ON THE PHEROMONES OF TIGERS: EXPERIMENTS AND THEORY

Pheromones are very well known in the insect world but such chemical messengers in mammals have been studied only recently. True, muscone and civetone, the scents of the musk deer and the civet cat, were thoroughly investigated quite a long time ago but it is only recently that chemical and other aspects of mammalian pheromones have attracted wide attention. Mech and Peters (1977, p. 321) comment that "the study of chemical communication came of age only a year or so ago, with the founding of the *Journal of Chemical Ecology*. The relatively delayed development of the field resulted from the extreme difficulty encountered in attempting to cope with its subject matter." In the preface to the same symposium Müller-Schwarze and Mozell (1977) mention that only six mammalian pheromones had been identified chemically prior to 1976.

Since then a few more mammalian pheromones have been investigated and the ecological aspects have been revealed at a deeper and interlinked level. As may be expected, trappers, hunters, and poachers had a certain insight into some of these communicatory signals and their way of life called for immediate application of this knowledge. In his biography of a poacher Speakman (1961, p. 153) described a technique of rabbit catching which we would today consider as applied science of pheromones. The poacher said, "In the spring when the buck rabbits were full of the urge to breed I would catch a doe and skin it, scrape up the loose earth from the little latrines that rabbits scoop out for themselves and rub the earth round inside the skin. Then I covered my gin trap with that earth and knew I should catch a buck." The scientist generally has had less first-hand experience with animals in the wild, but recently an ever-increasing number of field ecologists have been visiting the wilderness and studying animals there.

Let us first describe, briefly, some of the ecological aspects of the tiger, a most secretive animal. Known until recently only through legendary anecdotes and hunters' tales, the study of the tiger is a virgin field of research. The first scientific study of the tiger in India was published by Schaller (1967). The accumulated experience of hunters and woodmen could be summed up in a very few words—the tiger is a nonsocial, nocturnal animal having acute night vision and sense of hearing but with slight or no olfactory powers. Here we will review the relevant social-asocial, territorial, and olfactory aspects of the tiger and then present the results of our studies and the implications thereof.

Schaller (1967) found that the territories of females may overlap part of the territory of a male. Sometimes two females and cubs would temporarily associate to form a loose group.

Smith (1978) and other members of the Smithsonian team have now radio-collared 50 tigers in Chitawan, Nepal, and this long-term project is likely to yield the most reliable data on the territory of tigers. It is already known that an outsize male tiger allows seven tigresses within his territory. The young males tend to

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leave the home range and inhabit the suboptimal regions at the boundary of the forest. That young male tigers are forced to do so was also the view of experienced foresters in India (Carrington-Turner 1967). However, one tigress in Chitawan has relinquished part of her territory to her daughter (Smith 1978).

At present it may be tentatively assumed that the tiger has a certain more or less well-defined territory or hunting ground. Does it mark its territory with urine or feces as many other animals do?

Tigers emit a strong musky odor, the source of which is a certain "anal gland" (Schaller 1967). The term "anal" is incorrectly used since the "anal gland" does not open into the anus but its secretion, a white fatty fluid, is passed through the urinary channel. Any sample of urine will contain a certain small amount of this fluid but sometimes a white jet of the fluid issues at an angle when the tiger or tigress raises a hind leg. We shall refer to this fluid as the marking fluid (MF). In this context we may note the description of Mech and Peters (1977) who studied urine marking in wolves. They found that the urine ejected while the leg of the wolf was raised (RLU) was the most important scent mark. Urine excreted in the squatting posture or scats was less important. The authors followed wolves over 240 km and observed 584 RLU. The nature of the terrain and vegetation inhabited by the tiger in geographical India precludes the possibility of such a detailed or accurate study. In the wild Schaller (1967) could see the ejection of this marking fluid on only three occasions.

A pertinent question which can be raised in this context is, How strong is the olfactory power of a tiger? Jim Corbett, the famous hunter and naturalist (Corbett 1947), stated that tigers have no sense of smell. However, he only meant that tigers do not hunt their prey by following their scent. Stuart-Baker (1920) stated that tigers do have a rather poor sense of smell and furnished some data which were rather anecdotal. He observed that his pet leopard cubs were less able than dogs or civets to follow odor trails. Other old-time observers asserted a limited power of houndlike sense of smell to the tiger, i.e., the ability to follow a spoor.

In the study of pheromones we are concerned with the ability of perceiving the smell of the urine or the musky odor therein. Schneider (1930) and Leyhausen (1952; see Schaller 1967) studied the details of "flehmen," a characteristic gesture of many animals. In members of the cat family the animal "grimaces" with the tongue hanging out when it smells its own urine or that of another conspecific. This observation supports the possibility of communication by pheromones.

Since 1976 we (Brahmachary and Dutta 1979) have been working on the supposed pheromone of tigers by taking advantage of the opportunities offered by Khairi, a pet tigress. Prof. S. R. Choudhury, ex-professor of Dehra Dun Forestry College, India, and the present Director of the Tiger Project in Orissa, has raised this tigress since early cubhood with the help of Miss Nihar Nalini, his cousin. The tigress, now a little more than 4 yr old is allowed free run in the house which is located in a large fenced-in, partly wooded compound. Twice she was let loose in the forest for a few days.

We have taken advantage of the opportunity of observing flehmen and collecting the MF of Khairi. We analyzed the MF of three other fully mature (more than 10 yr old) tigers (2 females, 1 male).

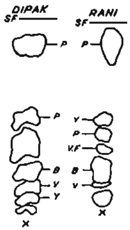

FIG. 1.—Comparison of the chromatograms of MF of two adult tigers. *P* = pink; *Y* = yellow; *B* = brown; *V* = violet; *V.F.* = very faint.

We established the following facts. (1) Steam distillate of the material contained the odor. (2) The distillate could elicit the flehmen response from Khairi. (3) The distillate rapidly lost its odor (although in its natural form the odor lasts for days and days). (4) The distillate contains ammonia and more than one amine. (5) At least one amine appears to be common to all the four tigers (referred to as "tigeramine" henceforth). (6) The characteristic "tigeramine" is absent in the golden cat (Brahmachary and Chattopadhyay 1978), house cat, and in a young lioness of Zaire, Central Africa (Brahmachary, unpublished).

The distillate was analyzed chemically with the help of thin layer chromatography and paper chromatography (Whatman 1 and 3 MM). The distillate is highly alkaline but it can be rendered into the hydrochloride form with HCl. Free HCl was removed by heating. The residual salts were dissolved in water and spotted on the thin layer or Whatman paper and run with the solvent *n*-butanol-acetic-acid-water (4:1:1). The chromatogram was developed with ninhydrin stain. Marker (standard) ammonium chloride was used to determine the position of the ammonium chloride in the distillate after the chromatographic run. When this part of the paper was cut out and rendered alkaline, a distinct odor of ammonia was perceptible. Other ninhydrin-positive spots were located after cutting out a longitudinal strip of the paper and staining it with ninhydrin. These pieces of paper also emitted a distinct odor after being rendered alkaline. Figure 1 shows the comparison of MF chromatograms of different tigers. In all the samples of MF collected from Khairi in 1976 and 1977, only two amines and ammonia were visible. Beginning in September, 1978, more samples were studied and new amines were noticed.

In all samples from all four tigers, one amine having the highest R_f value was always present. The "tigeramine" was eluted and subjected to ultraviolet and mass spectrometry. Ultraviolet spectroscopy indicated an aromatic, i.e., benzenoid ring. The fragments in mass spectrometry showed that the molecular ion has the molecular weight of 121 and the characteristic fragments of molecular weight 30 and 91 suggest CH_2 NH_2 and C_6 H_5 CH_2, respectively. Electrophoresis on Whatman paper with standard mono- and diamines revealed that it migrated with monoamines. These facts suggest that the formula for tigeramine is $C_6H_5CH_2CH_2NH_2$, i.e., phenylethylamine. Standard β-phenyl-ethylamine (Sigma Chemical Co.) was run as a reference on the chromatographic paper and it migrated with the tigeramine obtained from Khairi. In the recent samples taken from Khairi, one amine moved very close to putrescine, a diamine, in both electrophoretic and chromatographic runs. Chromatographically, a similar spot has been found in other tigers as well.

Thirty-one different parameters have been used to establish "biochemical fingerprints" of human beings (Strauss 1960). These parameters are the concentrations of substances occurring in urine, saliva, and sweat. The relative concentrations, or pattern, varies from person to person but is characteristic for any person (or identical twins).

The absence of tigeramine in the cat and golden cat and the variation in the chromatographic pattern of amines of adult tigers (D and R) suggest the hypothesis that phenylethylamine is a biochemical fingerprint at the species level, and other amines, in different combinations or relative intensities, might characterize the individuals.

On the basis of the above facts we can formulate the following theory. Assuming that the amines are really responsible for the odor, the lipids seem to be only vehicles and/or fixative agents. With the help of thin layer chromatography we separated the lipid into several components but we did not pursue this line any further because we realized that the pheromone was not a lipid. Albone et al. (1977) analyzed the lipids of the lion MF and also reported the presence of putrescine and cadaverine.

The tiger's ecology and ethology have evolved a pheromone system adapted to its needs. The crucial condition is that the pheromone must last for a long time—the exact opposite of, say, an alarm substance of ants or the sexual attractant of female moths. Since an odor molecule cannot be long-lasting, the fixative in the form of lipids is an indispensable adaptation. A further, though minor, mechanism to ensure its persistence might be the comparatively high molecular weight of the "tigeramine." Theoretically the upper limit at which an odor molecule can volatilize is ~ 300. Castoramine from the beaver is known to have a molecular weight of 234.

Preliminary experiments indicate that the fatty smelly fluid from a gland in the anal region of the rat snake contains amines (Brahmachary, unpublished). This anal secretion is believed to play a role in sexual recognition and attraction in snakes. The similarity with the tiger MF is marked. Lipids serve as fixatives of the scent which has to be long-lasting in order to be useful for these creatures, answering to the needs of their way of life. Oldak (1976) reports that the essence of odor in certain snakes is ether extractable and apparently the odor is a lipid; but because he did not try to separate the amines by steam distillation he may have missed them. The snake leaves a trail on the ground and the tiger does indeed mark shrubberies, tree trunks, rocks, etc., by ejecting the marking fluid. In the hottest part of summer in one of the hottest regions of India one of us (RLB) noticed that the leaves of a *Butea* sapling carried the odor of the marking fluid for at least 10 days. In the same region Schaller (1967) later confirmed that in summer the odor would last for weeks on tree trunks. One of us (RLB) also found that the odor would not be easily washed from the lower sides of a clump of leaves (on which tiger MF had been sprayed) kept hanging under an open tap overnight. Thus even in rainy seasons the scent would not immediately vanish. Marmosets increase marking in the rainy season (Epple 1974) and tigers may do likewise. The long-lasting scent probably acts as information for the animal or other conspecifics after a lapse of time. This may be a territorial pillar marking with aggressive connotation for other tigers (or invitation for the other sex) or simply a landmark for its own orientation. In any such case the pheromone system probably contains several bits of information: It is a tiger's mark, it is of a particular tiger, it is of a particular sex, and finally it is the "status" of the sex, such as estrus. Hormones in the urine probably code the sexual identity and status (as discussed below). There may also be volatile lipids which are sexually stimulating, as in the case of preputial glands of rats and mice (Gawienowski 1977). It is for the present a reasonable tentative hypothesis that the common amine indicates the "tigerness" and the other amines, in different combinations or different combinations of intensities, characterize the individual. Thus, for example, the chemical odor signs might be equivalent to name plates like Mr. John Brown, Mr. Robin Brown, Miss Susan Brown, etc. The Mr. and Miss part will correspond to hormones or other substances like pheromones and the amines will answer for the Christian and family names, the common amine being the equivalent of "Brown."

In experiments performed with zoo animals and with Khairi, it was noticed that the tigers could perceive the smell of MF from a short distance only, say 1–2 ft. This is probably the lower limit since all the experiments were performed in the daytime and in the presence of various distractions. Possibly in nature and particularly at night the sense of the tiger is more acute and the animal may smell the marking posts from a slightly greater distance. The long-lasting odor may well be an adaptation to ensure perception by an animal with weak olfactory powers. Even while passing by well-trodden cross roads, landmarks, etc., the tiger may very well miss the odor, but if it lasts for days and days the chances of noting it are high. Even if the odor is missed one day, it may be perceived the next day or the day after when the animal passes by the landmark at a still shorter distance. In any case there must be a functional value of marking which is a very strong biological instinct.

Another aspect that needs further study is the effect of sex hormones in the urine of a tiger on conspecifics of the opposite sex. Not only sniffing and grimacing but also extensive licking of areas urinated upon by other tigers have been observed on such encounters. Such perception of sex hormones may stimulate (i.e., sexually) members of the opposite sex. The female in the pre-estrus stage, in particular, might get a fillip toward attaining the estrus stage. The pheromones will, in that case, be a guide to the location of hormones.

Finally, there is of course the possibility that secretion of a strongly odorous marking fluid is nothing more than an evolutionary legacy.

In the fox, mongoose, and lion, resident bacteria very definitely play a role in the production of pheromones (Albone et al. 1977). This is probably the case with the tiger. In all these cases, as well as in the human skin and vagina certain bacteria convert the metabolites into odorous compounds. The human vagina (Michael et al. 1974) like the rhesus monkey vagina (Curtis 1971) produces certain compounds such as acetic acid, propionic acid, etc., the cycle of which coincides with that of estrus. In the female rhesus these substances act as a sexually attracting pheromone.

The relationship of these bacteria to their hosts is therefore a very interesting study from the evolutionary point of view. A sort of symbiosis has developed whereby the products of bacterial origin are used by the host animals as important communicatory signals in their social lives. These bacteria may be said to be salogenic (as opposed to pathogenic). A very well-known phenomenon is the symbiotic association of mammals and bacteria and/or protozoa the latter of which enable the mammal to break down cellulose. Such salogenic bacteria, that is, those which are positively beneficial, and their host mammals are so interdependent that the system of pheromones could have evolved.

ADDENDUM

We are now studying the pheromones of a pair of leopard cubs we are raising in the garden of Calcutta Snake Park. The smell of leopard urine is similar to that of tiger urine. Phenylethylamine is present in very early stages of cubhood. The major part of the odor is due to a nonamine fraction now under investigation.

Very relevant to the discussion on the tiger's olfactory ability is the observation of one of us (RLB) at the Lion camp of George Adamson at Kora, Kenya; namely, that a free-ranging lioness made a beeline to a point at a distance of about 30 ft. (where a wild lion had urinated earlier) and then sniffed and flehmened.

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