CHARACTERIZING THE SCENT AND CHEMICAL COMPOSITION OF PANTHERA LEO MARKING FLUID USING SOLID-PHASE MICROEXTRACTION AND MULTIDIMENSIONAL GAS CHROMATOGRAPHY–MASS SPECTROMETRY–OLFACTOMETRY

Supplementary Note, Figures and Tables

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Supplementary Note. Additional information on the utility of solid phase-microextraction and multidimensional-gas chromatography-mass spectrometry-olfactometry for research focused on semiochemicals.

Interfacing solid phase-microextraction (SPME) and multidimensional chromatography with olfactometry provides a unique opportunity to address the knowledge gaps in chemical sensory analysis development for lion MF and to identify the chemicals responsible for the characteristic odor of lion MF. The emissions of volatiles from MF (defined here as a simultaneous and mixed excretion of MF and urine) was analyzed in totality, MF was not separated from urine, in order to improve understanding of the perceived odor of gases emitted from lion MF. We did not analyze fecal excretions, a common form of scent-marking, because in lions defecation can be done at random. This is indicating its potentially lower order in the hierarchy of scent-markings. Although the scope of this study was limited to lion’s MF, the same approach could be used for other species, excretions, environments, and with behavioral studies. Once odor and odor-causing compounds in territorial markings are known, this knowledge can be exploited to determine the effects of specific compounds on animal behavioral. Future studies can develop behavioral assays and compare changes in chemical composition of scent-markings to the behavioral responses during the introduction of the odorous compounds identified in this study to lions. This unique and novel methodology combining SPME and MDCG-MS-O could be used to further understand the way animals perceive scent-markings, and potentially prevent the eradication of many endangered species.

Chemosensory cues play a large role in the reproductive behavior and proliferation of many species. Understanding the role of odors in scent-markings has proven to be integral in the conservation research of a plethora of endangered species. The focus of this work has been to increase reproduction in and out of captivity. Odors within scent-markings have been proven to influence male ejaculation in various animals including giant pandas (Ailuropoda melanoleuca), Drosophila melanogaster and Pieris rapae, Zosterisessor ophiocephalus and Gobius niger, Gallus gallus and Microtus pennsylvanicus. Males tend to ejaculate in the presence of competitive males in an effort to preserve their genetic influence and survival within their species. In the case of giant pandas it is hypothesized that chemosensory cues from potential rivals “increase male pandas' sexual motivation towards females, and enhance their territorial behavior”2. The lack of competition in captive environments can potentially be inhibiting reproduction of endangered species unless knowledge of chemosensory cues is expanded2.

Often, studies are able to equate behaviors with scent-markings, but do not identify specifically the roles of individual compounds in animal behavior nor attempt to understand how the animals are perceiving these scents. The ability of elephants to detect cyclohexanone in musth has led...
scientists to suspect that some musth signal messages in elephants may be single compounds. More research on the roles of individual scents and chemical compounds within markings is needed in order to gain an understanding of the influence each has on eliciting behaviors.

Solid phase-microextraction is a solvent-free, one step sampling/sample preparation technique that has been limitedly used in the sample preparation of large mammalian scent-markings. Since its conception in the late 1980s, it has proven to be one of the superior sample preparation techniques available for analytical work in the area of fundamental analytical chemistry, environmental analysis, pharmaceutical, food and forensic analyses. SPME is a reusable technique that combines sampling and sample preparation and is suitable for laboratory and field environmental work. The SPME process is facilitated on a polymeric coating that has a high affinity for organic compounds. SPME has been used for sampling of volatile compounds in air, livestock odor, breath of animals, volatiles inside rumen, volatiles emitted by decaying animal mortalities, and insect-induced plant volatiles among other applications. Enrichment associated with SPME often leads to significantly improved method detection limits and elimination of artifacts from solvents compared with other sampling and preparation methods.

Multidimensional-GC-MS-O is one of the most advanced methods for simultaneous chemical and sensory analysis, enabling volatile organic compound separation and isolation of odor-active compounds. Precise and advanced capabilities to detect trace levels of components is due to its multi-column system which allows for a better separation and identification of volatiles many of which are odorous. The olfactometry is enabled by a sniff port which gives odor panelists an opportunity to characterize each separated compound as it is being eluted through one of the selected GC columns. This feature allows for the determination and verification of compounds through chemical (GC column retention times, MS spectral matches) and, simultaneous odor matching confirmation using trained odor panelists and published scent-to-compounds link libraries. There is limited working knowledge of how mammals process odor signals. Therefore, the human nose is considered ideal in understanding odor perception in animals because the human sense of smell is capable of distinguishing and recognizing a diverse range of characteristics of volatile compounds. Headspace-SPME and MDGC-MS-O was used in the identification of VOCs from Panthera tigris altaica MF. This use of SPME in conjunction with MD-GC-MS-O allowed for aroma recognition and chemical confirmation of 2-AP, which was previously considered one of the characteristic odor compounds of P. tigris tigris MF, but could not be identified previously using solely chemical analysis with GC-FID and GC-MS. The objectives of this study were to: 1) develop a novel method for the simultaneous chemical and scent identification of lion MF in its totality, 2) identify the characteristic odorants responsible for the overall scent of lion MF as perceived by human panelists, and 3) compare the existing library of known odorous compounds characterized as eliciting behaviors in animals in order to understand their functionality in lion behavior.

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| MDGC-MS-O Mode | Extraction Time | Mean # of Odorous Compounds Identified | STD DEV | RSD% |
|----------------|-----------------|--------------------------------------|---------|------|
| No Heart-Cut (NHC) | 1 min | 0 | 0 | 0 |
| | 1 h | 5.33 | 0.47 | 8.84 |
| | 24 h | 17.3 | 1.25 | 7.2 |
| Heart-Cut (HC) | 1 min | 1 | 0 | 0 |
| | 1 h | 10 | 0.82 | 4.41 |
| | 24 h | 24 | 0.82 | 3.4 |

**Supplementary Table S1.** Effects of extraction sampling time on number of odorous compounds detected. Effect of extraction time (1 min, 1 h, and 24 h) on the number of odorous compounds able to be detected using NHC and HC modes.
| No | RT (min) | Top 5 Ions and Their Relative Intensities | Odor Descriptors Observed by Panelists | Measured Odor Intensity |
|----|----------|-----------------------------------------|--------------------------------------|------------------------|
| 1  | 2.75     | 43(99),58(49),41(28),39(24),72(23)     | Cardboard, medicinal, body odor, rancid, foul | 30                     |
| 2  | 3.18     | 133(99),73(62),73(32),132(30),59(27)   |                                      |                        |
| 3  | 3.53     | 57(99),44(33),41(15),58(14),39(11)     |                                      |                        |
| 4  | 3.72     | 76(99),44(16),32(14),78(7),38(4)       |                                      |                        |
| 5  | 4.13     | 44(99),56(75),41(60),43(51),57(46)     |                                      |                        |
| 6  | 5.72     | 30(99),70(5),44(4),41(4),27(3)         |                                      |                        |
| 7  | 6.16     | 43(99),71(67),41(16),114(15),27(11)    | Herbaceous, plastic                 | 80                     |
| 8  | 6.27     | 43(99),71(57),41(12),70(8),14(8)       |                                      |                        |
| 9  | 6.57     | 81(99),80(90),39(22),53(22),42(20)     |                                      |                        |
| 10 | 7.62     | 43(99),72(81),57(70),41(64),85(29)     |                                      |                        |
| 11 | 7.75     | 43(99),72(42),41(19),71(15),39(15)     |                                      |                        |
| 12 | 8.03     | 69(99),55(93),98(68),42(68),56(65)     |                                      |                        |
| 13 | 8.39     | 81(99),82(26),53(16),138(14),39(7)     |                                      |                        |
| 14 | 8.48     | 57(99),86(40),71(33),55(26),56(17)     | Urinous, sour, animal               | 30                     |
| 15 | 8.59     | 67(99),54(90),82(90),41(70),81(65)     | Chemical, cardboard, medicinal, wheat |                        |
| 16 | 9.20     | 94(99),67(85),66(20),95(6),68(4)       |                                      |                        |
| 17 | 9.84     | 41(99),54(68),27(59),55(54)            |                                      |                        |
| 18 | 10.64    | 128(99),113(50),99(23),85(13),129(8)   | Herbaceous, dirt, nutty, earthy     | 80                     |
| 19 | 10.79    | 58(99),135(59),91(49),134(40),196(6)   |                                      |                        |
| 20 | 11.80    | 122(99),121(82),42(74),39(33),67(23)   |                                      |                        |
| 21 | 12.21    | 73(99),83(26),126(16),111(10),127(1)   | Herbaceous, musty, grassy, earthy, dirt | 100                |
| 22 | 12.71    | 97(99),154(21),98(21),45(5),99(7)      | Herbaceous                          |                        |
| 23 | 13.36    | 58(99),43(73),71(27),59(24),57(14)     | Herbaceous                          | 30                     |
| 24 | 13.46    | 43(99),41(99),57(79),55(55),44(54)     |                                      |                        |
| 25 | 14.03    | 77(99),106(95),105(95),70(76),202(1)   |                                      |                        |
| 26 | 14.19    | 83(99),55(73),98(34),139(16),140(2)    | Citrus, lemon, fruity               | 80                     |
| 27 | 14.24    | 55(99),83(88),43(87),29(48),98(46)     |                                      |                        |
| 28 | 14.71    | 95(99),81(43),124(24),79(20),55(15)    |                                      |                        |
| 29 | 14.79    | 43(99),56(78),41(61),29(57),57(50)     |                                      |                        |
| 30 | 14.99    | 58(99),41(5),59(4),43(3),42(3)         | Herbaceous, cucumber                | 60                     |
| 31 | 15.46    |                                      |                                      |                        |
| 32 | 15.61    | 73(99),58(79),74(5),59(3),60(1)        | Foul, burnt                         | 15                     |
| 33 | 16.15    | 43(99),55(99),41(94),56(82),69(73)     | Herbaceous, potato, nutty           | 30                     |
| 34 | 16.25    | 59(99),31(42),41(42),27(18),29(18)     |                                      |                        |
Supplementary Table S2 continued

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 34 | 17.29 | 43(99),73(33),55(21),41(20),44(20) | Cardboard, green pepper, herbaceous, plastic |
| 35 | 17.70 | 55(99),41(97),43(84),69(62),57(58) |
| 36 | 18.22 | 58(99),41(5),43(4),59(4),42(3) |
| 37 | 18.86 | 132(99),133(84),118(21),117(17),130(12) | Medicinal, grassy, herbaceous |
| 38 | 19.28 | 71(99),43(74),56(55),27(54),89(52) |
| 39 | 20.01 | 57(99),41(69),43(58),55(52),67(42) |
| 40 | 20.68 | 55(99),69(82),57(75),83(71),56(67) | Waxy, butter |
| 41 | 21.62 | 96(99),95(88),39(56),38(14),29(14) | Medicinal, chemical |
| 42 | 22.06 | 55(99),69(79),56(69),57(68),83(66) |
| 43 | 22.14 | 43(99),41(83),55(68),67(48),84(45) |
| 44 | 22.74 | 30(99),99(80),42(78),41(72),43(69) |
| 45 | 24.51 | 192(99),91(24),165(22),119(16),65(15) |
| 46 | 24.73 | 83(99),82(28),153(25),55(19),156(19) |
| 47 | 25.75 | 149(99),177(21),76(14),65(12),150(12) | Citrus, lemon |
| 48 | 26.27 | 135(99),107(38),164(12),136(10),95(10) |
| 49 | 26.64 | 117(99),90(25),89(11),118(94),116(58) |
| 50 | 26.96 | 105(99),77(65),182(48),51(23),181(80) |
| 51 | 28.47 | 170(99),169(60),141(24),115(15),171(13) |
| 52 | 28.93 | 60(99),44(72),17(70),43(26),16(14) |
| 53 | 29.08 | 95(99),67(76),152(54),96(53),55(47) |
| 54 | 29.64 | 114(99),91(53),65(15) |

Abbreviations: No-Number; RT-Retention Time

**Compounds in bold is the characteristic compound 3-methyl cyclopentanone

**Supplementary Table S2.** Summary of all unconfirmed peaks in the chromatogram of *P. leo* MF. Compounds were listed by identifying markers: the top five ions, odor descriptors observed by panelist, and retention time.
| No | RT (min) | Compound Name | CAS | R. Match (%) | Match (%) | Top 5 Ions and Their Relative Intensities | Odor Descriptors Observed by Panelists | Measured Odor Intensity | Published Odor Descriptors | Published Odor Detection Threshold (ppb) | Surrogate Odor Activity Value (PA/ODT) | Andersen and Vulpius (2009) | Cited relevance to behavior |
|----|---------|---------------|-----|--------------|-----------|------------------------------------------|---------------------------------------|----------------------|-------------------------------|------------------------------------------|----------------------------------|---------------------------------|----------------------------------|
| 1  | 1.48    | Trimethylamine | 75-50-3 | 99           | 99        | 58(99), 59(68), 30(32), 42(25), 28(10)  | Foul, fishy, rancid                    | 100                  | Fishy, oily, rancid, sweaty | 3.70-16.00E-01d                        | 1.10E+07                        | X                              |                    |
| 2  | 1.70    | Acetaldehyde  | 75-07-0 | 85           | 82        | 44(99), 43(57), 42(17), 41(7), 40(2)    | Pungent, chemical, ethereal, and musty | 100                  | Pungent, ethereal, fresh, lifting, penetrating, fruity and musty | 1.50-12.00E+01d                        | 1.08E+04                        | Locomotion, Taste aversion, Anxiety |                    |
| 3  | 2.01    | Acetone      | 67-64-1 | 89           | 81        | 43(99), 58(64), 15(23), 42(9), 27(6)    | Solvent, ethereal, apple, pear         | 5.00E+05d            | 5.74E-01                        | X                              | Rattus rattus, Homo sapiens, Mus musculus |                  |
| 4  | 2.56    | 2-Butanone   | 78-93-3 | 94           | 82        | 43(99), 72(25), 29(17), 27(8), 57(8)    | Etherial, diffusive and slightly fruity with a camphoraceous nuance | 5.00E+04d            | 5.67E+01                        | X                              | Sexuality                       | Panthera leo |                  |
| 5  | 3.50    | 2-Pentanone  | 107-87-9 | 81           | 81        | 43(99), 29(22), 27(11), 57(19), 86(15)  | Sweet, fruity, ethereal, wine, banana, woody | 7.00E+04d            | 9.12E-01                        | X                              | Concentration                  | Odocoileus virginianus |                  |
| 6  | 4.78    | 3-Hexanone   | 589-38-8 | 90           | 65        | 43(99), 57(83), 71(51), 29(51), 27(36)  | Sweet, fruity, waxy, rum, grape         | X                              |                                |                                |                                  |                    |
| # | Value | Compound | Odor | Ratios | Bioactivity | Species |
|---|-------|----------|------|--------|-------------|---------|
| 7 | 5.18  | Dimethyl disulfide<sup>a</sup>,<sup>b</sup> | Foul, rotten, vegetable | 94(99), 79(57), 45(48), 46(25), 47(19) | 60 | 1.60-120.00E-01<sup>d</sup> | Oviposition inhibition, Attraction, Sniffing |
|   |       |          |      |        |             | Anopheles coluzzii<sup>12</sup>, Carolia perspicillata<sup>13</sup>, Rattus rattus<sup>14</sup>, Delia radicum<sup>15</sup>, Glossophaga sonicina<sup>16</sup> |
| 8 | 5.36  | 3-Methylbutanal<sup>a</sup>,<sup>b</sup> | Ethereal, aldehydic, chocolate, peach, fatty | 44(99), 43(93), 41(90), 58(81), 29(46) | 87 | 2.50-3.00E+02<sup>d</sup> | 8.40E+02 | X | Attraction |
|   |       |          |      |        |             | Harmonia axyridis<sup>16</sup> |
| 9 | 6.36  | 3-Penten-2-one<sup>a</sup>,<sup>b</sup> | Fruity, acetone, phenolic, fishy | 69(99), 41(96), 43(59), 39(55), 84(27) | 91 | 1.53E+00<sup>d</sup> | 1.83E+05 |
| 10 | 8.59 | 3-Methylcyclopentanone | Urinous, sour, animal | 67(99), 54(90), 82(90), 41(70), 81(65) | 84 | 3.00E+00<sup>d</sup> | 1.67E+04 | X | Aggregation, Inhibited behavior, Excitation |
|   |       |          |      |        |             | Locusta migratoria manilensis<sup>18</sup>, Culicoides nubeculosus<sup>19</sup>, Agrotis ipsilon<sup>20</sup> |
| 11 | 8.78 | Heptanal<sup>a</sup>,<sup>b</sup> | Fresh, aldehydic, fatty, green, herbal, wine-lee ozone<sup>b</sup> | 44(99), 43(79), 70(75), 41(60), 55(52) | 85 | 3.00E+00<sup>d</sup> | 1.67E+04 | X |

**Supplementary Table S3 Cont’d**
| No. | Value | Compound     | Molar Vol | Odor Description | pEBA | pEBA 100% | Stimulus Description | Species | References |
|-----|-------|--------------|-----------|------------------|------|-----------|----------------------|---------|------------|
| 12  | 9.49  | Cyclohexanone a,ᶲ | 108-94-1  | 82               | 86   | 55(99),42(75),98(45),41(37),39(36) | Minty, acetoneb | 1.20E+02  | 2.80E+03 | X | Attraction, Locomotion, Stimulation, inhibition | *Mus musculus* a,  *Hyphantria cunea* b, *Steinernema feltiae* b, *Steinernema carpocapsa* b, *Steinernema kraussei* b, *Heterorhabditis bacteriophora* b |
| 13  | 9.56  | Octanal a,ᶲ | 124-13-0  | 81               | 77   | 43(99),29(90),41(90),44(74),57(65) | Aldehydic, waxy, citrus, orange peel, green, fattyb | 7.00E+01d | 1.00E+04 | X | Immobility | *Mus musculus* a |
| 14  | 10.39 | 2,5-Dimethyl pyrazine a,ᶲ | 123-32-0  | 97               | 91   | 108(99),42(99),39(42),40(29),81(16) | Nutty, potato, cocoa, earthy, taco shell, animal, | 8.00-18.00E+02 | 3.47E+02 | | Fear, Freezing, Aggression | *Mus musculus* a, *Locusta migratoria manilensis* a |

*Notes:*
- pEBA: Potency of eliciting Behavioral Activity
- pEBA 100%: Potency at 100% of the stimulus
- Stimulus Description: Behavioral response of the species
- Species: Host species to which the stimulus is applied
- References: Additional species and references for the behavioral responses.
| No | Concentration | Compound | p-value | Sensory Descriptions | Sex Attraction |
|---|---|---|---|---|---|
| 15 | 11.49 | 2-Nonanone<sup>a</sup> | 821-55-6 | 94 | 43(99), 58(91), 41(29), 71(22), 57(22) | Fresh, sweet, green, weedy, earthy, herbal<sup>b</sup> | 0.05E-2.00E+02<sup>a</sup> | 1.05E+04 | Sex attraction | Leptonycteris curasoae<sup>5,6</sup>, Rattus norvegicus<sup>6</sup>, Aegorhinus superciliosus<sup>7</sup>, Dendrosoter protuberans<sup>8</sup>, Cheiropachus quadrum<sup>9</sup>, Ahasverus advena<sup>9</sup> |
| 16 | 11.58 | Nonanal<sup>a</sup> | 124-19-6 | 94 | 57(99), 41(96), 43(82), 29(74), 44(69) | Waxy, aldehydic, rose, fresh,orris, orange peel, fatty, peely<sup>b</sup> | 2.00E-02<sup>a</sup> | 4.66E+07 | X | Sexual attraction | Lycaenides argyrognomen<sup>2</sup>, Gravid culex quinquefasciatus<sup>3</sup>, Sitotroga cerealella<sup>2</sup>, Ephestia cautella<sup>2</sup>, Plodia interpunctella<sup>3</sup>, Galleria mellonella<sup>4</sup>, Theraphosa spinipes<sup>5</sup> |
| No. | Value | Compound | Purity | Retention Time | Odor | Concentration | Taxonomy |
|-----|-------|-----------|--------|----------------|------|---------------|----------|
| 17  | 12.98 | Acetic acid<sup>a</sup> | 3-13-<br>7 | 96 | 81 | 59(99),43(9<br>3),31(92),60<br>(46),29(37) | Sharp, pungent; sour, vinegar<sup>b</sup> | 6.00E+00<sup>a</sup> | 3.62+04 | Oestrous, Estrus, Attraction, Flight | **Bos Taurus**<sup>36,37</sup>, **Vespa maculifrons**<sup>38</sup>, **Drosophila melanogaster**<sup>39</sup> |
| 18  | 13.94 | Benzaldehyde<sup>a</sup> | 100-<br>52-7 | 97 | 95 | 77(99),105(92),106(90),51(51),50(32) | Strong, sharp; sweet, bitter; almond, cherry<sup>b</sup> | 3.50E+02-<br>3.50E+03<sup>d</sup> | 3.59E+03 | Oviposition, Defensive, Aggression, Alarm recruitment | **Veromessor andreii**<sup>40</sup>; **Scaptotrigona aff. Depilis**<sup>41</sup>, **Nearctic messor**<sup>42</sup>, **Bombyx mori**<sup>43</sup> |
| 19  | 14.46 | Linalool<sup>a</sup> | 78-<br>70-6 | 86 | 70 | 41(99),43(9<br>9),71(90),55<br>(64),52(64) | Citrus, grassy, green, herbaceous | Citrus, orange, floral, terpy, waxy, lavender rose<sup>b,e</sup> | 6.3E+01<sup>d</sup> | 1.60E+03 | Alarm recruitment, Attraction | **Vespa maculifrons**<sup>38</sup>, **Bombyx mori**<sup>43</sup>, **Colletes cunicularius**<sup>44</sup>, **Corythucha cydoniae**<sup>45</sup>, **Mus musculus**<sup>46</sup> |
| 20  | 14.52 | 1-Octanol<sup>a</sup> | 111-<br>87-5 | 91 | 72 | 43(99),56(9<br>7),41(93),55<br>(89),29(63) | Waxy, green, orange, aldehydic, rose, mushroom<sup>1</sup> | 1.10E+02-<br>1.30E+02<sup>d</sup> | 3.03E+03 | X | Foraging, Alarm recruitment, Sensory Perception | **Microplitis croceipes**<sup>47</sup>, **Apis dorsata**<sup>48</sup> |
| 21  | 15.71 | Butyrolactone<sup>a</sup> | 96-<br>48-0 | 88 | 82 | 42(99),28(7<br>5),41(58),29<br>(47),27(41) | Creamy, oily, fatty, caramel<sup>b</sup> | | | | **Appetite, Vomiting, and Temor Suppression, Estrus** | **Papio anubis**<sup>49</sup>, **Sus scrofa**<sup>50</sup>, **Bos Taurus**<sup>50</sup> |
| 22 | 16.01 Acetophenone\textsuperscript{a,k} | 98-86-2 | 97 | 88 | 105(99),77(86),51(41),130(33),43(21) | Sweet, pungent, hawthorn, mimosa, almond, acacia, chemical\textsuperscript{b} | 6.5E+01\textsuperscript{d} | 6.75E+03 | Anti-attraction, Attraction, Responsiveness Dendroctonus frontalis\textsuperscript{51}, Microplitis croceipes\textsuperscript{59}, Mus musculus\textsuperscript{53}, Dendroctonus brevicomis leConté\textsuperscript{54} |

| 23 | 16.83 Dodecanal\textsuperscript{a,t} | 112-54-9 | 91 | 90 | 41(99),57(99),55(82),43(76),82(63) | Plastic, waxy | 30 | Soapy, waxy, aldehydic, citrus, green, floral\textsuperscript{b} | 2E+00\textsuperscript{d} | 7.68E+04 | Physiological Responses Culex quinquefasciatus\textsuperscript{55} |

| 24 | 19.94 Phenylethyl alcohol\textsuperscript{a,s} | 60-12-8 | 83 | 67 | 91(99),92(56),65(22),12(22),39(12) | Floral, rose, dried rose, flower, rose water\textsuperscript{b} | 1.70E+01\textsuperscript{h} | 1.21E+04 |

| 25 | 21.29 Phenol\textsuperscript{a,t} | 108-95-2 | 96 | 95 | 94(99),66(39),65(27),39(24),40(12) | Phenolic, plastic, rubber\textsuperscript{b} | 5.90E+03\textsuperscript{a} | 1.27E+03 | X Estrus, Oestrus, Sexuality Idea leucoe\textsuperscript{6}, Bos Taurus\textsuperscript{57}, Mamestra brassicae\textsuperscript{57}, Bubalus bubalis\textsuperscript{58} |

| 26 | 22.32 4-Methyl phenol\textsuperscript{a,s} | 106-44-5 | 92 | 92 | 107(99),108(82),77(23),27(20),39(19) | Waxy, herbaceous, butter, sour, animal, barnyard, urinous | 60 | Phenolic, narcissus, animal, medicinal, mimosa\textsuperscript{a,s} | 5.50E+01\textsuperscript{d} | 1.28E+05 | Sexuality, Estrus, Oestrus, Diestrus, Sexual attraction Bubalus bubalis\textsuperscript{59}, Alces alces\textsuperscript{50}, Glossina spp\textsuperscript{61,63}, Stomoxys calcitrans\textsuperscript{64}, Equus Callabus\textsuperscript{56,66}, Bison bison bison\textsuperscript{57} |

| 27 | 22.74 2-Piperidinone\textsuperscript{a,t} | 675-20-7 | 85 | 75 | 30(99),99(80),42(78),41(72),43(69) | | | | | |

**Supplementary Table S3 Cont’d**
Supplementary Table S3 Cont’d

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28  | 26.64 | Indole$^a$ | 120-72.9 | 95 | 82 | 117(99), 90(25), 89(11), 18(94), 116(58) | Animal, floral, moth ball, fecal$^b$ | 1.40E+04$^d$ | 2.95E+01 | Sexuality, Age differentiat | Mus musculus$^{68,69}$ |

*Abbreviations: No-Number; R. Match-Reverse Match; RT-Retention Time; CAS-Chemical Abstract Service Number
**Compounds in bold are characteristic compounds and are displaying percentage matches from SHC-Cryo mode
'Odor descriptors observed by panelists do not match the published odor descriptors for this compound
$^c$ Compound does not have published odor descriptors, but odor associated with this compounds was detected by panelists
$^f$ No odors were detected by panelists, but odor descriptors have been published for this compound
$^‡$ No odors were detected by panelists and no odor descriptors have been published for this compound
$^a$ Odor descriptors observed by panelists match the published odor descriptors for this compound
$^b$ Compounds verified with the retention time and ion confirmation match of standards
$^c$ Good Scents Company$^{70}$
$^d$ Flavornet$^{71}$
$^e$ Leffingwell$^{72}$
$^f$ Indoor Air Quality Engineering: Environmental Health and Control of Indoor Pollutants$^{73}$
$^g$ Detection thresholds for phenyl ethyl alcohol using serial dilutions in different solvents$^{74}$
$^h$ Measurement of Odor Threshold by Triangle Odor Bag Method$^{75}$

**Supplementary Table S3.** Total VOC composition of *P. leo* marking fluid and its relationship to behavior
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Supplementary Figure S1. Lion Marking Fluid. Marking fluid and urine mixture released unto the floor of the indoor enclosure by a male in a squatting downward position. The urine appeared to be yellow in color and the marking fluid had a whitish coloring.
| Fiber Type                                      | Size (diameter x length) | Target Analyte Description                                      |
|------------------------------------------------|--------------------------|-----------------------------------------------------------------|
| **50/30 µm Divinylbenzene/Carboxen/ Polydimethylsiloxane** | 23 gauge x 2 cm          | Broad range of analytes; Flavor compounds; Volatiles and Semi-volatiles; C3-C20 (MW 40-275) |
| 50/30 µm Divinylbenzene/Carboxen/ Polydimethylsiloxane     | 24 gauge x 1 cm          | Broad range of analytes; Flavor compounds; Volatiles and Semi-volatiles, C3-C20 (MW 40-275) |
| 65 µm Polydimethylsiloxane/ Divinylbenzene                | 24 gauge x 1 cm          | Volatiles; Amines; Nitro-aromatic compounds (MW 50-300)         |
| 75 µm Carboxen/ Polydimethylsiloxane                     | 24 gauge x 1 cm          | Volatile/ low molar mass analytes; Biogenic volatile organic compounds (MW 30-225) |

**Fiber type selected for the rest of the study**

**Supplementary Table S4.** SPME fiber type selection. Fiber types tested for extraction efficiency of characteristic *P. leo* scent marking odor compounds.