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Determining an optimal method for the detection of odorous volatile organic compounds in tiger marking fluid in an effort to aid conservation

Abstract

Chemical communication plays an integral part of conserving tigers. Tigers, elephants, lions, and many other mammals use marking fluid (MF) and other excrements as means to communicate with each other and their ecosystem.

This research focuses on understanding which compounds eluted in tiger (MF) contribute to the overall odor of MF. Specifically, which compounds are responsible for behavioral responses. This study collected MF samples from four, sixteen year old Panthera tigris tigris from South Khayerbari Tiger Rescue Center in West Bengal, India. In this work, MF has been studied to gain a perspective on how tigers utilize MF for territorial and reproductive communication. Specifically, this study expanded upon previous research methodologies that characterized the odor of MF, of Panthera tigris tigris, based on thin-layer chromatography (TLC). TLC was utilized for the separation and identification of lipid compounds. Alkali was added to the TLC paper for the identification of compounds, specifically 2-acetyl-1-pyrroline (2-AP). This research study compared the concepts of TLC against solid-phase microextraction (SPME) and multidimensional gas chromatography mass spectrometry-olfactometry (mdGC-MS-O) for detection of odorous volatile organic compounds. TLC analysis of the samples was performed at the University of Calcutta and mdGC-MS-O work was performed at Iowa State University.

The mdGC-MS-O technique uncovered 19 odorous compounds including 2-AP, suggesting that 2-AP is not the only contributing odor to the smell of Panthera tigris tigris MF.

Background

Scent marks are excretory social signals placed on a variety of objects in the environment in the absence of the receiver and are generally detected much later in the absence of the signaler (Gosling et al. 2001; Bossert and Wilson, 1963). It has been acknowledged that communication is a process in which animals use their sensory organs to send and receive information throughout their ecosystems (Forrester, 2008). Introductory studies using nasal identification and TLC were able to identify 2-AP as the characteristic odor of Bengal tiger MF (Brahmachary et al. 1990). Previous studies have utilized GCMS technology to analyze the constituents of scent marks to infer their purpose in animal communication, but have never used an MDGCMS-O to identify key odorous constituents of these markings to determine which specific compounds are contributing the highest odor intensities to markings.

Main Objective

Identify characteristic odor and composition of territorial marking fluid to improve understanding of the role of Bengal tiger semiochemicals in conservation

Specific Objectives

A. Use simultaneous chemical and sensory analysis to identify compounds responsible for the characteristic odor of marking fluid
B. Develop comprehensive library of compounds and odors making up marking fluid.
C. Compare previous methods for odor and chemical identification of tiger MF with novel sensory and chemical analysis (mdGC-MS-O), i.e., determine if 2-Acetyl-1-Pyrroline being solely responsible for the characteristic odor of marking fluid.

Materials and Methods

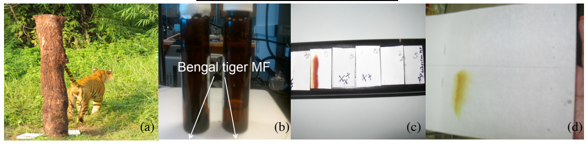

Figure 1. (a) Bengal tiger spray marking (b) Bengal tiger MF in the 40mL collection vials; (c) TLC, alkali, 2% KI application; (d) 2,4 dinitrophenyl hydrazine stain identification

Method 1 (Brahmachary et al. 1990)

- Marking fluid collected from four Bengal tigers at the South Khayerbari Tiger Rescue Center
- MF transferred into 40mL vials with 5mL of hexane for preservation during transportation
- Thin Layer Chromatography performed to separate the lipid compounds
- Hydrochloric acid added to acidify the aroma and prevent volatilization
- Alkali added for aroma identification
- 2% KI was added to cleave the reactive methyl ketone group of the 2AP molecule
- 2,4 dinitrophenyl hydrazine stain was deposited for amine detection

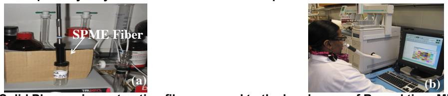

Figure 2. (a) Solid Phase microextraction fiber exposed to the headspace of Bengal tiger MF; (b) mdGC-MS analysis of Bengal tiger MF using SPME fiber

Method 2 (Simone Soso)

- Headspace extraction performed on previously collected MF from Bengal tigers (N=4)
- Solid Phase Microextraction and mdGC-MS-O analysis
- Compared odor detection findings with Flavornet database for accuracy

Results

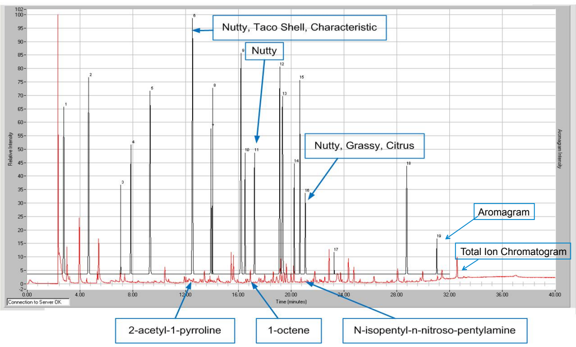

Figure 3. Identification of selected odorous compounds emitted from Bengal tiger (N=4) marking fluid (total compounds103). Odor characterization and chemical identification performed by simultaneous chemical and sensory analysis of VOCs.

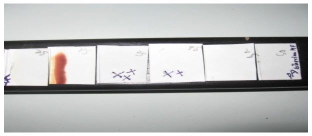

Figure 4. Color and odor identification of 2-acetyl-1-pyrroline after 2,4 dinitrophenyl hydrazine stain and alkali addition

Conclusions

1) Preliminary findings reveal that solid phase microextraction and simultaneous chemical and sensory analysis appears to be a more efficient method for ascertaining potential odorous compounds responsible for the characteristic odor of Bengal tiger MF
2) Using SPME and mdGC-MS-O, 19 odorous compounds identified, 3 of them share similar odor descriptions and all may influence the characteristic odor of the MF
3) Method 1 (Brahmachary et al 1990) and Method 2 (Simone Soso) can be used for aroma conformation of 2-AP nutty aroma of 2-acetyl-1-pyrroline

References

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Gosling, L. M., Roberts, S. C. & Peter J. B. Slater, J. S. R. C. T. S. a. T. J. R. 2001. Scent-marking by male mammals: Cheat-proof signals to competitors and mates. *Advances in the Study of Behavior.* Academic Press.
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