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LIPID COMPONENTS IN ANAL SCENT SACS OF THREE MONGOOSE SPECIES (*Helogale parvula*, *Crossarchus obscurus*, *Suricata suricatta*)

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Abstract—Anal sac secretions of three social mongoose species (*Helogale parvula*, *Crossarchus obscurus*, and *Suricata suricatta*) were chemically analyzed by means of gas chromatography–mass spectrometry. Compounds with high molecular weights (greater than 250) were found to distinguish the three species. Differences in composition suggest a possible species-specific role in scent marking. Male *H. parvula* had vitamin E present in the anal sac, whereas it was absent in the females. This suggests a possible sex-specific function of vitamin E in *H. parvula*. No differences in chemical composition were found between male and female *S. suricatta*. Ten compounds were found in female *C. obscurus* that were not present in the male.

Key Words—Mongoose, *Helogale parvula*, *Crossarchus obscurus*, *Suricata suricatta*, anal gland, lipids.

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INTRODUCTION

Mongooses are small to medium-sized carnivores that have a diverse array of social structures, from a solitary existence as in the small Indian mongoose, to a highly cooperative social structure as in the dwarf mongoose. Pocock (1916) related the size of mongoose scent sacs with the particular social structure they possess: gregarious mongooses have larger scent glands than more solitary mongooses, suggesting that more social species mark more frequently than more solitary species. Although this hypothesis has not been tested, it merits investigation. Pocock (1916) further described the morphology of the scent sacs of mongooses as distinctly different than other carnivores. There are several depressions and folds in the glands, which contain small glandular pits with several secreting pores. Secretions from these glands are thick waxy brown foul-smelling substances (Decker, personal observation).

Mongooses have very characteristic and stereotypical postures of scent marking (Gorman et al., 1974). Behavior patterns vary with the type of object being marked, especially with regard to its height above the ground (Rasa, 1973). Preferred objects for marking by dwarf mongooses are smooth, upright, horizontal, or sloping branches (Rasa, 1973). Such objects are marked from a "hand-stand" position (Gorman et al., 1974). Anal drag is a posture in which the animal strokes its anal surface across a very low or flat object. Rasa (1973) reports the anal drag to be used exclusively in allomarking. In the "leg lift" posture, the animal lifts its leg toward a low object. Again, this is most commonly used in allomarking (Rasa, 1973) but occasionally is used to mark low-lying objects such as the food dish.

Several functions of scent marking have been proposed (for reviews see Ralls, 1971; Eisenberg and Kleiman, 1972; Johnson, 1973; MacDonald, 1979). Conover and Gittleman (1989) have classified these potential functions into six general categories: (1) identity—information regarding individual, group, or sexual identification (Rasa, 1973; Gorman, 1976); (2) status—information on dominance status (MacDonald, 1979; Erlinge et al., 1982); (3) reproductive status—related to courtship and breeding (Gorman, 1980; Gorman and Trowbridge, 1989); (4) spatial information—serves a territorial function (Peters and Mech, 1975; Kruuk, 1978); (5) temporal information—reveals when individuals were at a particular location (Rasa, 1973); and (6) foraging—serves a bookkeeping function by informing an individual of whether it has previously looked for food in a particular area (Henry, 1977; MacDonald, 1979).

The objectives of this study were to establish methods of collection and analysis of mongoose secretions, to identify potential biologically active compounds present in the scent secretion of three species of mongooses, and to propose a potential function of scent marking in these mongooses.

METHODS AND MATERIALS

Materials. Solvents were chromatographic grade quality purchased from J.T. Baker Chemicals Co., Phillipsburg, New Jersey. Derivatizing agents were purchased from Supelco, Inc. (Bellefonte, Pennsylvania), Applied Science (State College, Pennsylvania), Aldrich, Inc. (Milwaukee, Wisconsin), Sigma Chemical Co. (St. Louis, Missouri), and Pierce Chemical Co. (Rockford, Illinois).

Collection. Anal gland secretions were collected from three species of social mongooses (*Helogale parvula*—two males, two females; *Crossarchus obscurus*—one male, two females; and *Suricata suricatta*—one male, one female) from the National Zoo, Washington, D.C., by swabbing the anal sacs with sterile cotton swabs. All chemical analyses were conducted at the Center for Environmental Biotechnology, University of Tennessee.

Extraction. The secretion was immediately extracted with a modified Bligh and Dyer solvent system (White et al., 1979), which consists of chloroform, methanol, and a phosphate buffer in a ratio of 1:2:0.8. The sample was left in the solvent system for a minimum of 4 hr. Subsequently, equal amounts of water and chloroform were added to the samples separating them into aqueous and organic phases. The aqueous layer was discarded, and the organic (lipid containing) phase was transferred to Teflon-lined screw-capped test tubes and dried under a stream of nitrogen at room temperature.

Lipid Isolation. Silicic acid columns were prepared using 0.5 g Unisil (100–200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pennsylvania), activated at 100°C for 60 min and preextracted in a minimal volume of chloroform. Total lipid was applied to the top of the columns in a minimal volume of chloroform. Sequential washes of 5 ml of chloroform, acetone, and methanol eluted nonpolar, glyco-, and phospholipids. Each fraction was dried under a stream of nitrogen.

Mild Alkaline Methanolysis. The mild alkaline methanolysis procedure (White et al., 1979) was utilized to prepare methyl esters of the ester-linked fatty acids of each lipid fraction.

Gas Chromatography (GC). Dry methyl esters of nonpolar, glyco-, and phospholipids were dissolved in isooctane containing the internal standard of methyl nonadecanoate. Samples of 1 μl were injected onto a 50-m nonpolar, cross-linked methyl silicone fused silica capillary column (0.2 mm ID, Hewlett Packard) in a Shimadzu GC-9A GC. A 30-sec splitless injection at 270°C was used. Hydrogen at a linear velocity of 35 cm/sec was the carrier gas with a temperature program starting with an initial temperature of 100°C. The temperature was then increased at a rate of 10°C/min to 150°C. At 150°C the temperature was increased 3°C/5 min to 282°C. An equal detector response was assumed for all components. Peak areas were quantified with a program-

mable laboratory data system (Nelson Analytical 3000 Series Chromatography Data System, Revision 3.6).

Gas Chromatography–Mass Spectrometry (GC-MS). Tentative component identification prior to GC-MS was based on comparison of the retention times with authentic standards. GC-MS analysis was performed on a Hewlett Packard 5996A GC-MS fitted with a direct capillary inlet utilizing the same chromatographic system except for use of a helium carrier gas and the temperature program, which started at 100°C and increased to 280°C at 3°C/min for a total analysis time of 60 min. The electron multiplier voltage was between 1800 and 2000 V, the transfer line maintained at 300°C, the source 280°C, and analyzer 250°C, and the GC-MS was autotuned with DFTPP (decafluorotriphenylphosphine) at *m/z* 502 with an ionization energy of 70 eV. The data were acquired using the Hewlett Packard 6/VM data system. Compounds were identified using the GC-MS library, which provided an 80% or greater probability of being correct for all compounds.

Nonpolar Lipid Fractionation. Preliminary analyses suggested the importance of the heavier compounds in distinguishing the species. Therefore, to achieve greater separation of those compounds with high molecular weights (greater than 250), the nonpolar lipid portion was subsequently fractionated. In this procedure, silicic acid columns were prepared using 1.0 g Unisil (100–200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pennsylvania) activated at 100°C for 60 min and preextracted with chloroform. Sequential washes of 10 ml of hexane, hexane–diethyl ether (90:10), and hexane–diethyl ether (80:20) eluted the compounds with smaller molecular weights (<250) so that better separation of the larger compounds (molecular weights >250) could be achieved. Ten microliters of BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide] were added to the fraction containing compounds with high molecular weights (>250). These samples were subsequently analyzed on the GC-MS. The temperature program initially began at 50°C for 1 min. The temperature was then raised 30°C/min until it reached 150°C. Subsequently, the temperature was raised 2°C/min to 250°C, then 5°C/min to 300°C with a final time of 30 min.

Statistical Analyses. A phenogram was generated from a similarity matrix using the Numerical Systems Program (Rohlf et al., 1982). Principal component analysis was performed to summarize variation among taxa.

RESULTS AND DISCUSSION

The Bligh-Dyer extraction was very effective in revealing a larger spectrum of compounds than previous analyses of carnivore scent secretions (Gorman et al., 1978; Davies et al., 1988; Apps et al., 1988, 1989). The primary difference between this study and previous studies is the choice of solvent systems used

to extract the compounds. Previous analyses have used *n*-hexane as the solvent, and this is only effective in revealing the straight-chain hydrocarbons, whereas the Bligh-Dyer system is effective in revealing more complex compounds.

An unweighted paired group cluster analysis showed differences among species in only the nonpolar lipid fraction (Figure 1). *Helogale parvula* males and females grouped together. Similar patterns were also detected in *C. obscurus* and *S. suricatta*. Separation of individuals within a single species was not apparent with the glyco- and phospholipid portions of the samples, and are therefore not reported here.

Although the sample sizes in this study are too small to assess significant differences in the chemical composition of the anal sacs between sexes and among species, we feel that it is worthwhile to present these differences as a descriptive measure.

Intraspecific Comparison of Helogale parvula. Sixty-six compounds were revealed in the neutral lipid fraction of *H. parvula* (Appendix 1). Other studies of carnivore scent secretions have revealed between 10 and 30 compounds (e.g., Gorman et al., 1978; Davies et al., 1988; Apps et al., 1989). This may suggest the complexity of scent marking in this species.

Eleven additional compounds were found in the dominant male *H. parvula*, which were not found in the subordinate male or females (see Appendix 1). Although Rasa (1973) has reported no differences in frequency of marking between dominant and subordinate males, the additional chemicals found in the dominant male suggests that the scent secretions of the dominant male may serve an additional or different function.

Vitamin E was present in the anal sac of male *H. parvula* and was absent in the female *H. parvula*. This compound may be sex specific in *H. parvula*.

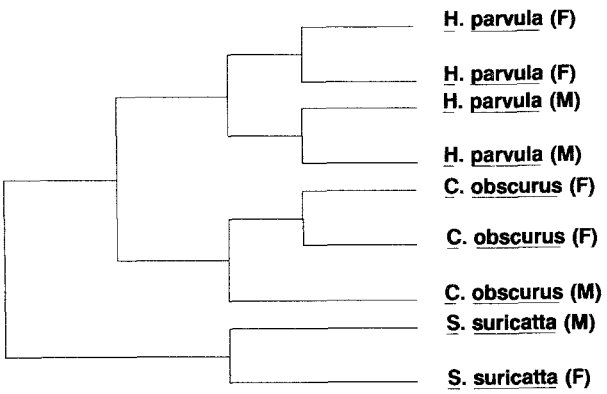

FIG. 1. A phenogram illustrating the similarity in nonpolar lipids among *Helogale parvula*, *Crossarchus obscurus*, and *Suricata suricatta*. M = males; F = female.

Although many studies (e.g., Silverstein et al., 1966) have shown that a combination of several chemicals are necessary for biological activity, some studies of carnivores have identified single compounds to be unique to the males (e.g., Apps et al., 1989). This may suggest that single compounds can play an important sex-specific role in some cases. A detailed study of the dwarf mongoose by Rasa (1973) has shown that they traverse their home range in 25–30 days. These mongooses were able to detect scent secretions from themselves or other mongooses for up to 30 days after the scent was deposited (Rasa, 1973). Rasa suggests that the primary function of anal marking, as animals traverse their home range, is to provide information on individual identity and to "date" the mark. Rasa also found that another mongoose can detect differences in intensity of a mark up to 1 hr after it has been deposited.

Intraspecific Comparison of Crossarchus obscurus. Eleven additional compounds were found in relatively large quantities in *C. obscurus* females, which were not found in *C. obscurus* males (Figure 2). Very little is known about the behavior and ecology of *C. obscurus* (for a review see Goldman, 1987). However, these results may suggest that the females rely more heavily on scent marking than do the males.

Intraspecific Comparison of Suricata suricatta. Few qualitative differences were found between the male and female meerkat (Figure 3). This may suggest

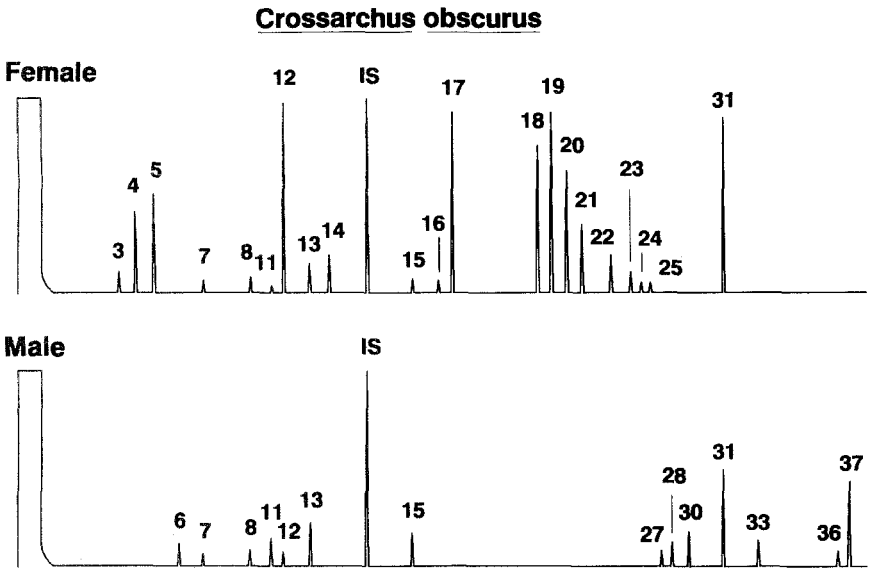

FIG. 2. Chromatogram of male and female *Crossarchus obscurus*. Numbers above peaks correspond to those in Appendix 1, where compounds and relative quantities of each are given. IS = the internal standard of methyl nonadecanoate.

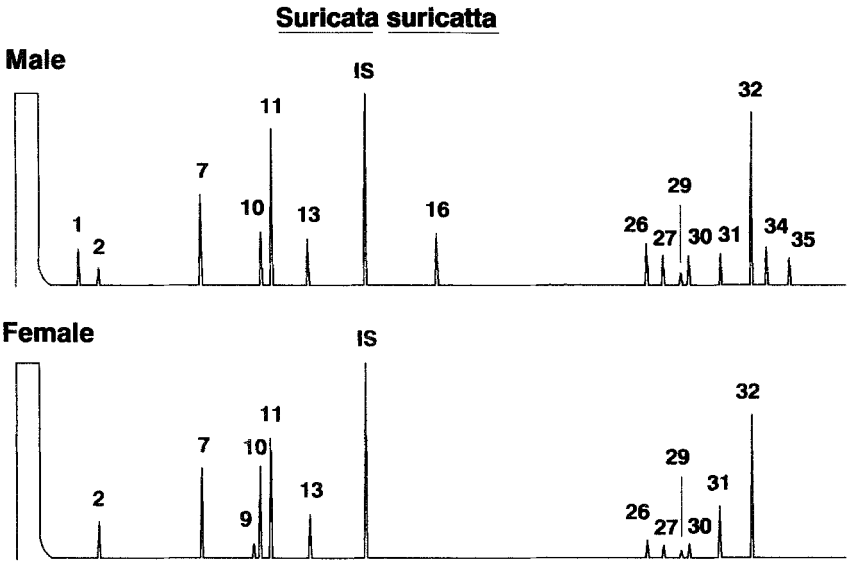

FIG. 3. Chromatogram of male and female *Suricata suricatta*. Numbers above peaks correspond to those in Appendix 1, where compounds and relative quantities of each are given. IS = the internal standard of methyl nonadecanoate.

that the function of scent marking in male and female meerkats does not differ. Additionally, Moran and Sorensen (1986) found no marked differences in the frequency of marking between male and female meerkats. Furthermore, large individual differences in scent marking were not tied consistently to sex or age.

Interspecific Comparison. Principal component analysis summarized 85% of the variation in the first two components (Figure 4). Figure 4 reveals that males and females of the same species are more similar to each other in the chemical composition of their scent sacs than with individuals of different species. Additionally, the species are clearly different from each other. If the "loadings" are overlayed onto the "scores" in Figure 4, we can see which compounds are best able to maximally separate the species. For example, cholesterol (denoted as sterol a) distinguishes the meerkats, the polybranched trienoic acid (denoted as 2464a) distinguishes the kusimanse, and a variety of compounds (18:0, sterol b, 713a, and 713d) distinguish the dwarf mongooses. The compounds that distinguish the species are relatively heavy compounds (molecular weights greater than 250) and, therefore, suggest a function that promotes long-term signaling of the scent mark. Female *C. obscurus* had a unique profile of compounds that were not present in the male (Figure 2). Few differences were found between male and female *H. parvula* or between male and female *S. suricatta* (see Figure 3 and Appendix 1).

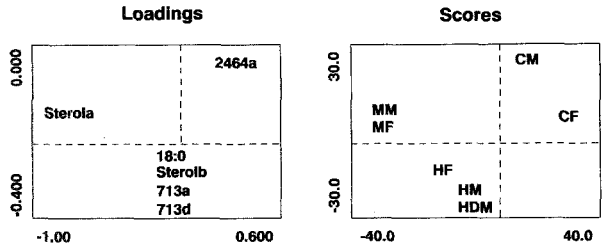

FIG. 4. Results of principal component analysis. "Loadings" represent those compounds which were maximally able to separate the species. "Scores" represent the relative separation of the species. CM = *Crossarchus obscurus* male; CF = *Crossarchus obscurus* female; MM = *Suricata suricatta* male; MF = *Suricata suricatta* female; HM = *Helogale parvula* male; HDM = *Helogale parvula* dominant male; HF = *Helogale parvula* female. 2464a = an unknown polybranched trienoic acid; sterol a = cholesterol; 18:0 = an 18-carbon saturated carboxylic acid; sterol b = cholestanol; 713a = pentadecanol; 713d = heptadecanol.

Three previous studies have chemically analyzed the anal sac secretions of mongooses. Gorman et al. (1974) found a series of short-chained (C_1–C_{10}) saturated carboxylic acids in the small Indian mongoose (*Herpestes auropunctatus*). Similar compounds were found in the yellow mongoose, *Cynictis penicillata*, by Apps et al. (1989). Hefetz et al. (1984) found heavier compounds, C_{10}–C_{22}, in the Egyptian mongoose, *Herpestes ichneumon*. Our results are most similar to those of Hefetz et al. (1984). Differences in results, to some extent, may be due to differences in collection regimes and analysis of secretions.

The short-chained (C_1–C_{10}) highly volatile fatty acids have been found to be present in the scent secretions of most carnivores analyzed (e.g., Gorman et al., 1974; Albone and Perry, 1976; Preti et al., 1976). These compounds are relatively simple and do not have a large number of possible structural isomers as opposed to more complex molecules (i.e., those with larger molecular weights). Since many animals have short-chain fatty acids in their scent secretions, it seems unlikely that these serve a unique communicative function. The short-chain fatty acids probably function in directing the animal to the scent, while the heavier compounds are actually the communicative part of the scent. The fact that animals actually go up to the scent to smell it (Decker, personal observation), lends some support to this argument.

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APPENDIX 1. RELATIVE QUANTITIES (PICOMOLES) FOR NONPOLAR LIPID FRACTION OF FATTY ACIDS PRESENT IN ANAL SACS OF DWARF MONGOOSE (*Helogale parvula*), MEERKAT (*Suricata suricatta*), AND KUSIMANSE (*Crossarchus obscurus*)[a]

#	Compound	(MW)	HDM	HM	HF	CM	CF	SM	SF
1	unknown	(?)	0	0	0	0	0	1,844	0
2	br pentadecanol	(229+)	28,399	35,924	5,113	0	0	717	1,771
3	unknown	(?)	0	0	0	0	1,335	0	0
4	br 2Me13:0	(214+)	7,929	2,740	3,528	0	6,378	0	0
5	br 2Me13:0	(214+)	4,960	5,203	217	0	620	0	0
6	16:1w9c	(256)	2,080	2,551	245	1,750	0	0	0
7	16:0	(258)	45,133	32,221	8,029	3,576	3,545	1,321	1,295
8	17:0	(272)	3,362	2,773	741	293	1,045	0	0
9	br 1Me18:0	(285+)	3,884	6,668	1,299	0	0	0	934
10	br heptadecanol	(258+)	8,483	8,487	3,153	0	0	2,257	1,687
11	i18:0	(300)	28,936	19,774	6,055	1,475	251	6,332	4,220
12	18:1w9c	(284)	3,647	3,809	0	398	10,747	0	0
13	18:0	(286)	10,009	8,897	2,568	2,862	1,157	2,653	1,958
14	unknown	(?)	0	0	0	0	1,478	0	0
15	20:0	(314)	1,634	1,340	617	2,024	1,175	0	0
16	unknown	(?)	0	0	0	0	789	2,962	0
17	?-methyl, ?-hydroxy benzene	(108+)	0	0	0	0	22,600	0	0
18	1,1-phenyl 1 propene	(194)	0	0	0	0	41,177	0	0
19	br benzene	(78+)	0	0	0	0	24,287	0	0
20	br benzene	(78+)	0	0	0	0	3,655	0	0
21	?-phenyl pentane	(148)	0	0	0	0	2,161	0	0
22	?-phenyl pentane	(148)	0	0	0	0	719	0	0

APPENDIX 1. CONTINUED

#	Compound	(MW)	HDM	HM	HF	CM	CF	SM	SF
23	?-phenyl pentane	(148)	0	0	0	0	817	0	0
24	?-phenyl pentane	(148)	0	0	0	0	1,027	0	0
25	?-phenyl pentane	(148)	0	0	0	0	4,826	0	0
26	unknown	(?)	7,365	3,563	1,806	0	0	1,367	859
27	26:1	(364)	7,776	23,380	8,203	161	0	1,098	254
28	26:1	(364)	0	0	0	846	0	0	0
29	26:1	(364)	5,490	10,697	3,036	0	0	329	61
30	26:1	(364)	28,456	76,044	24,779	469	0	2,713	409
31	polybr trienoic acid	(125+)	2,242	6,086	4,358	7,762	17,292	1,367	3,504
32	Cholesterol	(386)	406,422	529,061	133,481	0	0	4,599	3,964
33	21 dihydroxy desmosterol	(402)	10,927	9,969	2,733	261	0	0	0
34	28:1	(392)	0	0	0	0	0	1,967	0
35	28:1	(392)	0	0	507	0	0	540	0
36	29:1	(406)	0	0	0	335	0	0	0
37	29:1	(406)	0	0	0	3,881	0	0	0
	12:0	(202)	7,135	4,497	924	0	0	0	0
	br 2Me13:0	(214+)	3,319	2,547	0	0	0	0	0
	br 2Me13:0	(214+)	802	3,844	0	0	0	0	0
	br 2Me13:0	(214+)	4,346	6,974	375	0	0	0	0
	br 2Me13:0	(214+)	8,656	6,974	375	0	0	0	0
	br 13:0	(215+)	3,587	2,091	0	0	0	0	0
	a13:0	(230)	433	2,091	461	0	0	0	0

br 2 Me14:0	(229+)	1,394	2,164	133	0	0	0	0
br 2 Me14:0	(229+)	2,664	4,936	799	0	0	0	0
i14:0	(244)	38,104	19,456	3,551	0	0	0	0
br 14:0	(230+)	5,559	5,932	2,378	0	0	0	0
2,9 dimethyl 14:0	(258)	34,045	25,434	0	0	0	0	0
14:0	(230)	7,831	3,776	1,030	0	0	0	0
15:0	(244)	3,815	3,257	625	0	0	0	0
a15:0	(258)	38,657	17,101	2,746	0	0	0	0
pentadecanol	(231)	112,620	81,677	13,981	0	0	0	0
i16:0	(272)	123,541	57,501	0	0	0	0	0
br16:1	(255+)	10,637	2,749	559	0	0	0	0
16:1w7c	(256)	233	10,392	323	0	0	0	0
hexadecanol	(244)	50,274	33,985	5,629	0	0	0	0
br hexadecanol	(244+)	2,487	3,677	0	0	0	0	0
br hexadecanol	(244+)	6,576	3,740	0	0	0	0	0
br hexadecanol	(244+)	3,193	2,101	0	0	0	0	0
br 17:0	(271+)	643	4,032	1,071	0	0	0	0
br 17:0	(271+)	2,736	3,421	1,082	0	0	0	0
a17:0	(286)	20,683	9,018	2,321	0	0	0	0
heptadecanol	(258)	578,134	47,940	13,322	0	0	0	0
18:2	(282)	18,401	8,997	2,178	0	0	0	0
18:1w7c	(284)	1,359	9,785	935	0	0	0	0
octadecanol	(270)	9,233	4,389	1,315	0	0	0	0
a19:0	(302)	2,628	0	276	0	0	0	0
nonadecanol	(326)	18,355	6,194	2,160	0	0	0	0
22:0	(342)	2,561	0	605	0	0	0	0
26:1	(364)	7,849	0	0	0	0	0	0
28:1	(424)	2,562	1,950	0	0	0	0	0
28:1	(424)	2,967	1,468	0	0	0	0	0
28:1	(424)	918	0	607	0	0	0	0
28:1	(392)	0	0	1,256	0	0	0	0
Cholestanol	(250+)	98,573	18,442	1,442	0	0	0	0
Vitamin E	(379)	8,782	5,495	0	0	0	0	0

APPENDIX 1. CONTINUED

#	Compound	(MW)	HDM	HM	HF	CM	CF	SM	SF
	Unknown	(?)	4,599	0	0	0	0	0	0
	Unknown	(?)	2,815	0	0	0	0	0	0
	Unknown	(?)	233	0	0	0	0	0	0
	Unknown	(?)	3,044	0	0	0	0	0	0
	Unknown	(?)	2,960	0	0	0	0	0	0
	Unknown	(?)	2,032	0	0	0	0	0	0
	Unknown	(?)	2,860	0	0	0	0	0	0
	Unknown	(?)	5,824	0	0	0	0	0	0
	Unknown	(?)	701	0	0	0	0	0	0
	Unknown	(?)	167	0	0	0	0	0	0
	Unknown	(?)	4,599	0	0	0	0	0	0
	Unknown	(?)	8,126	5,985	0	0	0	0	0
	Unknown	(?)	5,989	2,377	0	0	0	0	0
	Unknown	(?)	0	319	204	0	0	0	0
	Unknown	(?)	0	0	505	0	0	0	0
	Unknown alcohol	(17+)	5,950	4,123	0	0	0	0	0
	Unknown alcohol	(17+)	9,308	4,749	0	0	0	0	0
	Unknown alcohol	(17+)	7,321	5,049	0	0	0	0	0

[a] # = peak numbers corresponding to Figures 2 and 3. Those compounds without peak numbers are only present in *H. parvula* and are listed at the end of this appendix; MW = molecular weight; HDM = Dominant *H. parvula* male; HM = *H. parvula* male; HF = *H. parvula* female; CM = *C. obscurus* male; CF = *C. obscurus* female; SM = *S. suricatta* male; SF = *S. suricatta* female. Fatty acids are designated as a total number of carbon atoms : number of double bonds with the position of the double bond nearest the aliphatic (w) end of the molecule indicated. This is followed by the suffix c for *cis* and t for *trans* configuration of monoenoic acids. The prefixes i, a, or br indicate iso-, anteiso-, or branched (position undetermined). Mid-chain branching is indicated by the number of carbon atoms from the carboxyl end of the molecule and Me for the methyl group (10Me 16:0 is a 17-carbon fatty acid).

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