrated behaviourally. These include maternal-to-filial signals (Rasmussen 1995), female-to-female estrous state signals (Slade 1999), male-to-male musth-related signals (Rasmussen 1988, 1998; Rasmussen *et al* 1997b; Scott *et al* 1997), a variety of male-to-female signals (Perrin and Rasmussen 1994; Perrin *et al* 1996; Schulte and Rasmussen 1999a, b), and female-to-male signals (Rasmussen *et al* 1996b, 1997a). Only the latter two have been characterized chemically. Remarkably, a single compound, (Z)-7-dodecenyl acetate (Z7-12:Ac), has evolved as a female-to-male preovulatory pheromone. Full activity or reception apparently requires transporter proteins and/or cofactors. In contrast to the single female-produced pheromone, male Asian elephants in musth produce a remarkably varied repertoire of musth-specific chemical signals. Some of the mostly volatile emissions are functional as singlets and others as blends. This review focuses on the evolution of these two categories of chemical signals in the context of the behavioural ecology of the elephant in south-east Asia.

2. Evolution

Chemical signals of *E. maximus* have been shaped from ancient eras to recent millennia by ecological constraints including major, sometimes rapid, climatic events and more subtle, often vegetation-related changes. The earliest elephantid species originated in Africa. Skeletal remains

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first appeared in late Miocene deposits dating back about 7 million years (Maglio 1973). Even though the genera *Loxodonta* and *Elephas* diverged 5 million years ago, recent genetic studies have demonstrated that modern-day Asian elephants (*E. maximus*) and African elephants (*Loxodonta africana*) (Paenungulates) belong to a similar clade, one of the six superorder clades of endemic African mammals (Stanhope *et al* 1998). There is strong support for a mutual Asian and African elephant clade; similarity is demonstrated at all five phylogenetic loci of highly disparate functions (four disparate nuclear protein coding genes and a tandem alignment of the 12s and 18s mitochondrial region) (Stanhope *et al* 1998). By the early Pliocene, three genera were recognized in Africa: *Mammuthus*, *Elephas*, and *Loxodonta*. During the Miocene, Pliocene, Pleistocene, and Holocene periods, climatic changes of great magnitude occurred on the African continent (Shoshani and Tassy 1996). Elephants gradually migrated out from Africa to the European and Asian continents. *Elephas* and *Mammuthus* rapidly radiated into northern climates during the Plio–Pleistocene (Maglio 1973). During the evolutionary process, they attained sufficient adult size to withstand all predator attacks except man (Eisenberg 1981). There is evidence that the Asian elephant, *E. maximus* evolved from *E. hysudricus* found in the Shivaliks of south Asia (Maglio 1973). With climate changes *Elephas* disappeared from Africa and several species of *Elephas* became extinct in Europe. By the late Pleistocene in Europe, two species – *Mammuthus* and *Elephas* – were evident. Concurrent with the Pleistocene glaciation in the northern hemisphere in Europe and Asia, *E. maximus* migrated southward and extensively inhabited south-east Asia (Maglio 1973). By six thousand years ago, the range of *E. maximus* was extensive, stretching from the Tigris–Euphrates Basin, eastward up the Yangtze-Kiang and probably up into northern China (Sukumar and Santiapillai 1996).

Ecological factors – temperature (absolute and variation-range), day length, moisture (water availability and humidity), and terrain (including vegetation and its degree of seasonality) – affect the short-term survival and ultimately the long-term survival or evolution of the species. In the recent evolutionary past, especially in northern Asia, elephants of the taxa *Elephas* and *Mammuthus* were subjected to severe inter-seasonal temperature differences. For example, mammoths dwelling in North America and Eurasia coped with summers of varying temperatures and long, low-temperature winters of short day length. Apparently this proboscidean reproduced on a seasonal basis, with a long gestation period. Births were timed to occur during the warm spring flush of new protein-rich growth (Guthrie 1990). Possessing thick hair and perhaps very thick fat (Haynes 1991), these mammoths apparently weathered the harsh winters and did not migrate far (Haynes 1991); for large mammals, especially subadults, a long-distance migration is bioenergetically expensive (Haynes 1991). In contrast, most present-day habitats of the Asian elephant are in warm tropical environments. These tropical environments also have high moisture availability during most seasons. Moisture availability, in the forms of drinkable water and vegetation types, affects elephant migration and daily life patterns. In southern climes, longer grasses became available and *E. maximus* adapted to feeding on coarse plant material. Such dietary changes affected the chemistry of the chemical signals.

Ecological factors also influenced the development of structural and physiological characteristics of elephants. Although elephants had already achieved large mass, evolutionarily recent elephants in their adaptations to an increasingly tropical environment developed a number of additional structural characteristics. They required more complex dentition as the vegetation comprising the diet changed from low tundra plants to long grasses and browse-like shrubs. To cope with the tropical environment, skin became thicker, ears acquired more surface area, and thick fat and hair were selectively lost. Like other arctic mammals, mammoths and early *Elephas* in the north were presumed to have a variety of hair and related glands (Brown and McDonald 1985). With changes in body structures in the southward migrating elephants, the methods of dispersion of chemical signals were affected. As sebaceous glands with their lipid secretions and body hairs with their substrate effects were reduced, other methods were required to distribute and prolong signal lifetime.

One of these methods was the modification of a facial apocrine sweat gland, the specialized temporal gland. This gland, especially in male Asian elephants, has an interesting evolutionary history. Paleolithic engravings suggest that mammoths may have had larger temporal glands (Pocock 1916). The temporal gland and perhaps other hair-associated glands may have been a major vehicle for chemical signals during synchronous, herd-like reproduction. Male woolly mammoths were presumed to undergo a seasonal rut; the temporal gland presumably sent signals broadcasting male condition and reproductive fitness to females and dominance position to males. In the past, bigger temporal glands in elephants may have been the dominant releasers of chemical signals, and both sexes might have utilized such signals. With the diminished size of the temporal gland, we postulate that urine became increasingly more important as a medium for dispersing chemical signals, initially by females (the pre-ovulatory pheromone) and eventually by males in musth. Now the temporal gland is primarily functional in males as a signalling mechanism before and during musth (Rasmussen and Schulte 1998). Present day female elephants check the temporal glands of males. Although at times females exhibit temporal gland secretions (Krishnan 1972; Sukumar 1994) and indeed specific chemicals (Rasmussen 1988), no chemical signaling function has been assigned.

3. Social structure

The adaptive behaviour of elephants, ultimately the product of natural selection, produced the present-day structure of Asian elephant society and also affected the evolution of chemical signals. While the genetic endowment of Asian elephants sets the range of responses allowable in a particular environment, their ultimate adaptation is governed by behaviour. An intimate entwining of chemical signals and behaviour is required to ensure successful functioning.

The significant changes in behaviour and culture that are presumed to have occurred as elephants migrated south-east may be an important evolutionary force. Circumstantial evidence of calving time, optimized for maximum mild weather conditions and protein-rich food supply, suggests that female *Mammuthus* and *Elephas* in the north synchronized their estrous cycles to coincide with peak male reproductive capacity; there was a rut-dominated reproduction where synchronous ovulation by most females coincided with peak reproductive activity by most males. Present-day female groups of *E. maximus* have evolved a system based on individualistic or small-family-unit estrous synchrony (Eisenberg *et al* 1971). Presently, there are remarkable similarities between the Asian elephant and some whales; in four species of matrilineal whales, there is evidence for cultural heritage, i.e., the traditions that affect behaviour are a decisive force in evolution (Whitehead 1998). Like whales, present-day elephant social structure is generally matrilineal. There is considerable and continual transgenerational passage of information (Eisenberg 1981). Such information includes tool use, location of food and water resources, and defensive awareness. A dominant matriarch guides her female compatriots and young offspring, including males up to the early stages of sexual maturity (Kurt 1974; Sukumar 1994; Sukumar and Santaipillai 1996). The social organization of the female herd serves as a protective device and provides a social milieu for young elephants to mature and learn adult roles (McKay 1973). Especially inventive female leaders, possessing multi-generational knowledge, are able to significantly influence the adaptability of their familial group members. Adaptation and awareness are finely tuned by sophisticated sensory systems, particularly tactile, auditory, and chemosensory systems.

Males also possess sensory awareness, but male social structure is much more loosely organized (Jainudeen *et al* 1972). Dominance hierarchies are functional, especially during musth. Our data on chemical signals provide evidence that part of necessary social communication among males is facilitated through chemical communication (Rasmussen *et al* 1997b).

When the synchronous reproduction of large herds of elephants was gradually replaced by smaller, genetically related female groups and loosely organized, mostly solitary males, mechanisms had to be developed to ensure reproduction. One of these mechanisms is chemical signals and/or pheromones. The transmission of chemical messages about sex-specific reproductive status facilitates this process. These signals provide physiological or hormonal state information about the female or male emitter. Clearly physiological condition influences secretions/excretions and their composition and relative quantities. Behaviours such as dominance interactions within either female or male subgroups may also influence behaviour and physiology. Initially, a female-to-male chemical signal system would be of top priority. As modern female elephant society developed and became composed of small groups that were genetically related, it became of primary importance for males to know when a particular female was reproductively active. For females living in close proximity within a familial herd, a female-to-female signal would be desirable. Such chemical signals would affect behaviour or be primer pheromones affecting hormone levels.

There are several physiological and anatomical factors that affect female emissions and reception. From a physiological and hormonal perspective, the estrous cycle of female elephants is long, yet the fertile period is short. Thus, timing and synchrony are critical for effective fertilization. During the long female estrous cycle (12–18 weeks), several hormonally and pheromonally distinct periods are evident. Based on events related to ovarian function, changes in serum hormonal concentrations help define distinct follicular, pre-ovulatory, and luteal periods during the estrous cycle. The interrelationships between pheromones/chemical signals and hormones are set against an estrous cycle uniquely possessing two luteinizing hormone peaks and regularly alternating high/low serum progestin concentrations (5α-pregnane-3,20 dione is the dominant progestagen and the easily measured progesterone only about 20%) (Schwarzenberger *et al* 1997; Hodges 1998). Pheromonally, (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac), a female-to-male specific chemical signal, functions as an attractant and reproductive-timing signal; this urinary compound is not detectable during the luteal phase, is measurable during the follicular stage, and is in high concentration during the pre-ovulatory period (Rasmussen *et al* 1997a). Not only are there differential responses by female elephants to urine from musth and non-musth males, dependent on the hormonal status of the female receiver (Rasmussen and Schulte 1998), but females differentially respond to estrous and non-estrous urine dependent on their own hormonal status (Slade 1999). Further research in these areas could provide fundamental useful information about female elephant society.

In addition, the specialized anatomy of the female includes extensive mucous-producing glands along the long urogenital tract. Thus, multi-level chemical messages directed toward conspecifics originate either from these glands or in the copious amounts of urine from the kidney, as blood ultrafiltrate, that flush along the tract. This urine contains information on several operational levels including species, femaleness, individuality, signals to offspring, signals to other females, and, most specifically, a quantitative discrete signal to males.

Chemosignals are emitted via somewhat similar routes in males and females, but with considerable differential emphasis. In females, urine and associated mucus are apparently the dominant sources of at least reproductive information to conspecifics. In females, although emissions are observed post-parturition and during stress, the temporal gland has not yet been demonstrated to be a source of female-emitted chemical signals. The degree that females exchange physiological information via breath-to-breath signalling in comparison to males also needs further research.

One fascinating area of research in chemical signals is the role of the interdigital glands for either male tracking of females or the exchange of information between females. We have observed in captivity the reunion of familiar females after a separation period often results in initial frequent checks to the cuticular region (Slade 1999; L E L Rasmussen and T E Goodwin, unpublished).

Although male society is more loosely knit, older males influence the behaviour of younger ones in a somewhat indirect fashion. Young male calves stay within the female natal group until they are 5–6 years old (Eisenberg *et al* 1971). Their behaviour differs somewhat from that of females. They do not participate in allomothering or even interact as much with younger calves. Prior to sexual maturation, they still remain on the fringes of the natal group, but wander further and exhibit more independence (Sukumar 1994; Sukumar and Santiapillai 1996). As young and middle teenagers, they strike out increasingly on their own and thus increase their chance of encountering older bulls or bulls in musth. The recognition of the state of musth or non-musth of these neighboring males is important to their coexistence.

The extent that musth varied during evolution certainly was a contributing factor to the development of chemical signals. Among male Asian elephants, multilevel messages are especially evident during musth. Behavioural patterns change and dominance-ranking interchanges can occur. During this musth period, there is evidence that the chemosenses play a major role in affecting the progression of musth, the relationship of males to males, and males to females. Dramatically elevated testosterone concentrations not only affect behaviour, but also have been demonstrated to alter the type and quantity of emitted compounds functioning as chemical signals. Self-induced reduced food intake, sometimes to the point of starvation, also alters metabolism and affects chemical signal emissions (Rasmussen and Perrin 1999; Schulte and Rasmussen 1999b). Fat reserves serve as metabolic fuel. Our recent data on several measures of fat metabolism indicate definitive alterations during musth (Rasmussen *et al* 1997b; Rasmussen and Perrin 1999). The high serum androgen levels and the altered metabolism affect not only the emission of chemical signals, but also their reception.

Thus, male elephants impart chemical messages of not only maleness, but also the musth or non-musth condition. Such chemosignals are in part signals of body condition and yet may also carry an important direct reproductive message. Temporal gland secretions (TGS), urine, and breath are all vehicles for these chemical signals. Male urine contains messages of species, sex (maleness), individuality, and, for the male, musth status (Rasmussen 1988; Rasmussen *et al* 1997b; Scott *et al* 1997). Especially secretions from the temporal gland of the Asian elephant carry specific messages, i.e., an “in musth” signal; Asian males only secrete during musth. Specific subgroups of the chemical emissions from TGS and presumably urine during musth have specific directionality as chemical messages toward certain subgroups of males, females, or young.

4. Chemical signals

A variety of physically and chemically different molecules are available as chemosignals for elephants. The specific chemical identity of a compound or combinations of compounds that are bioactive among the multiplicity of compounds released is of primary importance. Perhaps of equal importance is what additional compounds bind to bioactive compounds. These additional compounds may be in the original effluvia, such as urine or temporal gland secretions, or in mucus near the neuroreceptive cells prior to signal transduction. Such binding may affect specificity, release rate, or lifetime of the bioactive molecule. In addition, internal physicochemical factors, such as electrolyte levels and pH, affect the chemistry and the potential bioactivity of compounds. For example, our research has demonstrated that during musth, serum magnesium levels are lowered, while serum pH is elevated (Rasmussen and Perrin 1999).

Physical environmental factors, during evolution and now, affect chemical signals. After emission, the chemical signals released by elephants can be altered or stabilized. In addition, durations or lifetimes of emitted chemicals can be affected by humidity, rain, or temperature. The duration of the signal is dependent not only on the type of compound released, but also on its reaction to the water environment. The processes of hydrolysis or desiccation, which influence signal lifetimes, are affected greatly by the degree of water in the environment. Although hydrolysis may hasten chemical degradation, dry windy conditions can accelerate volatilization and thus the disappearance of a chemosignal. pH also has considerable importance in determining odour, especially with regard to acidic or basic compounds. For example, with increasing acidity, a volatile odoriferous base such as an amine will increasingly form involatile salts, thus contributing less to the perceived odour. Conversely, odourous acids, such as volatile fatty acids, will increasingly form salts with increasing alkalinity.

The increased ultraviolet exposure in the open terrain of fields and grasslands significantly affects the chemical stability of certain molecules present in animal exudates and effluvia, thus curtailing their lifetimes as chemical signals. Structural aspects of trees, including the height and density of the canopy, the presence and density of tree foliage and undergrowth (such as shrubs), and atmospheric conditions (Garstang *et al* 1995) including wind (Bossert and Wilson 1963), affect the lifetimes and transmissions of chemical signals. Canopy shelter in Asian forests may prolong chemical signal life. Even emissions from leaf or scrub foliage, especially if photoreactive, can affect the lifetime of chemical signals.

Thus the chemical composition of signals apparently changed with the metabolic adaptations to warmer climates. Molecules designated as chemical signals in colder climates, such as lipids and proteins, might take on roles as carriers, while more volatile substances might become more important functionally in warm, humid climates.

Focus here is on two identified chemosignals in Asian elephants: (i) a single urinary compound, a female-to-male pre-ovulatory pheromone, and (ii) a set of signals emanating from temporal gland secretions of males in musth. The first is a single compound, unidirectional signal to the opposite sex. The second is a medley of different chemical messages to different responders.

4.1 *Pre-ovulatory pheromone*

Social and chemical evidence support the concept that a female-emitted, male-received pre-ovulatory pheromone was of primary concern as elephant society evolved following migration into tropical south-east Asia. (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) from female pre-ovulatory urine may have been the first chemical signal to evolve in recent times. A conserved pathway exists for acetate synthesis via fatty acids. Long-chain fatty acids are abundant in elephant serum, with palmitic acid (hexadecanoic acid) being the dominant fatty acid (Duncan and Garton 1968; McCullagh 1973). We can hypothesize that acetate could be synthesized in liver through chain shortening and via β-oxidation of fatty acids in the presence of a $\Delta 11$-desaturatase.

As females were freed up to become more individualistic hormonally, all males did not have to compete at the same time. Competition became less fierce. Experience, body condition, and the ability to sustain musth became factors in male reproductive success. Thus, males, subsequent to females, were freed by climatic changes to expand and alter their time of mating. It became necessary for males to find individually cycling females among many groups, asynchronous with each other, but often exhibiting intragroup synchrony. Thus, within a particular familial group, one or more females might be approaching ovulation. Although hormonally there is a three-month cycle, there are hints that this cycle could be shortened by the presence of a male and his pheromones (V Krishnamurthy, personal communication).

The identification and quantitation of *Z*7-12:Ac provides an outstanding example of the evolution of a chemical signal carrying a specific biological message (i.e., the timing of reproduction) to a conspecific. As ovulation approaches, female urine elicits increasingly high frequencies of flehmen responses from males (Rasmussen *et al* 1982, 1986), suggesting the presence of a specific chemosignal. In a natural environment, these responses are clearly linked to reproductive behaviour. In a captive setting, after fifteen years of laborious purifications, we demonstrated that the active pheromone is a single compound, *Z*7-12:Ac. It is released in increasing concentrations in female urine as ovulation approaches (figure 1). Male chemosensory responses increase in parallel to the increased amounts of this urinary compound, and pre-mating behaviours are observed (Rasmussen *et al* 1997a). What initiates the synthesis at a specific time, the location of the synthesis, and the biochemical route are unknown. Maximum bioresponses by males are quantitatively linked to the concentrations in females of high serum luteinizing hormone, low serum progesterone, and increased urinary *Z*7-12:Ac. Interestingly, studies in Myanmar with work camp elephants demonstrated societal constraints on these male responses. In these field tests, the presence of dominant males reduced the bioresponses of subdominant males (Rasmussen *et al* 1997a).

The chemical and biochemical constraints on *Z*7-12:Ac as a chemical signal tie in nicely with its behavioural and ecological considerations. The specific requirement for the *Z* isomeric form of 7-dodecenyl acetate and the concurrent negligible response to the *E* isomer imposes configurational restrictions for the reception of the message. Thus, males of other species, or *E. maximus* females, will not respond to this signal. The role of proteins reinforces the specificity. Full bioresponse by males is only obtained when the acetate is presented in a medium of control urine (Rasmussen *et al* 1997a). If selected urinary proteins are separated from the acetate by Sephadex G-25 fractionation, male bioresponse is reduced, again suggesting that other compounds, i.e., proteins, are important for the full efficacy and accuracy of the pheromone. Our recent data demonstrate that *Z*7-12:Ac binds loosely and non-specifically to several urinary proteins including albumin with a unique N-terminal. Binding to such a protein will facilitate the

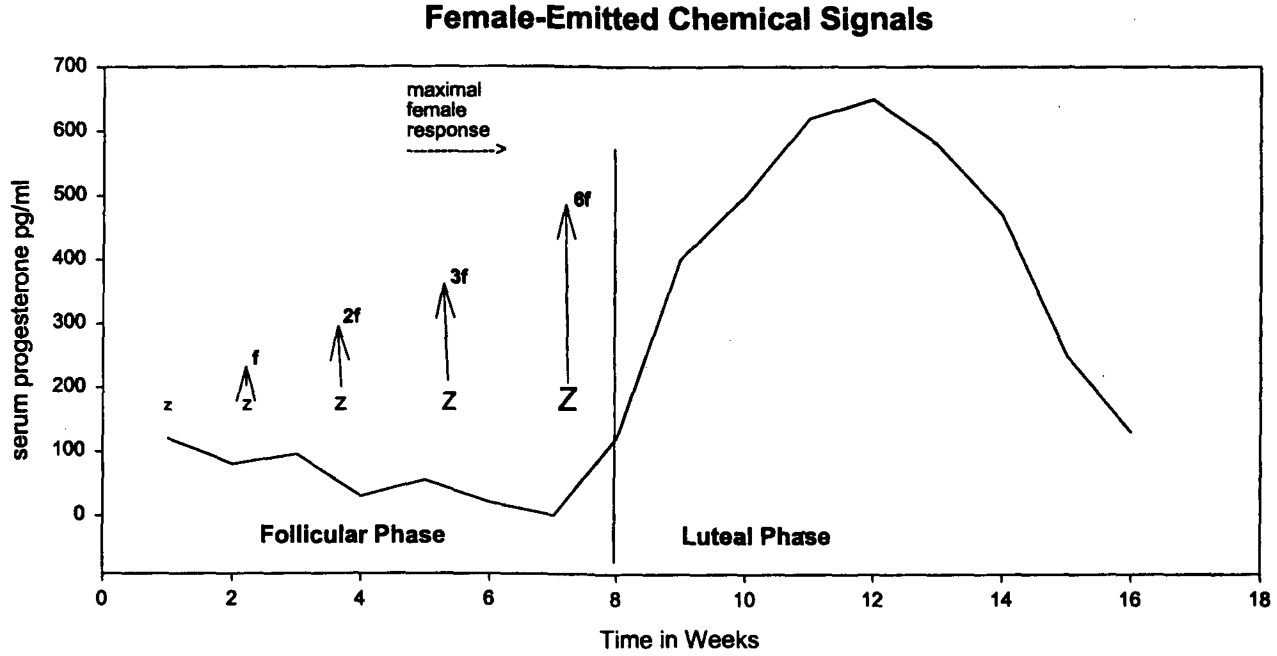

Figure 1. Female-emitted chemical signals in relationship to the estrous cycle. Graph shows serum progesterone levels throughout follicular and luteal phases of the estrous cycle. Also shown is the mating message toward males via the pheromone (Z)-7-dodecenyl acetate (**Z**). The increasing concentration of **Z** is indicated by the vertical arrows and by increased point size of **Z**, and the male response by the number of flehmens (**f**). The status messages from females toward other females occur in estrous urine. Females respond the most to estrous urine during the follicular stage (responses to luteal urine do not change over the cycle). They also perform the maximum number of genital checks, tail slappings, and aggressive acts during the follicular period.

transport of the pheromone and eventually signal transduction. Such protein transfer and specificity may be extremely important in precise species recognition during mating.

The chemical fate of Z7-12:Ac in the natural environment is an important aspect to its functioning as an elephant reproductive pheromone with a specific and time-dependent message. Free Z7-12:Ac in urine probably undergoes a gradual uni-directional hydrolysis to its corresponding alcohol. Its degradation reduces the signal duration of the pheromone. In the wild or in outdoor elephant enclosures, native urine or urine plus test substances is subjected to potential hydrolysis, oxidation, and ultraviolet radiation. Protein carriers can prolong effective pheromone lifetimes by reducing these degradative processes; enclosure of small molecules such as Z7-12:Ac by proteins can reduce its volatility and prolong its lifetime. Conversely, the message lifetime will be shortened if the protein is quickly degraded. If the protein degrades, its unfolding exposes the acetate to hydrolysis and then to potential oxidation. A urination event by a pre-ovulatory female elephant is usually a single event in time and location; females do not continuously emit urine; rather 99% is expelled in a single spot, although some dribbling may occur for several metres, especially when mucus is expelled prior to ovulation. These urine spots with quantitative amounts of the pheromone are effective in providing temporal information to male elephants. Such information allows males to locate females at their most receptive period.

Recent observations during bioassays demonstrate repetitive and comparative assessment by male elephants between a sample of Z7-12:Ac and a physically present anestrous female; in addition, the frequency of erections by solitary males during assays of Z7-12:Ac reinforces its integral involvement in the actual mating process.

The function and rationale for the dramatically and quantitatively increased clitoris-directed, under-body tail flicking by female Asian elephants during the pre-ovulatory estrous period as described recently by Slade (1999) is uncertain. This increased tail flicking occurs coincidently with increasing concentrations of the released urinary pheromone Z7-12:Ac, increased male flehmens, and increased urogenital checks by female conspecifics. Presumably either or both types of chemical signal could be disseminated in this manner.

4.2 *Musth chemosignals – ketones and bicyclics*

Diminished importance of rut and reduced competition for food probably reduced seasonal male–male competition. It became necessary to locate individual females and identify when they were able to ovulate to ensure successful mating. In addition, recognition of individual males and the degree to which an individual male was a potential competitor became important. Chemical signals from other males were needed to provide several types of information, probably the most important being the musth or non-musth condition. As male–male encounters were usually with only one or two males, individual information and the recognition of the exact stage of musth of a particular male was essential. The degree of musth of a potential competitor was necessary information in determining whether to challenge or retreat. Our research has demonstrated that some of this information is relayed in volatile form through emissions from the temporal gland (Rasmussen *et al* 1990; Perrin *et al* 1996; Rasmussen 1998).

Therefore, for male Asian elephants, the temporal gland is an important source of chemosignals. Apparently, only during musth in Asian elephants do males secrete from their temporal glands. Our research has redefined from a chemical perspective the stages of musth that were described behaviourally thousands of years ago in Hindu writings (Edgerton 1931). First, it has become apparent that these TGS have a duality in origin and a plurality in types of chemical compounds. There are components of the secretions that are apocrine in origin and others that originate in the blood circulatory system.

The unique temporal gland and its released substances serve as a specific example of an emission releasing a variety of chemical compounds, apparently sending differently directed messages (toward specific groups of conspecifics) of varying durations. The secretions from the temporal gland of the Asian elephant carry specific messages, i.e., an "in musth" signal which Asian males only secrete during musth. These messages may have differential time components. Released lighter volatile compounds may gain ready access to the olfactory system and, if requirements for cofactors are met, may function in male self-awareness; less volatile compounds may reinforce this message through the vomeronasal organ system via flehmen. Males flehmen to their own urine almost exclusively during musth (Rasmussen *et al* 1984). Males exhibit interest in their own temporal area, especially during pre-musth and early musth (Rasmussen *et al* 1984). Captive females in estrus may touch male TGS at times; at other times these same females may avoid secretions (L E L Rasmussen, unpublished data). In addition, young elephants and some sub-dominant females usually avoid certain volatile blends of musth TGS (Perrin *et al* 1996). Prior to overt TGS, a particular emitted compound or type of compound (e.g., 2-butanone) may inform both males and females of impending musth (Rasmussen *et al* 1997b). Other emitted chemicals with short lifetimes inform young bulls or noncycling young females of the nearby and/or recent presence of a dominant musth bull (Perrin *et al* 1996). This broadcast may also include chemical compounds that comprise messages of medium duration.

Other medium duration messages may be emitted in the urine. For example, estrous females may detect persistent urinary messages of a musth bull (Schulte and Rasmussen 1999a). Often similar compounds are released via several different effluvia. Messages in the TGS and urine tend to be multi-purpose and multi-duration, whereas those in the breath are of short range and short duration. An emission such as breath may be a very close-range, short-duration message during courtship to females or during male–male dominance interplay.

The compounds emitted include a wide spectrum of chemical types, e.g., proteins, steroids, and volatiles. Many are metabolic byproducts or excess concentrations of functioning compounds and, as such, represent honest signals. For example, during musth the temporal gland releases between 13·6 and 2781·3 ng/ml of testosterone and many volatiles (Rasmussen *et al* 1984, 1990, 1996a). This compares with a testosterone level in African non-musth TGS of 1·0 ng/ml (Rasmussen *et al* 1996a); 2·8 μg/ml is an incredibly high concentration of released testosterone, several-fold higher than the highest serum level reported (Rasmussen *et al* 1990). In addition, during musth, protein concentrations in the TGS were only 25 mg/ml ($n = 1$) (Easa 1987), yet urinary proteins, especially albumin, are elevated during musth (H Coeckx and L E L Rasmussen, unpublished data). None of these relatively non-volatile compounds (proteins and steroids) has demonstrated bioactivity as chemical signals, yet the higher urinary protein content may facilitate transport and/or reception of more volatile, less water-soluble signals. Other single, higher-molecular-weight lipids (potentially of a persistent nature, imparting long-term messages) released from the temporal gland have not yet been demonstrated to be chemical signals.

The release of multiple compounds of varying properties from the temporal gland creates the potential for multiple chemosignals imparting diverse messages (figure 2). We have already noted different behavioural responses by different types of conspecifics. Compounds of medium or high volatility generally are signals of medium or short duration, respectively (figure 2). Some musth signal messages may be single compounds, such as cyclohexanone (Perrin and Rasmussen 1994). Cyclohexanone (MW 98 kDa) with a boiling point of 161°C volatilizes slowly over a period of hours and thus is a relatively longer lasting signal than compounds of lower molecular weights. Cyclohexanone elicits flehmen responses from sub-dominant females, but males do not respond (Perrin and Rasmussen 1994). Although the database is small, response to cyclohexanone does not appear to be affected by the estrous state of the responder (although one of the highest responders, a sub-dominant female, was in luteal phase; a low responder was a non-cycler, and a pair of low responders had never been exposed to males). Most interestingly, cyclohexanone may also elicit the behaviour of clustering or star formation (females with offspring form a protective circle around young) (Eisenberg *et al* 1971; Rasmussen 1998).

The lifetime of ketones as chemical signals may depend on their interactions with other constituents. For example, fast and loose covalent binding of ketones as schiff bases (similar to binding of retinal in visual pigment) may occur (Mason and Morton 1984), or these ketones may be bound to olfactory or vomeronasal organ proteins at the cell surface, similar to the mechanism proposed for *Z*7-12:Ac. The metabolic origin of some ketones may explain their continued production and release during musth. Metabolic perturbations during

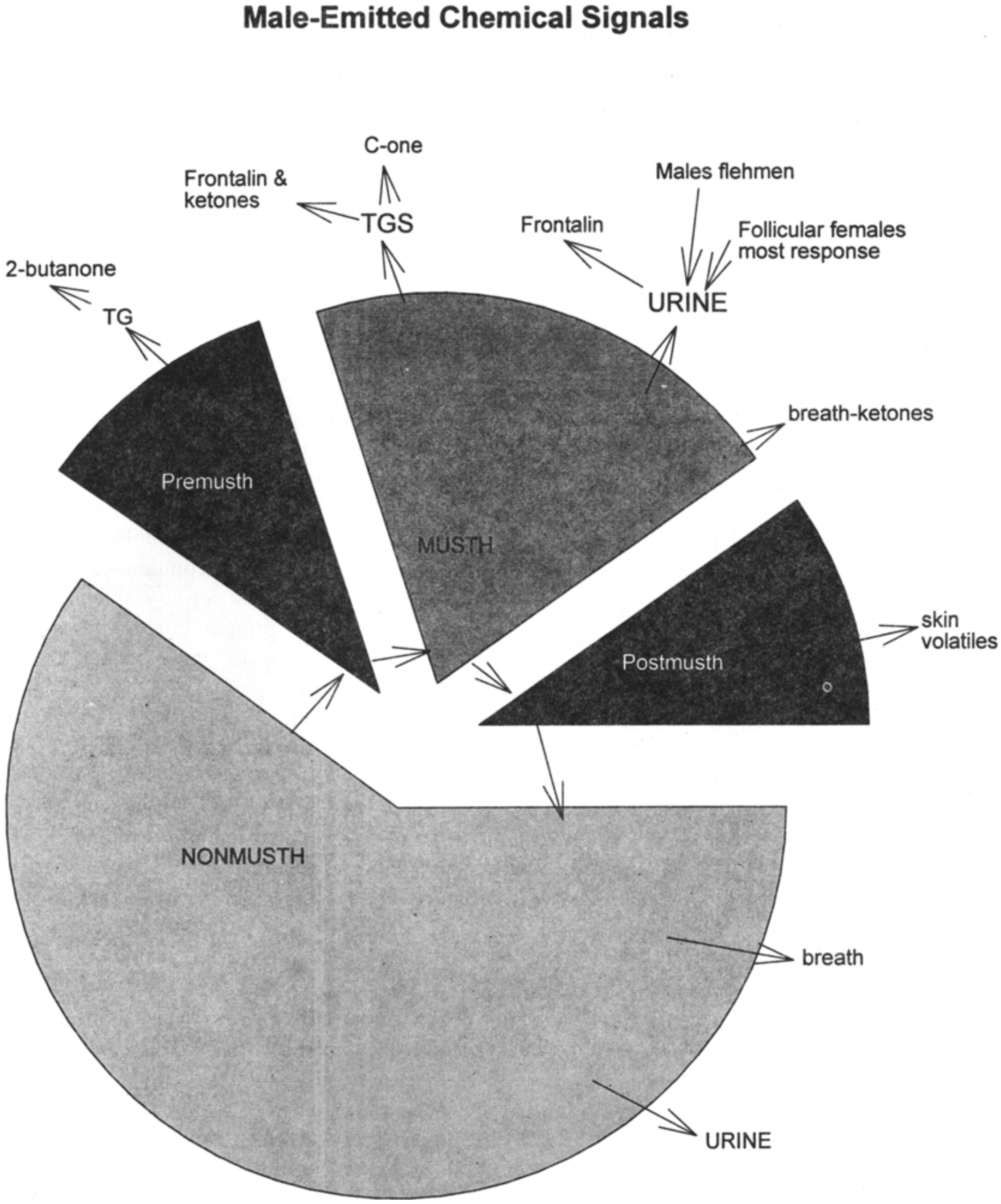

Figure 2. Male-emitted chemical signals in relationship to various stages of musth and non-musth. Starting clockwise in the premusth period, 2-butanone is released from the dry orifice of the temporal gland (TG). During musth, the TG begins secreting and releases two categories of compounds that have demonstrated bioactivity. A seven-ketone and frontalin mixture produces retreat by some females and young males. Cyclohexanone (C-one) does not elicit male responses, but some females flehmen frequently, and females with young often form clustes. Chemical signals in urine include frontalin; responses vary among conspecifics: young males flehmen, older males or males in musth ignore, and females may flehmen or retreat. Musth urine elicits both self-flehmens and flehmens by other males, as well as female responses. In contrast, non-musth urine elicits no responses or only greatly reduced ones. During musth, a variety of musth-specific ketones are released; these ketones are not seen in non-musth breath. During postmusth the volatiles from the temporal area are characteristic of volatiles emanating from the skin.

musth are evidenced by large elevations of triglycerides and concurrent increases in serum lipase, providing an increased source of fatty acids (Rasmussen *et al* 1997b). Decanoic acid, one of these fatty acids, could provide a ready source of a ketone such as 2-nonanone (L E L Rasmussen, unpublished).

Chemicals released in the temporal gland secretions of male elephants that function as messages may be composed of mixtures of several compounds. However, combinations with proteins, such as occurs with Z7-12:Ac may extend lifetimes. This may be especially true with compounds emitted in the urine during musth when the protein content of urine rises (L E L Rasmussen, unpublished). These multiple compounds may be compounds of homogeneous molecular weight and volatility or they may be of widely varying volatility. Some signals may be light molecular weight compounds and/or compounds that volatilize and dissipate more rapidly. For example, several volatile alcohols and aldehydes are characteristic of male Asian TGS, although no chemosensory function has been ascribed to this mixture (Perrin *et al* 1996). A mixture of seven ketones, an alcohol, a cyclic ketone, and frontalin that elicits behavioural avoidance is not only a complex mixture, but the physical properties of the compounds differ (Perrin *et al* 1996). The cyclic ketone cyclopentanone and frontalin may persist as the chemical signal for a longer duration than the lighter molecular weight straight-chain ketones.

Our recent data from samples obtained in 1998 at Mudumalai National Park in south India have reconfirmed that musth has distinct stages based on the emitted chemicals and to which conspecifics respond. Characteristic emissions occur during particular stages of musth whether an elephant is in a sanctuary in the US, a zoo in Europe, or a work camp in India (L E L Rasmussen and V Krishnamurthy, in preparation). 2-butanone signals pre-musth, indeed before visible secretions. The unpleasant odour of 2-nonanone is indicative of late musth, and a series of quantitative changes in other ketones take place throughout musth, dependent on changing metabolism. We are currently investigating the role of the bicyclic frontalin among these ketonic mixtures.

5. Concluding remarks

In this exploration of the evolution of chemical signals in the Asian elephant we have demonstrated and postulated that the development of chemical signals occurs both concurrently and at times after behavioural and ecological changes. With the migration of elephants to habitats of less severely demarcated seasons, synchronized rutting periods, seasonal female estrus, and simultaneous maximum breeding efforts by males were reduced. Females could no longer rely on this annual herd synchrony to attract males. Individual female elephants had a definitive need to attract males. We have postulated that initially a female-to-male pheromone arose to provide this attractive information. Females from small family groups (sometimes only a single individual) sent out multi-dimensional messages to wandering, often solitary males. These messages notified males of their presence and of approaching estrus. Strategically, it made sense to have a several-weeks-long attraction period to ensure that males were in the area at ovulation and to allow primer pheromones to reach males, perhaps stimulating androgenic hormones and resulting in increased male dominance tendencies and libido, thus facilitating mating. The pre-ovulatory pheromone, (Z)-7-dodecenyl acetate, appears in the urine at the end of the luteal phase and gradually increases over the long follicular stage to peak in concentration prior to ovulation. Its concentration is linearly correlated with flehmen responses by males, but with imposed biochemical constraints required for full bioactivity. Its structural and functional convergence with lepidopteran sex pheromones is remarkable.

Within small female-oriented family units, it is advantageous to be aware of the estrous conditions of other females, especially the matriarch. Recent behavioural and chemosignal data have demonstrated a urinary/mucous female-to-female reproductive-state chemosignal that is apparently different chemically from the female-to-male pre-ovulatory pheromone. What role the observed increased frequency of under-body tail flicking has in the dispersion of either or both Z7-12:Ac and the as yet unidentified female-to-female chemical signal is relevant to our understanding of elephant society.

Males also needed to inform conspecifics of their physiological state, especially when they were in musth. As seasonal rut disappeared, competition among males became more individualistic and more dependent on the genetically controlled and environmentally influenced phenomenon of musth. Males developed systems for emitting multi-faceted chemosignals that were released primarily via the temporal glands, but also from other sources including urine and breath. Often particular blends of compounds produced maximum signalling efficacy. There is an ultimate evolutionary advantage to females who could accurately assess male reproductive fitness from male-emitted chemical signals, and to females or other males who could anticipate from such signals possible aggressive tendencies associated with musth. Thus, the Asian elephant has evolved a multi-directional and multi-dimensional chemical communicative system that helps maintain societal cohesiveness and functionality within familial and ecological units.

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