

Table 1. Comparison of the two organisms for their productivity

Enzyme source	P-HPH added in the reaction (g/l)	C-N-HPG produced (g/l)	Total HPG produced after decarbamylation (g/l)
<i>Agrobacterium</i>	5.0	2.3	2.3
<i>Pseudomonas</i> sp. H1	5.0	4.0	3.2
	50.0	22.0	—

Cell mass concentration of 2–5%, pH 8 and temperature 40°C.

hydantoin derivatives like hydantoin, hydroxy phenyl hydantoin and structural analogues like uracil on the formation of hydantoinase in the isolated micro-organism in comparison to uninduced cell mass. Under standard reaction conditions the concentration of N-C-HPG measured in the reaction mixture with the uninduced cell mass was 1.25 g/l whereas cell mass induced with hydantoin, hydroxy phenyl hydantoin and uracil showed concentration of 1.5, 1.4, 2.0 g/l, respectively. There was improvement in the enzyme activity due to induction with all the above compounds tested, with uracil showing the highest. From the study on the course of progress of reaction with time, conversion reaction proceeded till around 20–25 h, and further incubation did not result in any improvement in yield at 5 g/l of substrate concentration with 2% cell mass concentration. There are a few reports on the direct conversion of DL-P-HPH to D-P-HPG. Oliverly *et al.*⁷ have shown that the production of D-P-HPG may be achieved in one step using resting cells of *A. radiobacter*. A compara-

tive study along with *A. radiobacter* NCIM 2986 was conducted to examine the efficiency of the enzyme system. *Pseudomonas* sp. H1 was seen to have 1.7-fold more yield in comparison to *Agrobacterium* (Table 1). The enzyme seems to be different from that reported by Dong-Man Kim and Hak-Sung Kim⁶ who used hydantoin, dihydrouracil, DL-P-phenyl hydantoin and DL-P-HPH to induce the enzyme system in the isolated micro-organism, but found the enzyme to be a constitutive one, as the activity was similar in spite of addition of all the inducers. The optimum temperature and pH for the activity of the enzyme was more or less similar to those in case of substrate concentration at 10 g/l. Investigation of the activity with higher substrate concentration showed the enzyme to be economically promising for higher hydrolysis of the substrate. About 50% conversion is achieved at 50 g/l substrate concentration.

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ACKNOWLEDGEMENTS. We thank Dr Gautam Shah CPIATR, USA for advice and interpretation of IR results and Dr Baijayantimala Garnaik (NCL), Poona for help in interpretation of NMR results of the isolated compounds.

Received 9 April 1999; revised accepted 6 January 2000

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Behavioural and chemical aspects of scent marking in the Asiatic lion

Scent marking by the big cats was first described by Locke¹ who studied the tiger in Malay but it was Schaller who first clearly mentioned that tigers spray a mixture of urine and secretion from the anal gland upwards and backwards which presumably leaves odourous signals on salient environmental objects. This spraying was subsequently observed in detail by Smith *et al.*² in the wild and by Brahmachary *et al.*³ in captivity. Schaller's statement that the odourous secretion from the anal gland

is mixed with urine in both tiger⁴ and the African lion⁵ runs into a difficulty because the anal gland opens towards the anus and has no connection with the urinary tract⁶. Both male and female tigers spray with a high frequency^{3,7} but in the African lion it is predominantly a male activity⁵.

The present note is intended to document the behavioural and chemical aspects of scent marking in the Asiatic lion. This investigation was made in two enclosures at the Interpretation Zone,

Sasan-Gir, Gir National Park, India. Each enclosure has an area of 81 m² but occasionally the lions were released in the 420 ha fenced forest area where they could freely roam and even hunt prey. Thus, they were exposed to a more natural environment than that of most zoos. Observations were carried out during 2 h in the morning and again for 2 h in the afternoon when they were generally most active. The relative frequencies of urination and marking fluid (MF) and their location within the enclosures

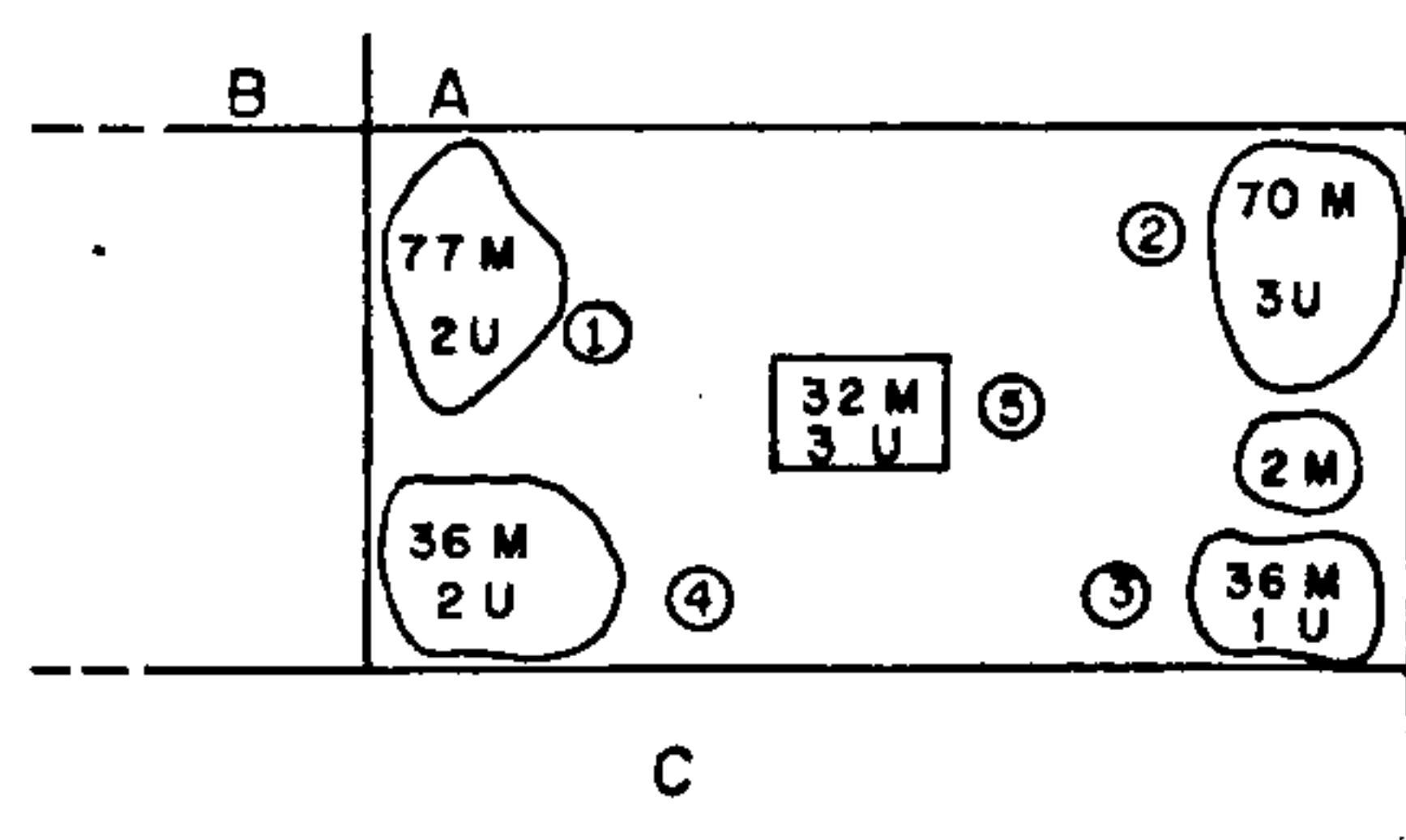


Figure 1. Marking and urination pattern of lion-1 in the enclosure A. The two enclosures A and B are almost identical but there is an artificial rock and a tree in the central part of A. C is the roof of the building from which observations were made. M, marking; U, urination.

were recorded during the day. Altogether 256 h of observation was made.

A mature male lion (lion-1, 7 years of age) in enclosure A sprayed MF 253 times while he urinated only 11 times. The distribution pattern of MF was non-random. Figure 1 shows that the two areas 1 and 2 were marked 77 and 70 times, respectively, i.e. they together account for ~57% of the total markings. The areas 3 and 4 were marked 36 times each (~14%) while the artificial rock with tree (area 5) was marked 32 times. Three lionesses roaming in the 420 ha area often visited the enclosure (which they could not enter).

During the same period a mature lioness in the same enclosure was never observed to spray but she urinated 176 times in a non-random manner; the pattern generally agrees with that of the male MF. 139 urinations (~78%) took place in areas 1 and 2 (92 and 47 respectively). When, later, this lioness exhibited overt oestrous or pre-oestrous behaviour such as rolling on her back to attract the attention of the male she was observed for 4 days (8 h of observation) when she sprayed 4 times. After the 4 days the behaviour mentioned above as well as spraying was no longer observed. During the 4 days the male in the same enclosure sprayed MF 60 times. Furthermore, 10 standing urinations and a single ordinary urination were also observed.

A second, older male in enclosure B was observed for 30 days (~120 h of observation). He sprayed MF 60 times as against two urinations. 43 markings (~71%) were made in two corners 1 and 3, only 2 markings were in area 2, and 6

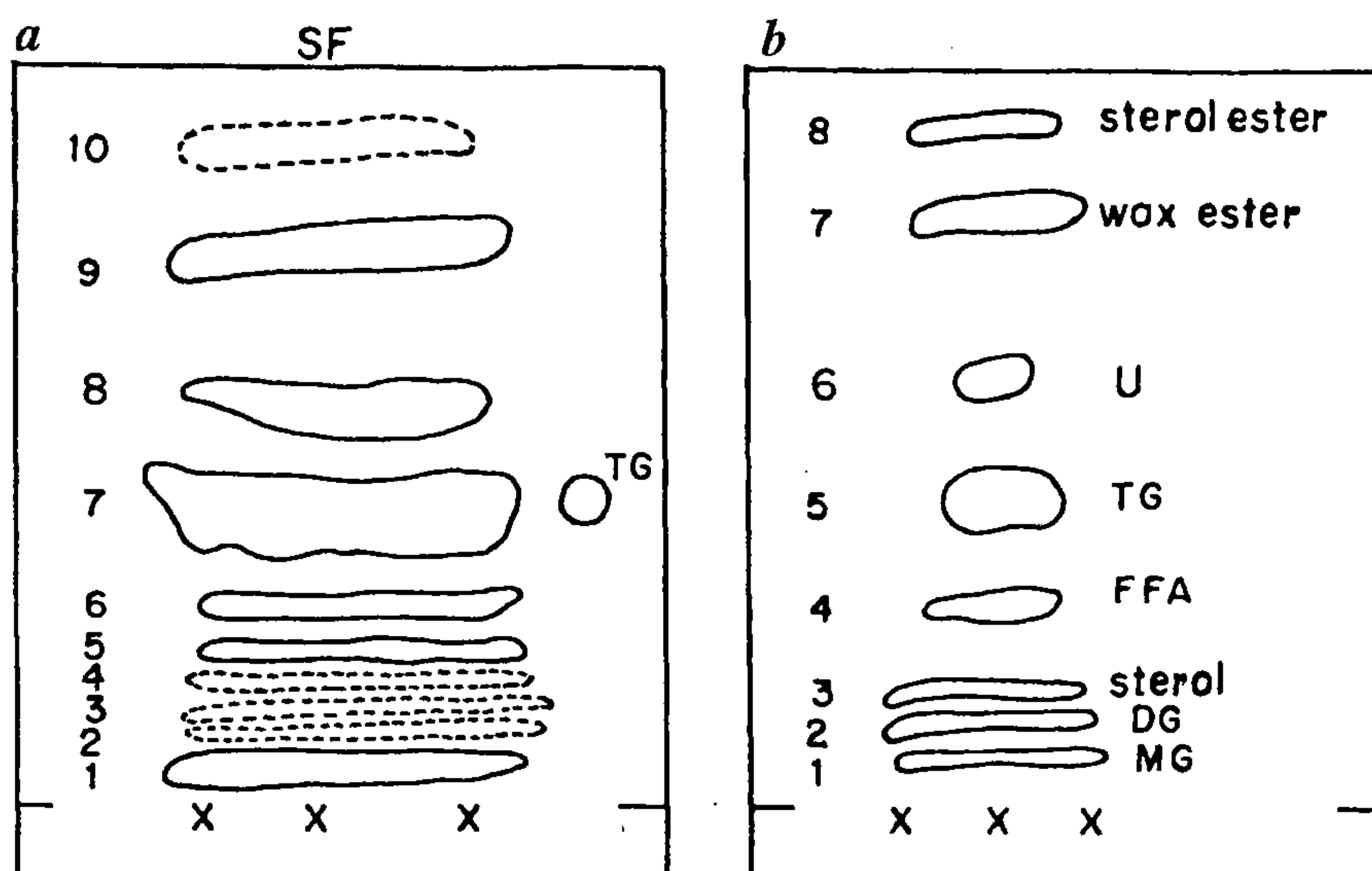


Figure 2. TLC lipid profile of (a) an Asiatic lion and (b) a tiger. Solvent - Pet ether:ether:acetic acid (90:10:1.5); MG, monoglyceride; DG, diglyceride; TG, triglyceride; FFA, free fatty acid; U, unknown; Broken lines indicate very faint spot.

markings were noticed in the central region.

The MF of lion-1 was collected in a clean tray and then transferred to a bottle containing hexane. Lipid from MF was extracted according to the method of Bligh and Dyer⁸ and the chloroform phase containing the lipid was concentrated. This was spotted on high performance thin layer chromatographic plates and developed with hexane:ether:acetic acid (90:10:1.5). The spots, visualized in I-vapour, were compared with the standard lipid profile in tiger MF (Figure 2).

After developing a paper chromatogram with *n*-butanol:acetic acid:water (4:1:1) and with pyrrole as a standard the aromatic amino acids were converted into Schiff bases (yellow or red) with the help of *p*-dimethylaminobenzaldehyde in conc. HCl. MF from the lion shows a single yellow spot, a violet spot coincides with that of standard pyrrole.

Four ml of anal gland fluid (AGF) was extracted from lion-1. The amines and amino acids in it were compared with those of the MF (of the same lion). 30–50 μ l of each sample (MF/AGF) was spotted for a comparative study. Two ninhydrin spots (on the paper chromatogram) of AGF were absent in MF and one spot of MF was absent in AGF. A putative free fatty acid (FAA) was detected from the remaining AGF

with the help of methyl-red staining after PC (solvent-100 ml methanol:1 ml NH_4OH). The corresponding spot was absent in the MF. Two other spots were found in the MF after very short runs which disappeared in longer runs, suggesting the presence of low molecular weight FFA.

Lion MF does not have the basmati rice odour (2-acetyl-1-pyrroline) which is characteristic of the tiger⁹. This odour disappears in strong acidic medium but lion MF has a pH of about 6, as in the case of the tiger⁷. Lion MF was made alkaline but even then no such odour was perceptible.

As in the tiger^{4,7}, leopard¹⁰ and cheetah¹¹, the ratio of marking to urination is very high in the lion. This and the distribution pattern of marking (non-random) are in conformity with the theory that MF has a functional role. However, both the tiger and tigress mark with high frequency⁷. While tiger-1 marked 3035 times in the course of 13 months the two tigresses with him marked 2703 and 1691 times, respectively. Tiger-2 marked 505 times in a period of 7 months during which two other tigresses within his enclosure marked 288 and 270 times, respectively.

However, when the lion-1 marked 253 times the lioness in the same enclosure did not mark even once. As against only 11 urinations by lion 1 the lioness urinated 176 times. The sites were dis-

tributed in a non-random fashion. Thus it is not impossible that urination of the lioness may bear informative connotations spread in her area. However during the brief period of oestrous the lioness was seen to spray mark. Trackers in Gir generally do not see lions and lionesses urinating but both the male and the female have been observed to spray. According to some trackers, lionesses spray only during 'courtship' stage.

The lipid chromatogram of MF of the Asiatic lion shows minor differences from that of tiger⁷. Sterol-ester is relatively less in the former. The volatile molecules like the amines and free fatty acids were putative pheromone molecules.

The MF of lion shows a single yellow Schiff base. The leopard MF also reveals a single yellow spot¹⁰ but both the tiger⁷ and cheetah¹² show two spots, yellow and red. The Schiff bases are thus a sort of species marker. Pyrrole occurs in all four animals.

It is reported that big cats spray a mixture of urine and anal gland secre-

tion^{2,5}. However, no connection between the anal gland and urinary tract has been noticed in the tiger⁵. The amount of fluid in the anal gland is too small to significantly contribute to the MF. Also a particular FFA of the AGF cannot be traced in the MF. Similarly, the differences in ninhydrin positive substances also point at separate pathways of extrusion of MF and AGF.

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ACKNOWLEDGEMENT. We thank Mr A. Sreevastav, former Conservator, Gir, and other staff for co-operation. We are grateful to Dr R. H. Sabapura for extracting AGF.

Received 4 June 1999; revised accepted 3 November 1999

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Palaeoenvironmental significance of *Botryococcus* (Chlorococcales) in the Subathu Formation of Jammu and Kashmir, India

Botryococcus is an oil-forming green alga of the order Chlorococcales. Colonies of this alga have been recognized

in the fossil records dating back to Precambrian^{1,2}. Modern *Botryococcus* occurs in freshwater lakes, ponds or

ditches in wide geographical regions that are normal or slightly alkaline with low humic content. It is also known to

Table 1. General stratigraphy of paleogene rocks in the study area

Upper Murree	Miocene	Monotonous grey sandstone and grey-brown to yellowish subordinate mudstone
Lower Murree	Upper Eocene Oligocene	Brownish-red sandstone and mudstone alternating with subordinate siltstone
-----? Disconformity-----		
Subathu group	Late Palaeocene-middle Eocene	Purplish-red shales and silt stone, oyster coquissites Olive green nummulitic shales and limestone Carbonaceous shales with coal seams (<i>Botryococcus</i> yielding horizon)
-----Unconformity-----		
Lain Bauxite Formation	?	Bauxite/laterite
Khargala Chert Formation	?	Chert Breccia
-----Unconformity-----		
Sirban limestone (= Great limestone/Vaishnodevi/Reasi limestone/Jammu limestone)	Precambrian	Cherty dolomitic and quartzite