

Fifty years of tiger pheromone research

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The results of our 50 years of tiger pheromone research have been summed up in the larger context of research in this field. In order to understand the perspective, tiger behaviour, such as that of territory and home range, the possible sources of tiger pheromone as revealed through observation, the chemistry of putative pheromones and the expression of ‘self’ and ‘non-self’ by individuals, the fixatives of pheromones and the possible mechanism of stamping of individuality in the pheromonal potpourri have been discussed. The implications of some of the putative pheromone molecules in genomics have been indicated. Our present limitations in understanding the essence of the problem and some future probable guidelines have also been discussed.

Keywords: Lipid, mammalian pheromone, tiger, volatiles.

THIS is a review on chemical communication in the tiger spanning the period 1964–2014 that captures the essence of a journey through a long and devious route to keep the spirit of worthwhile research in biological sciences executed by Indians working in India on Indian fauna. We have indicated the foggy concepts on scent marking starting from Schaller’s mistake in 1967 and lasting even up to recent time¹ and opened up a discussion that was fiercely opposed by most wildlifers up to late 1980s, who believed that the tiger has no sense of smell.

In 1964, one of us (R.L.B.) along with George Schaller, the initiator of modern tiger research in India, was observing a herd of spotted deer reacting to the smell of a tiger squirting on a shrub. This behaviour of the tiger was never recorded in the more than a century of blood sport literature, except once by Locke². Both Locke and Schaller considered that tigers (Locke saw this only in male tigers) leave some osmic message in this manner for other tigers to ‘read’. As the ‘squirting’ was rarely seen till about 1980s, the origin and chemistry of the fluid squirted was not well-understood and the theory remained largely unsubstantiated.

The complex and multidisciplinary nature of the problem took a more concrete shape after close observation on a pet tigress ‘Khairi’ of Simlipal forest, Odisha in 1976 by S. R. Choudhury and R.L.B. In order to gain a proper

perspective, the problem can be split here into the seven following points.

1. Concept of ‘ethology’ – the transmission of message from one individual to another.
2. Uncertainty of the concept of territory/home range in the tiger.
3. Unclear idea of the source of tiger pheromone.
4. Pheromonal message in the tiger is contradicted by the general notion that olfactory sense in the tiger is poor.
5. Nature of the putative pheromones.
6. How can the osmic (pheromonal) messages last long, i.e. what is the mechanism of ‘fixing’ in nature?
7. Stamping of ‘individuality signature’ in the pheromonal message.

According to Adamson³ and Adamson⁴, a lion encodes a message by scent marking that is decoded by another lion. Brahmachary and Dutta (1964–1987) made a preliminary attempt to unravel the facts of coding–decoding in tiger and leopard. The present authors have envisaged the totality of the problem and investigate the chronological order of developing the theme. They discuss the existing confusion related to the above points by their personal understanding and association with research on pheromone of tiger (*Panthera tigris tigris* Linné, 1758) – our national animal.

Origin and evolution of pheromones: significance and related intricacies from behavioural, anatomical and physiological perspective

Haldane and Spurway⁵ first applied Shannon’s information theory in the field of biology, namely decoding the message of the ‘dance language’ of honey bees. Wilson⁶ first used this approach on the pheromone trail of ants. Response to the chemical environment emerged at a primitive stage in evolution, e.g. all cell membranes respond to chemical stimuli to some degree. Chemical interaction between single-celled organisms probably played an important role in the evolution of multicellular organisms and from small to large animals.

Elephants and some moths share the sex pheromone (*Z*)-7-dodecen-1-yl acetate, illustrating a phenomenon of independent evolution of particular molecules as signals by species that are not closely related⁷. Such coincidences

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are a consequence of the common origin of life as basic enzyme pathways are common to all multicellular organisms, and most classes of molecules associated with metabolism are found throughout the animal kingdom. Dawkins⁸ introduced the concept of extended phenotype and 'pheromone' as extended phenotype may reach beyond the body itself and carry its signature. However, the presence or absence of a particular compound in the same taxonomic clade makes it more complicated to extrapolate the functional divergence and evolution of pheromone in big cat family – for example, feline, a major urinary protein (MUP) derived amino acid in domestic cat^{9,10} is unidentified in big felids, whereas cauxin, an MUP is present in remarkably high amounts in big cats like Asiatic lion, Sumatran tiger and clouded leopard, but absent in snow leopard and puma⁹.

Odour also provides an indicator for assessing genetic relatedness and genetic compatibility of potential mates¹¹. In the biological signalling systems, gaining specificity in pheromone signals could be achieved either by the evolution of a large, unique molecule or using a unique blend of a relatively simple-structured multi-component pheromone. In vertebrates, 'individual identification' is the most important message that is used in chemical communication, and there has been an increasing interest in determining whether individuals have unique chemical fingerprints or odour types¹². Mammalian responses commonly depend not only on the reception of the chemical signal, but also on intricate combinations of chemical and non-chemical (viz. visual and tactile) cues, the physiological state of the mammal and on its past experiences¹³. The olfactory systems of most animal species have the ability to detect, discriminate and distinguish different volatile and non-volatile molecules with a large variety of structures as different odours. Usually, potential prey (rat) can distinguish predator-specific signals (cat) through its neuroendocrine system¹⁴. However, the involvement of multi-component kairomone, mutual adaptation of both the species (rat and cat) in the same environment, makes the phenomenon of species-specific predator-prey relationship more complex to understand. The evolutionary link between signalling predator and potential prey still remains to be studied in pheromonal research. This ability comes from the combination of the range of characteristic olfactory receptor types having overlapping specificities¹⁵. The principal sources for odour production in mammals include excretion, secretion which the signaler itself synthesizes, but some components of pheromones may be collected or gained in other ways by a combination of materials of its own and other organisms like resident bacteria, and even from the environment. Above all, the metabolic energy expense for signalling must be maintained at such a level as is beneficial for survival. Excessive energy expenditure will be selected against in course of evolution through natural selection.

Fuzzy concept of territory and home-range

von Uexkuell¹⁶ formulated the concept of claiming a right to land tenure. He used two terms 'Heim' and 'Heimat' (both meaning home or homeland in German), which the latter-day ethologists replaced by territory and home-range; a generally accepted norm is to describe that part of the home-range which is 'actively defended' as territory¹⁷, though other definitions are also found in the literature. However, territorial defence need not always involve direct conflict; vocalization (such as roars) and pheromonal signals may prevent direct conflict. Thus, it can be conceptualized that staking territory/home range by pheromonal markings may be helpful for accessing resources or mates (receptive females for males and vice versa); likewise, markings might regulate encounters between conspecifics by warning rivals or inviting mates.

It was realized by hunter-naturalists of ancient times that tigers regularly patrol their land or kingdom. Lions, leopards, cheetah, etc. also do the same; but only the lion forms a pride, rather like wolves or wild dogs. Sankhala¹⁸ emphatically denied the concept of a territory in the tiger, but his semantic exercise is confusing. It is much better to accept the view that the concept of territory or spacing is an elastic one. Not all species defend territories. Kropotkin¹⁹ was of the opinion that the African hippopotamus is a non-territorial animal, because hundreds of them lay peacefully in close proximity. All recent observers disagree; sometimes they can have a fierce fight – as in the Nile, Uganda. In the River Ruindi, Congo, during daytime thousands of hippos used to lie in a close-packed assembly, almost spanning the narrow river and yet at night each mature animal staked a claim on the piece of land which it regularly patrols and marks with faeces (R.L.B., unpublished). The tiger, essentially a loner, sometimes tolerates other tigers in the vicinity, may consort with genetically related tigers at the boundaries and/or overlap in the respective tigers territories^{20,21}. It is not different from the elastic concept of human territories – private houses are exclusive, a club house or restaurant or a village green or town park is shared and there lies the concept of home-range. The equivalent of claiming stake on land tenure or private house by a notice board or name plate is marking by animals with faeces, urine, secretions from many specialized glands like anal glands, sternal glands, supraorbital glands, gular pouches, poll gland, morillo, temporal glands, skin glands, preputial gland, caudal gland, interdigital gland, etc. and marking fluid (MF, a terminology coined by our group to eliminate confusion about synonymous words like 'scent-marking', 'urine spraying', etc.)²².

Thousands of observations gathered over a period of 4–5 years on MF, sprayed by 12 tigers (male and female) in large enclosures at Nandan Kanan Zoological Park (NKZP), Odisha showed, indirectly but emphatically, that MF plays a vital role. On an average MF spraying is

significantly higher than ordinary urination (50:1 avg.); this must have evolved to serve some purpose; otherwise this wastage of energy would have been selected against. These data also strongly suggest that MF is strategically placed on such boundaries/areas that are near the neighbours' boundaries²³. For example, a boundary never marked for one year began to be frequently marked after a tigress was introduced beyond this fencing. A boundary separating two enclosures was frequently marked and the opposite boundary (far beyond which other tigers roamed) was marked much less. Observational data in one enclosure at NKZP spread over 23 tree locations for one year enable us to assert that the thickness of the trunk or spread of the canopy are irrelevant, the strategic location of the tree is the determining factor. The data also indicate that each of the four tigers (one male and three females) of this enclosure had consistently chosen to stake a slightly specific individual area within their common area, synonymously 'home-range' as evident from countour maps²¹. Males mark more in defiance of other males, i.e. 'defence against the same sex' strategy is evident; the females also do likewise. These data, perhaps the largest mass of marking data in captive conditions, are complementary to the marking and territorial data of the Smithsonian group, who worked in Chitwan, Nepal over several decades²⁴.

Marking fluid, the primary source of pheromone in tiger

Since Schaller²⁵ described marking as 'a mixture of urine and anal gland secretion', this phrase has been repeated by numerous authors, even as late as in 2006 by Thapar¹, though Ewer²² doubted it and Hashimoto *et al.*²⁶ showed that there is no connecting channel between the anal gland and urinary tract, while Asa²⁷ proved beyond doubt that the contents of the anal gland never mix with urine and mix only rarely with faeces. Albone²⁸, who first tried to unravel the chemistry of MF of the tiger and lion, had a confused concept of the same; the prejudice of 'a mixture of urine and anal gland secretion' clouded his views and his observations were limited by the captive conditions in Bristol zoo. In a personal communication, Albone²⁸ explained his view. It is clear from this description that he mistook MF for a mixture of urine and anal gland secretion and he thought that the spray was an ejection from the anal gland. This is really a description of the MF spray as mentioned earlier; anal gland secretion can never contaminate it and the anal gland is too small to contain that much fluid. On observing MF ejected hundreds of times by the pet tigress, Rajchoudhury and Brahmachary realized that it cannot mix with anal gland secretion, but in view of the prevalent prejudice, Brahmachary and Dutta²⁹ were confused even in 1980. Later, the group challenged the faulty concept in subsequent

publications by mentioning that MF is ejected upward and backward through the urinary tract of both male and female tiger. The mode is different from ordinary urination.

Tiger has a sense of smell

Each olfactory receptor of an individual can be stimulated by a range of particular different odour molecules depending on their molecular shape and the receptor active sites – and each kind of odour molecule stimulates a range of different olfactory receptors³⁰. If the pheromonal messages of MF convey information pertaining to territory, even in an elastic sense of a loose spacing mechanism, and also regarding the sex, then the tiger must have a fairly good olfactory sense in order to perceive the volatile molecules. This is indeed the case and, in addition, certain non-volatile molecules are 'perceived' through the vomeronasal organ (VNO) or Jacobson's organ. However, even in 1980s, the present authors faced stiff resistance from wildlife circles. This prejudice was largely due to the categorical statements made by Jim Corbett, the doyen of hunter-naturalists and several other hunter-naturalists suggesting an obtuse olfactory sense in the tiger; but there were also many remarks contradicting this opinion³¹. Close observation on tame or semi-tame tigers proved that they have a fairly keen sense of smell³² and the present authors noted the same with a tame tiger in which this faculty appeared at least by the age of five months. The sense of olfaction in tiger cubs seems to develop after the age of four months and in African lion cubs, at the age of six months. It is precisely then that the 'Flehmen' gesture (nose wrinkled, tongue protruded) first appears in juvenile big cats. Montagu *et al.*³³ reported that tiger has 713 functional olfactory genes as against 805 in the case of dog. So the tiger has nearly 90% of the dog's olfactory genes and it has 21 functional genes of V1r (for VNO) as against only eight for the dog. During 1930–1980 several researchers elucidated the function of Flehmen gesture and VNO.

Pheromonal molecules, their chemistry in tiger and other big cats

Since the late 1970's our group has been investigating MF and urine of the tiger, lion (African and Indian), leopard (Indian) and the cheetah. This work was constrained by the availability of techniques at our disposal – the simple ones like paper chromatography (PC), thin layer chromatography (TLC), various staining and spot tests, and old fashioned gas chromatography (GC) with packed columns. Later, gas chromatography mass spectrometry (GCMS) was available, though rarely, and that too was a far cry from automated solid phase dynamic extraction (SPDE) probe, enantiomer-specific columns with

multidimensional GCMS, etc. However, these sophisticated instruments too may not provide accurate results, and simple classical techniques of organic chemistry may be helpful for identification and quantification of these molecules.

Tiger MF was fractionated into fatty acid fraction, DNP-aldehyde fraction, amine fraction, etc.^{23,29,34} after steam distillation, which separated non-volatile material from the volatile fractions, the putative pheromone(s). One of the amines, phenylethylamine (PEA) detected in tiger urine/MF, warrants special interest. PEA is a common urinary product which was undetectable in many preliminary attempts of the late 1980s in our experiments with domestic cat and golden cat, while it was present in all tiger samples. Many years later, Liberles and his group reported that PEA in tiger urine is ~47 times more than that in the cat³⁵. The discovery of *taar4* gene, which is specific for smelling PEA, raises a challenging issue. PEA might be a pheromone in the tiger and a kairomone in the prey species 'in which case neural circuits activated by TAAR4 (the product of *taar4* gene) might be very different in predators and prey' (S. D. Liberles, pers. commun., 2014). In fact, Taar5 specifically detects mouse odour trimethylamine and causes species-specific behavioural responses. Burger *et al.*³⁶ identified more than 100 molecules in the tiger, including some detected by us (although the distinction between urine and MF was not mentioned clearly). Despite these advancements in technology, all compounds are still not detectable in all samples³⁷. In only 2 out of 36 samples from maned wolf all the 11 compounds were detected. One particular compound was detected in only 10 (out of 36) samples. We could easily collect samples of MF and urine from the pet tigress. We also devised an easy means of collecting MF from tigers in NKZP and had access to a pet cheetah in Namibia. Burger *et al.*³⁶ reported two hydrocarbons (HCs) – decane and undecane. In the 1980s Brahmachary and Dutta had an inkling of the existence of hexane and possibly octane in the headspace (just drawing air from MF-filled air-tight screw-capped glass bottle by a clinical syringe and putting it into the injection port of GC), but because of possible contamination in the solvents this work was not pursued. This was independently confirmed by Ballantine in Swansea in just one and not other samples despatched to him by the authors. Petroleum HC is present in various biological samples; undecane and tridecane occur in alarm pheromone of certain ants³⁸. Albone²⁸ cited many other examples in mammals.

Fresh MF and urine of the tiger (Indian, Amur or Siberian) and Indian leopard have a strong aroma due to 2-acetyl-1-pyrroline (2AP), the elusive compound that imparts fragrance to the best quality aromatic rice. Brahmachary and Dutta realized in the late 1970s that tiger aroma is not civetone (which bears an aroma resembling good rice), but its chemical structure has certain similarities with those compounds which impart fragrance to the

leaves of *Pandanus foetoides* (*P. amaryllifolius*) and Basmati, and many indigenous aromatic rice varieties. All three disappear after acid treatment and reappear after alkali treatment. Later it was found that they permanently disappear after KI treatment and 'toncil' treatment. Buttery *et al.*³⁹ discovered that the aroma of rice and *P. foetoides* leaves is due to 2AP. Later with the help of paper chromatography using two solvents, the authors came close to solving the problem. Finally with the help of two packed columns (Squalene and Carbowax 20M) and GC co-chromatography the tiger aroma was identified^{21,23,40} to be 2AP, the first example of occurrence of this compound in the animal world. Later, we traced this molecule in the aroma of *Maduca indica* (= *Bassia latifolia*) flower⁴¹ and in a particular strain ('sonamung' in Bengal) of mungbean, famous for its aroma⁴². Burger *et al.*³⁶ and Soso 43 also failed to detect it by sample enrichment probe (SEP)-guided headspace and from SPME GCMS. Soso also failed to detect it in the Siberian tiger and Bengal tiger respectively, using multi dimensional solid phase micro extraction GCMS (MD-SPME-GCMS), but after she brought a sample of the Siberian tiger to the University of Calcutta, Kolkata under an Indo-US exchange programme, 2AP was isolated with PC and identified with certainty. After that, she detected 2AP from MF of Siberian tiger in the US⁴³. 2AP was synthesized in the lab by heating proline and sugar at 170°C by Schieberle⁴⁴, Poddar-Sarkar *et al.*²³ could bring it down to 124°C and later to 105°C, but the rice plant biosynthesizes it at a temperature of about 30°C of autumn sunshine. It has now been discovered that the process is enzymatic (and not due to heat reaction, known as Maillard reaction) and probably a mutation of the rice *BADH2* gene allows accumulation of precursors of 2AP⁴⁵.

The cats have been classified under different categories such as *Felis*, *Neofelis*, *Panthera*, etc. and more recently, the lineages of different cats like lion, leopard, tiger, snow leopard, cheetah, etc. have been worked out. Certain characteristic morphological features such as the special hyoid bone of the tiger, have not been reflected in the genomic lineages. Moreover, these genomic studies yield different results, partly because some worked with mitochondrial DNA, others with nuclear DNA; but even on combining all the data contradictory conclusions have been drawn. Davis *et al.*⁴⁶ have been widely quoted, and Sul and Williams⁴⁷ have marshalled the data of four different investigators, including Davis *et al.*⁴⁶. The results are not consistent; sometimes lion and leopard have been grouped together as nearest kin, while in other cases tiger and snow leopard (rather than leopard in general) seem to be nearest kin. If we replace genomics by metabolomics, the result would be still more confusing. For example, 2AP characterizes tiger and leopard, and these two would be nearest kin in this respect. Similar views were also obtained for the presence or absence of cauxin and felinine.

More satisfactory is the lineage of different races of the tiger. It is generally agreed that the Amur tiger is close to Caspian, but more distant from the Indian race⁴⁸. The fact that 2AP occurs in both Indian (Bengal) and Siberian (Amur) race, suggests that 2AP must have evolved in the tiger at an early stage of evolution. Finally, we may point out that no work has been carried out on non-volatile pheromones of the tiger MF (transported to the VNO), which may yield different lineage. Recently, the discovery of gene *CES7* encoding cauxin, the MUP identified from 22 species of felid lineage sheds light on evolutionary diversification of the cat family and contributes evidences for 'Darwinian selection within and between cat lineage'. In addition, the presence of felinine as well as extremely low levels of cauxin in domestic and golden cats, with replacement of some specific amino acids of large non-volatiles, suggest their probable involvement not directly as a pheromone but as a carrier of low molecular weight pheromonal compounds.

On smelling MF, the prey species such as deer evince interest but they do not flee headlong. On the other hand, a whiff of the body smell of the prowling tiger (or lion) nearby impels immediate flight in the prey species. Moreover, tigers may rub their head on trees or the ground or on walls (in captivity) while in the sexually aroused state and this suggests putative pheromonal signals transferred to the corresponding substrate. Moreover, just before spraying MF the lion, as a rule, rubs his head on a tree or rock. This implies that two messages and/or signatures are left at two neighbouring spots. Fatty acids of C9–C24 have been identified by us from the mane of African lion. Recently, wild tigers from the Indian Sunderbans have been frequently straying into villages and recaptured, sometimes by live trapping and sometimes by tranquilizing them. The difficulties in the logistics of collecting body smell samples of tranquilized tigers by rubbing with clean cotton and then hexane extraction and GCMS analysis are enormous, but in three samples a number of methylated fatty acids and benzene derivatives like nitrobenzene, methyl benzaldehyde and hydroxyl benzaldehyde were identified, but we probably missed some polar compounds which might have been revealed with GCMS analysis of dichloromethane extract.

Mechanism of fixation

The smell of MF rapidly disappears if the heavier lipids are separated and these therefore act as fixatives. Over decades our attempts have led to the identification of 30–40 volatile compounds. As mentioned earlier, some fixatives must occur in the MF in order to delay the dissipation of the (volatile) pheromone molecules, for, otherwise the tiger would be forced to renew the markings at an impossibly high rate of frequency. Our early experiments with steam distillation clearly proved that heavier

lipids, triacylglyceride, diacylglyceride, free fatty acids, wax ester and cholesterol ester 'fix' the volatile osmic signals to a large extent²³. The cheetah spends more metabolic energy in the form of lipids as we have seen from our previous experiments, although its physiological system is less efficient for fixing volatiles which vanish within 24 h. It has been noted that even after 10 hot days, the smell of tiger MF on a leaf, though faint, was still perceptible to the human nose (R.L.B., unpublished). However, the information content of such old MF must have decreased with age with the loss of more volatile molecules, since the number of parameters ensuring proper identification of an individual also decreases. Also, we know that tigers and other animals renew markings. A particularly difficult problem is that of marking by the marshmangrove tigers of the Sunderbans, where the habitat is twice daily inundated by tidal waters. Marking here is almost pointless but nonetheless the marsh tiger, originally an immigrant from a northern clime, has not unlearned the basic instinct. To what extent these faint signals are functional is a difficult problem indeed.

Stamping of individuality signature

According to the accepted theory of pheromonal communication, the distinction between the residents of two neighbouring territories or the difference between the territory holder and the floating transient, the distinction between self and non-self must be evident in the pheromones of different animals. Distinctive individual features are evident at every level, in the face, in the voice, in fingerprints, in tissue rejection and histocompatibility complex, etc. The argument posed by Archibald Garrod as early as in 1882, that the mark of individuality of a human being might be imprinted in the urine and the large mass of data gained on quantitative differences in the contents of a number of compounds like amino acids, fatty acids, etc. in sweat, saliva and blood characterizes the individual. The ratios and proportions of 41 such parameters arranged in star-shaped lines are characteristic for each person and these ratios are largely independent of diet, minor illness, etc.⁵⁰.

Gorman⁵¹ reported that the ratios of a number of fatty acids are distinctive for each of the 24 mongoose studied. In *Lemur catta*, a more recent record based on GC of brachial gland secretions of ten animals proves this more rigorously⁵². It has been concluded that the lemur 'actually forms a mental representation of a specific individual by its scent'. In case of three tigers of NKZP, a mother and son duo, and a more distantly related tigress, among the 11 fatty acids of MF quantitatively estimated throughout the year, two, namely acetic acid (highly volatile) and octanoic acid (comparatively less volatile) were used as chemomarkers for discrimination⁵³. The clusters of mother and son are close, while for the third animal the

cluster was more distant; this finding corroborates the information available from Tiger Studbook data⁵⁴. Suppose only two out of 100 possible pheromonal components in tiger/leopard MF occur in significantly large quantities in, say, one particular tiger, the final yield of the combinations, represented in a single tiger id is a big number. A large number of compounds, various amines, aldehydes, fatty acids, etc. (only identified classes besides many unidentified compounds) would yield a vast number of combinations limited only by the acuity of the tiger's olfactory power. As pointed out by Voznessenskaya *et al.*⁵⁵, an animal (in this case rodent) finds it difficult to distinguish more than 5 to 6 animals by their smell. A tiger need not distinguish every individual transient tiger in his domain; the ability to distinguish self and a few known neighbours is enough. All strangers will be recognized as intruders without further distinguishing them as individuals.

Lacuna existing in pheromone research

We are as yet unable to answer the following questions.

What exactly are pheromone(s)?

(i) Volatile or non-volatile? Ethological observations strongly suggest that MF is the source of pheromones, but so far we have only agreed on two broad principles, namely that the molecules which are volatile are candidates for pheromones to be tackled by nasal epithelium and that some non-volatile molecules are expected to be signals for the VNO.

(ii) Which particular molecule or molecules actually function as pheromones in the tiger? Is the ensemble of molecules rather than one or two particular molecules responsible for pheromonal function?

We have no inkling as to whether any particular volatile compound of MF acts like muscone or castoreum (itself a mixture of compounds) as in the case of musk deer or beaver respectively. In Indian crestless porcupine (*Hystrix brachyura*), only cresol in both ortho and para isomeric forms, is identified as the principal component for pheromonal communication in both sexes. As little is known about non-volatile molecules, except presence of cauxin in urine (but not in MF) of Sumatran tiger⁵⁶, we cannot at present even ask that question in the context of VNO. The first alternative is quite likely. On the other hand, Apps *et al.*⁵⁷ subjected this problem to a searching analysis and concluded that the common compounds which feature in all or most of the species, such as fatty acids, aldehydes, ketones, amines, etc. cannot serve as pheromones because of the very fact that they are shared by all or most of the species. How can a tiger or lion distinguish these common

products from those of leopard, cheetah, hyena, etc? Apps *et al.*⁵⁷ were concerned mostly with the African wild dog (AWD) and posit that AWD has a characteristic prepuccial gland distinct from the sympatric species. Therefore, the characteristic secretions from this gland must be the functional pheromones. But in that case only the bearers of such special glands, like the camel with its poll gland or capybara with its morillo can utilize such pheromones. Furthermore, the temporal gland of the elephant secretes chemical compounds which may act as signals even in a moth, as discovered by Rasmussen⁷.

Also, from the point of view of genomics it is unlikely that all the sympatric species, say, the antelopes in the African Savanna, must have different molecules; it is more likely that quantitative differences of the same molecules play a role. The explanation for presence of the same compound, 2AP in MF of tiger (*Panthera tigris*) and leopard (*P. pardus*), co-existing in the forests of Central India, is lacking. Such compounds most likely help in discriminating individuals of the same species and it is possible that the same mechanism might also be responsible for discriminating species. Thus, at present we cannot explain how the tiger or lion distinguishes MF of conspecifics from those of sympatric species. However, the recent findings of del Barco-Trillo and Drea⁵⁸ explain that in 12 different species of lemurs, galago, etc. 'species signature' of urinary profiles is distinctive, namely that 'none of the total 74 volatile compounds were shared by all species and 70% of the compounds detected were expressed in only one species'. The scenario in the big cats may be the same or different.

The problem of overmarking

Overmarking is a common phenomenon in all or most animals. Tigers, like many other animals, overmark the marking by strangers (neighbours on transients) when they pass by the spots overmarked by the latter. It is as if one's signature is superimposed by another's and then the first individual stamps his/her signature once again on the jumbled, indistinct letters. That the animal can distinguish superimposed markings has been proved. Recent rigorous findings have been reported in the case of mongoose^{59,60}. It is thus to the interest of the territory holder to renew its mark on the same spot – to reassert its rights, as it were. That the animal, a rodent or a mongoose or a tiger has developed that much olfactory acuity is stunning.

Durability and degeneration of the pheromone molecules in nature

As these are mixtures of many compounds with differential volatility, while in contact with atmosphere their chemical nature is continuously changing under natural

oxidation, heat, hydration, etc. Then how could one individual identify or distinguish the exact reproductive status of the opposite sex, or the identity of an individual? Both of these are essential for candidates of pheromone. Our research on tiger pheromone has just placed us on the first step of a long, winding staircase and might be considered as one of the challenging arenas of Indian ethochemistry research.

Guidelines for future work

Some of the present lacunae are difficult to solve today. As regards field work, many more radio-collared tigers in India would throw light on the problems of territory, home-range and its overlaps, static interaction (use of same space by more than one animal at different time points) and dynamic interaction (use of same space by more than one animal at the same time), etc.

The olfactory sense in tiger cubs more than four months old has to be tested with different odorants and putative pheromone molecules in spacious zoos. Likewise, bioassay on adult tigers with different combinations of the 100-odd compounds identified in MF may shed light on this problem.

The chemical analysis of the MF of the South China tiger, the supposed ancestral line of tigers, is an important task ahead. The study of putative pheromone(s) from vaginal fluid of tiger is possible only in sedated tigers.

Future prospects of ethological research in India

The rich biodiversity of India offers a vast area for ethological and ecological research and yet till recently, these branches of science have largely been neglected. As early as in 1943, G. C. Bhattacharya, a self-taught naturalist working in Bose Institute in wartime Calcutta, published a paper on weaver ants (*Oecophylla smaragdina*), which compared favourably with the best investigations in Europe and USA at that time. As already mentioned, from 1960s ethoecological studies from a modern perspective were initiated and there has been a recent explosion of interest in young people, which augurs well for the future. From ant to elephant there is no dearth of species for studying in our country. Newer techniques such as detecting firing of neurons on binding of odorant/pheromone molecules, identification of molecules by GCMS, LCMS and MALDI-TOF-MSMS and tracking down the related gene for specific pheromonal components, including statistical evaluation of data and mathematical expression of behaviour, etc. are now available in some Indian laboratories. Thus, present-day research can be extended to various flora and fauna of our country and this means a multipronged attack, including old-fashioned natural history, modern biochemistry, molecular biology and many more interdisciplinary approaches. In fact, all

or most of these studies require close cooperation among scientific organizations, zoo authorities, and National Parks and Reserves of our country.

Certain flora or fauna serve as important ecological scaffoldings in their particular eco-niche and a multitude of fascinating investigations can be generated. This discipline of study is the cross-boundary subject – not botany or entomology or ornithology or mammalogy or chemistry, but etho-ecology as a whole. So the sky is the limit and in Tagore's words – 'Akash Bhara Surya Tara, Biswa Bhara Pran'.

1. Thapar, V., *Tiger: The Ultimate Guide*, Oxford University Press, 2006.
2. Locke, A., *Tigers of Trengganu*, Museum Press, London, 1954.
3. Adamson, J., *Born Free*, Collins, London, 1960.
4. Adamson, G., *My Pride and Joy*, Collins, London, 1986.
5. Haldane, J. and Spurway, H., A statistical analysis of communication in *Apis mellifera* and a comparison with communication in other animals. *Insectes Soc.*, 1954, **1**, 247–283.
6. Wilson, E. O., Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith) and the experimental induction of social responses. *Anim. Behav.*, 1962, **10**, 159–164.
7. Kelly, D. R., When is a butterfly like an elephant? *Chem. Biol.*, 1996, **3**, 595–602.
8. Dawkins, R., *The Extended Phenotype*, Oxford University Press, UK, 1983.
9. Miyazaki, M., Yamashita, T., Suzuki, Y., Saito, Y., Soeta, S., Tiara, H. and Suzuki, A., Major urinary protein of the domestic cat regulates the production of feline, a putative pheromone precursor. *Chem. Biol.*, 2006, **13**, 1071–1079.
10. Rutherford, S. M., Kitson, T. M., Woolhouse, A. D., McGrath, M. C. and Hendriks, W. H., Feline stability in the presence of selected urine compounds. *Amino Acids*, 2007, **32**(2), 235–242.
11. Penn, D. J., The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, 2002, **108**, 1–21.
12. Beauchamp, G. K. and Yamazaki, K., Individual differences and the chemical senses. *Chem. Senses (Suppl. 1)*, 2005, **30**, i6–i9.
13. Nyby, J. and Whitney, G., Ultrasonic communication by adult myomorph rodents. *Neurosci. Biobehav. Rev.*, 1978, **2**, 1–14.
14. Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. and McGregor, I. S., The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosci. Biobehav.*, 2005, **29**, 1123–1144.
15. Wyatt, T. D., *Pheromones and Animal Behaviour: Communication by Smell and Taste*, Cambridge University Press, UK, 2003.
16. von Uexkuell, J., *A Stroll through the World of Animals and Men Translated in Instinctive Behaviour* (ed. Schiller, C. H.), International Universities Press, Inc., New York, 1957, pp. 5–80.
17. Burt, W. H., Territoriality and home range concepts as applied to mammals. *J. Mammal.*, 1943, **24**, 346–352.
18. Sankhala, K. S., *Tiger*, Collins, London, 1978.
19. Kropotkin, P., *Mutual Aid: A Factor of Evolution*, Anarchism subject archives, 1902. Review by Paul Mattick, Western Socialist, Boston, USA, 1956.
20. MacDougal, C., *The Face of the Tiger*, Rivington Books, London, 1977.
21. Poddar-Sarkar, M. and Brahmachary, R. L., Pheromone of tiger and other big cats. In *Neurobiology of Chemical Communication*, (ed. Mucignat, C.), Taylor & Francis (CRS Press), 2014, pp. 407–461.
22. Ewer, R. F., *Ethology of Mammals*, Elek Science, London, 1968.

23. Poddar-Sarkar, M., Mammalian semiochemicals: chemical and behavioural aspects with special reference to tiger. PhD thesis, University of Calcutta, Kolkata, 1995.
24. Smith, J. L. D., McDougal, C. and Miquelle, D., Scent marking in free-ranging tigers, *Panthera tigris*. *Anim. Behav.*, 1989, **37**(1), 1–10.
25. Schaller, G., *The Deer and the Tiger*, The University of Chicago Press, 1967.
26. Hashimoto, Y., Eguchi, Y. and Arakawa, J. A., Historical observation of the anal sac and its glands in a tiger. *Jpn. J. Vet. Sci.*, 1963, **25**, 29–32.
27. Asa, C. S., Relative contributions of urine and anal-sac secretions in scent marks of large felids. *Am. Zool.*, 1993, **33**, 167–172.
28. Albone, E., *Mammalian Semiochemicals*, John Wiley, UK, 1984.
29. Brahmachary, R. L. and Dutta, J., On the pheromones of tigers: experiments and theory. *Am. Nat.*, 1981, **118**, 561–567.
30. Duchamp-Viret P., Chaput, M. A. and Duchamp, A., Odor response properties of rat olfactory receptor neurons. *Science*, 1999, **284**(5423), 2171–2174.
31. Perry, R., *The World of the Tiger*, Atheneum, New York, 1965, p. 260.
32. Raj Choudhury, S., *Khairi: The Beloved Tigress*, Natraj, New Delhi, 1999, p. 192; ISBN: 8185019711.
33. Montagu, M. J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2014, **111**(48), 17230–17235.
34. Brahmachary, R. L. and Dutta, J., Chemical communication in the tiger and leopard. In *Tigers of the World* (eds Tilson, R. L. and Seal, U.), Noyes, USA, 1987.
35. Ferrero, D. M. *et al.*, Detection and avoidance of a carnivore odor by prey. *Proc. Natl. Acad. Sci. USA*, 2011, **108**(27), 11235–11240.
36. Burger, B. V., Viviers, M. Z., Bekker, P. I., Roux, M. le, Fish, N., Fourie, W. B. and Weibchen, G., Chemical characterization of territorial marking fluid of male Bengal tiger. *Panthera tigris*. *J. Chem. Ecol.*, 2008, **34**, 659–671.
37. Goodwin, T. E. *et al.*, Hemiterpenoids and pyrazines in the odorous urine of the mottled wolf (*Chrysocyon brachyurus*). In *Chemical Signals in Vertebrates 12* (eds East, M. L. and Dehnhard, M.), 2013, pp. 171–184.
38. Hoelldobler and Wilson, *The Ants*, Springer, Berlin, 1990, p. 732.
39. Buttery, R. G., Ling, L. C. and Juliano, B. O., 2-Acetyl-1-pyrroline: an important aroma component of cooking rice. *Chem. Ind.*, 1982, **4**, 958–959.
40. Brahmachary, R. L., Poddar-Sarkar, M. and Dutta, J., The aroma of tiger and rice. *Nature*, 1990, **344**, 26.
41. Midya, S. and Brahmachary, R. L., The aroma of *Bassia* flower. *Curr. Sci.*, 1996, **71**(6), 430.
42. Brahmachary, R. L. and Ghosh, M., Vaginal pheromone and other compounds in mung bean aroma. *J. Sci. Ind. Res.*, 2002, **61**, 625–629.
43. Soso, S. B., Poddar-Sarkar, M. and KozielJacek, A., Determining an optimal method for the detection of odorous volatile organic compounds in tiger marking fluid in an effort to aid conservation. Iowa State University digital repository, USA, 2012.
44. Schieberle, P., Quantitation of important roast smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J. Agric. Food Chem.*, 1995, **50**, 2442–2448.
45. Kovach Michael, J., Calingacion Mariafe, N., Fitzgerald Melissa, A. and McCouch, S. R., The origin and evolution of fragrance in rice (*Oryza sativa* L.). *Proc. Natl. Acad. Sci. USA*, 2009, **106**(34), 14444–14449.
46. Davis, B. W., Li, G. and Murphy, W. J., Supermatrix and species tree methods resolve phylogenetic relationships within the big cats, *Panthera* (Carnivora: Felidae). *Mol. Phylogenet. Evol.*, 2010, **56**, 64–76.
47. Sul, S.-J. and Williams, T. L., Big cat phylogenies, consensus trees and computational thinking. *J. Comput. Biol.*, 2011, **18**(7), 895–906.
48. Driscoll, C. A. *et al.*, A postulate for tiger recovery: the case of the Caspian Tiger. *J. Threat. Taxa*, 2012, **4**(6), 2637–2643.
49. Eaton, R., *The Cheetah – The Biology, Ecology and Behaviour of Endangered Species*, Van Nostrand and Reinhold, New York, 1974.
50. Strauss, B. S., *An Outline of Chemical Genetics*, W. B. Saunders, UK, 1960.
51. Gorman, M. L., A mechanism for individual recognition by odour in *Herpestes*. *Anim. Behav.*, 1976, **24**, 141.
52. Pelagi, E. and Dapporto, L., Beyond odor discrimination: demonstrating individual recognition by scent in *Lemur catta*. *Chem. Senses*, 2006, **31**, 437–443.
53. Poddar-Sarkar, M. and Brahmachary, R. L., Can free fatty acids in the tiger pheromone act as an individual finger print? *Curr. Sci.*, 1999, **76**(2), 141–142.
54. Siegfried, S. and Müller, P., International Tiger Studbook, Zoologischer Garten, Leipzig, 1992.
55. Voznessenskaya, V. V., Parfyonova, V. M. and Zinkevich, E. P., Individual odortypes. In *Chemical Signals in Vertebrates 6* (eds Doty, R. L. and Müller-Schwarze, D.), Plenum Press, New York, 1992, pp. 503–508.
56. McLean, L., Hurst, J. L., Gaskell, J. C., Lewis, J. C. M. and Beynon, R. J., Characterization of cauxin in the urine of domestic and big cats. *J. Chem. Ecol.*, 2007, **33**(10), 1997–2009.
57. Apps, P., Mmualefe, L. and Mc Nutt, J. W., A reverse engineering approach to identifying which compounds to bioassay for signaling activity in the scent marks of African wild dogs (*Lycanopictus*). In *Chemical Signals in Vertebrates 12* (eds East, M. L. and Dehnhard, M.), Springer, USA, 2013.
58. del Barco-Trillo, J. and Drea, C. M., Socioecological and phylogenetic pattern in the chemical signals of strepsirrhine primates. *Anim. Behav.*, 2014, **97**, 249–253.
59. Ferkin, M. H. and Pierce, A. A., Perspectives on over-marking: is it good to be on top? *J. Ethol.*, 2007, **25**, 107–116.
60. Jordan Neil, R., Mwanguhya, F., Furrer, R. D., Kyabulima, S., Rüedi, P. and Cant, M. A., Scent marking in wild banded mongooses: intra-sexual overmarking and competition between males. *Anim. Behav.*, 2011, **81**, 43–50.

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