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Talking Defensively, a Dual Use for the Brachial Gland Exudate of Slow and Pygmy Lorises

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Introduction

On the ventral side of the elbow of the both the slow (*Nycticebus bengalensis*, *N. coucang*) and pygmy (*N. pygmaeus*) lorises, one can perceive a slightly raised, fairly hair-free but barely visible swelling, termed the brachial gland (Figure 12.1). Observers of captive lorises have found that when the animal is disturbed during capture and handling, the gland secretes about 10 microliters (μl) of a clear, strong-smelling liquid in the form of an apocrine sweat. Typically, male and female lorises assumed a defensive position with head bent downward between uplifted forelegs, like a miniature prize fighter in a clinch, while imparting gland exudate to the head and neck (Fitch-Snyder, 1996). The lorises frequently licked their own brachial gland regions, and also wiped these glands against their heads. The gland is active in lorises as young as 6 weeks (Fitch-Snyder, unpubl. data).

Early observers of the loris concluded that the gland contains a form of toxin, basing this conclusion primarily on reports from individuals on the receiving end of painful, slow-healing bites. Lorises have a specialized needle-like, oral tooth comb used for grooming, and the close association with this comb and the licking of the gland made it a natural assumption to visualize the comb as a device for injecting brachial gland derived toxic secretions. Although the design of advanced venom delivery architecture (injecting poison hypodermically through a sharp-pointed tube) has evolved numerous times in vertebrates (i.e., gila monsters, stingrays, stonefish, snakes), offensive and defensive toxins are seldom found in mammals. The platypus and water shrew are the mammals most well documented in the use of this strategy. Thus, the loris would be a singular example of a primate that uses a toxin, has a specialized device for injecting it, and that loads its “sting” in a secondary manner by retrieving the toxin from a part of the body not associated with the toothcomb. Specialized teeth on the lower jaw of the loris have been shown to be effective in conducting liquid upward (Alterman, 1995).

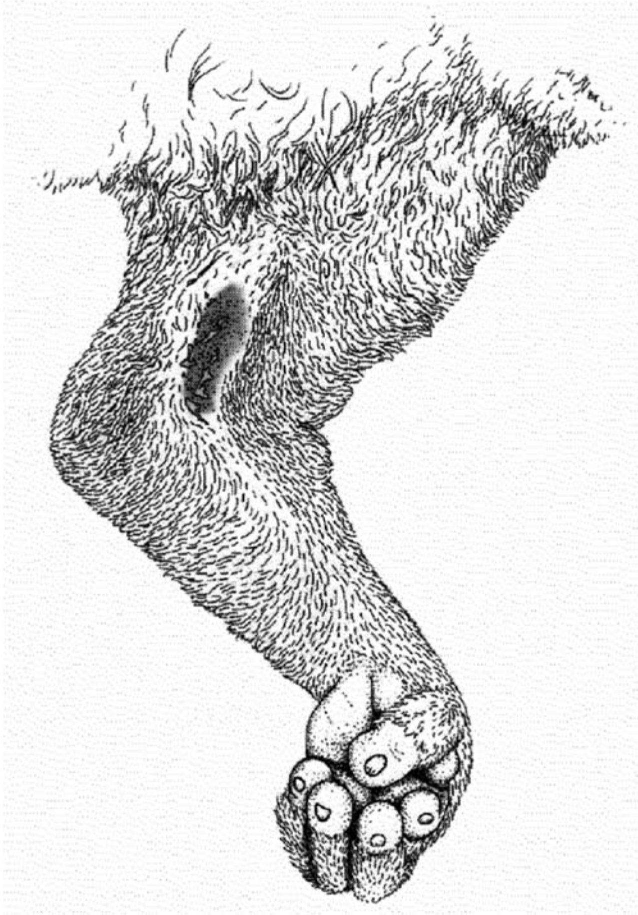

FIGURE 12.1. A drawing of the brachial gland by Helga Schulze

While lorises are predatory as well as frugivorous, their prey consists of insects and vertebrates so small relative to lorises that no venom should be needed to kill or immobilize these prey prior to being ingested by lorises. Little is known about predation of lorises and whether their bite is used as a defensive weapon against predators. They are unable to fend off predators with their bites, and they have been captured and killed by orangutans (Utami & van Hooff, 1997), snakes (Wiens & Zitzmann, 1999), and hawk-eagles (*Spizaetus cirrhatus*) (C. van Schaik, 2005). In contrast, the bites they inflict on social enclosure mates are common and severe. These wounds show a large affected area with a loss of fur, prolonged edema, are slow-healing, and often are life-threatening (Rasmussen, 1986).

The toxin hypothesis has lead to a number of attempts to characterize the protein contents of the gland, beginning with Alterman & Hale (1991), Alterman (1995),

and, most recently, Krane et al. (2003). Recognizing that lorises are a prosimian species whose biology is not fully understood, we re-examined different components of exudate from both the pygmy and slow lorises (including both the volatile low molecular weight metabolite and non-volatile higher molecular weight protein fractions) and present two hypotheses for the dual use of the gland. In addition to being a defensive toxin reservoir, the strong-smelling glandular secretion displays all the components necessary for it to play an important functional role in olfactory communication.

Methods

Brachial gland samples were obtained from a colony of male and female slow lorises (*Nycticebus bengalensis*) (Groves, 1998) and pygmy lorises (*Nycticebus pygmaeus*) housed at the San Diego Zoo, and *N. bengalensis*, *N. coucang coucang*, and *N. pygmaeus* housed at the Singapore Zoo. Samples (from four individuals of each species) were obtained from fluid collected on the surface of the gland using capillary action in 2-μl micropipettes, and stored in glass vials at −20°C until analysis.

Volatile and semi-volatile compounds were extracted (35 min) from the vapor surrounding the micropipette using a solvent-free solid phase matrix extractor (SPME) containing a 65–μm polydimethylsiloxane fiber (Supelco, Bellefonte, PA). Fiber contents were analyzed by capillary GC-MS, with a Hewlett-Packard 5890 Gas Chromatograph-5970 MSD, controlled by HP/UX Chem Station software. The GC used a Supelco 60- m 0.25- mm ID, low-polarity SPB-octyl column (Supelco, Bellefonte, PA) operated isothermically at 75°C for 9 min followed by a 1.6°/min ramp gradient up to 210°C. A splitless injection was used with an injection temperature of 250°C. Helium was used as the carrier gas with a 7-psi column head pressure. Relative retention times and fragmentation spectra of peaks obtained by GC-MS were matched with those of known standards for identification. The remaining, scientifically unnamed compounds were used for analysis because they possessed a reproducible retention time and mass fragmentation pattern and could be classified according to chemical function. These compounds were assigned a number based on their column retention time.

Samples (5 μl) of exudate oil were diluted in methanol, centrifuged, and examined for non-volatile polar compounds with a Perkin-Elmer Sciex API-III instrument (Alberta, Canada) modified with a nanoESI source from Protana A/S. Medium-sized palladium-coated, borosilicate glass capillaries from Protana A/S were used for sample delivery. The instrument was operated in the negative (–) mode with ISV voltage set to 600 V, IN voltage set to 110 V and the ORI voltage set to 90 V. A curtain gas of ultra pure nitrogen was pumped into the interface at a rate of 0.6 L/min to aid evaporation of solvent droplets and to prevent particulate matter from entering the analyzer region.

On-line LC-MS of brachial gland samples dissolved in 0.1% TFA to a concentration of approximately 1 mg/ml were performed on a Phenomenex Jupiter

C-18 reverse phase column (150 × 1.0 mm, 5 μ, 300 Å); a gradient solvent A (0.1% TFA) and solvent B (90% acetonitrile in 0.1% TFA) were used at a flow rate of 50 μl/min. The gradient formation of a 1% gradient from 0–90% acetonitrile/0.1% TFA over 90 min was achieved using an Applied Biosystems 140B solvent delivery system. Electrospray mass spectra were acquired on a PE-SCIEX API 300 LC/MS/MS system with an Ionspray atmospheric pressure ionization source. Samples (25 μl) were injected manually into the LC-MS system and analyzed in the positive ion mode. Full scan data were acquired at an ionspray voltage of 4600 V and the orifice potential was set at 30 V over an ion range of 600–3000 m/z with a step size of 0.2 amu. Data processing was performed with the aid of the software package Biomultiview (PE-SCIEX, Canada).

Brachial gland fluid in the capillary was reconstituted with 10 μl of water by leaving the capillary side down in water. A portion of the reconstituted sample was added to 10 μl loading buffer, boiled for 5 min, and loaded onto an 18% tris-glycine gel (Novex cat. no EC6505). The gel was run for 60 min (running buffer tris 1.52 g, glycine 7.2 g, SDS 0.5 g, water 500 ml), stained (trichloroacetic acid 10 g, sulfosalicylic acid 10 g, coomassie blue 0.2 g, water 80 ml) for 120 min, and destained (methanol 10%, acetic acid 7.5%) overnight. A second portion was reduced prior to loading on the 18% tris-glycine gel.

After separation on the gel the α- (lower band) and β- (upper band) peptides were eluted from the gel and the amino terminal ends sequenced. Edman sequencing was perfomed on an Applied Biosystems PROCISE 494HT Protein Sequenator, using the Division of Biological Sciences Protein Sequencing Facility on the University of California, San Diego campus, Matthew Williamson, facility operator.

We analyzed 76 protein sequences obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein&itool=toolbar). We used the program CLUSTAL-X (Thompson et al., 1997) to align the sequences, followed by visual inspection of the resultant alignment for errors. The final alignment consisted of 159 amino acid sites. A copy of the full sequence alignment can be obtained by emailing Dr. Bryan Grieg Fry at bgf@unimelb.edu.au. Phylogenetic trees were reconstructed using the maximum parsimony (MP) and neighbor-joining (NJ) (Saitou & Nei, 1987) methods. MP heuristic searches were conducted by implementing random stepwise taxon addition with TBR branch swapping and the PROTPARS weighting scheme (Felsenstein, 2001), which takes into account the number of changes required at the nucleotide level to substitute one amino acid for another. NJ searches were conducted using amino acid p distances, as the simple p distance generally gives better results in phylogenetic inference than more complicated distance measures for minimum evolution methods such as NJ (Takahashi & Nei, 2000). Statistical reliability was assessed using 100 and 1000 bootstrap replications for MP and NJ searches, respectively. In order to simplify sequence nomenclature and to minimize confusion, we refer to proteins by their NCBI accession number in the text and figures.

Results

When examined by GC-MS, brachial gland exudates contained a complex mixture of volatile and semi-volatile compounds. A representative profile for the *N. pygmaeus*is shown in Figure 12.2. A total of 212 different compounds were observed in two individual pygmy loris samples. The results from these were pooled and are listed in Table 12.1. Also shown in Table 12.1 is the proportion (averaged) of the compound present. SPME matrix peaks and other artifacts are not listed in Table 12.1. Not all compounds were definitively identified by name, but based on fragmentation pattern and GC column retention time could still be recognized as unique compounds. In Table 12.1, these compounds are referred to as numbers, the numerical value reflecting each individual compound's relative retention time on the SPB-35 GC column. Minor amounts of a wide variety of aromatic compounds were identified, consistent with dietary absorption from a frugivorous species, and a concurrent difficulty in complete metabolism of this chemical class of compounds. The remaining identified compounds were a series of C_4– C_7 aldehydes, ketones, and acetates.

The compounds present in the brachial gland exudate from a *N. bengalensis* are listed in Table 12.2. There were 68 different compounds in the profile, about half of which were found in the pygmy loris using an identical method of analysis. A comparison of Tables 12.1 and 12.2 indicate that 33 of the 68 *N. bengalensis* compounds (48%) were unique to this species and not detected in the larger table of data from the pygmy loris. A disproportionately large signal of m-cresol is characteristic of slow loris scent, which, when mixed with other compounds, may contribute to the reported strong odor from this gland. It does not appear as if the dominant component of the exudate oil itself is a low molecular weight

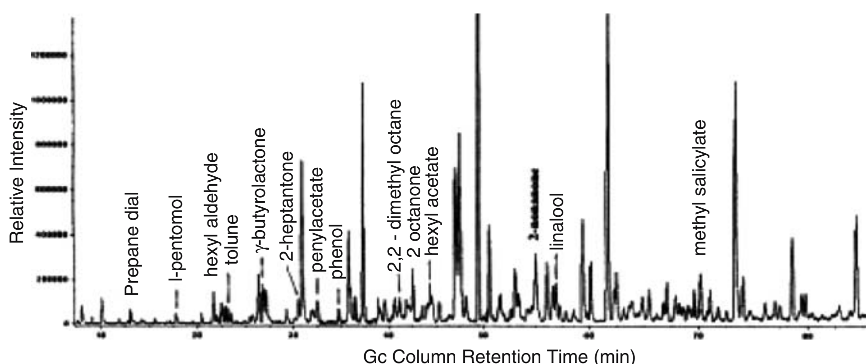

FIGURE 12.2. GC-MS profile of the volatile and semi-volatile components of pygmy loris (*N. pygmaeus*) brachial gland exudate. Peaks are expressed according to relative abundance. The x-axis is the time of elution from the GC column and the y-axis is the relative intensity of each peak

TABLE 12.1. Volatile and semi-volatile compounds found in pygmy loris (*N.pygmaeus*) brachial gland exudate as determined by GC-MS. Compounds are identified by relative area, followed by either the name (if known) or the relative retention time (if the identity has not yet been confirmed).

Name or Identification No.	Relative Amount
methanol	50
3,4-dimethyl heptane	1
0.952	5
methyl salicylate	103
ethanol	22
m-xylene	2
0.960	7
1.248	13
acetic acid	23
0.643	2
0.963	1
1.256	26
n-butane	21
phenol	75
0.966	36
1.261	96
isoprene	15
p-xylene	22
p-cymene	5
1.272	26
chloroform	3
0.678	35
0.973	8
1-dodecene	2
1-butanol	10
0.684	250
0.974	9
1.286	26
0.255	1
0.687	11
acetophenone	3
1.303	60
0.260	110
0.691	65
0.983	1
1.307	36
2-methylbutyral-dehyde	3
styrene	36
0.986	9
1.317	20
1,2-propanediol	32
o-xylene	2
0.989	7
1.325	6

TABLE 12.1. (Continued).

Name or Identification No.	Relative Amount
n-valeraldehyde	27
0.724	12
0.992	7
1.339	49
butyric acid	5
0.727	10
0.993	3
1.348	53
0.292	8
0.737	20
3-nonanone	25
1.354	1
methyl butyrate	124
0.742	7
2-nonanone	44
1.358	9
0.312	14
1-heptanol	35
limonene	12
1.361	1
0.318	12
0.747	37
1.002	20
1.363	24
1-heptene	4
0.755	22
1.013	13
1.365	24
0.342	5
0.761	29
1.016	12
1.378	247
0.346	6
benzaldehyde	21
benzoic acid	10
1.389	9
1-pentanol	23
0.762	40
1.025	25
1.400	75
0.372	1
0.767	15
1.030	2
1.411	12
0.375	14
6-Me-5-hepten-2-one	6
linalool	13
1.424	17
isovaleric acid	1
0.771	4
1.027	14

TABLE 12.1. (Continued).

Name or Identification No.	Relative Amount
1.432	9
0.392	2
2,2-dimethyl octane	62
nonyl aldehyde	35
1.437	10
0.397	32
0.774	13
1.047	8
1.447	43
3-hexanone	26
0.777	6
1.056	7
1.465	36
ethyl butyric acid	2
0.782	66
1.060	2
1.485	2
hexyl aldehyde	72
0.785	43
1.071	220
1.499	36
n-valeric acid	45
0.790	15
1.078	21
1.501	20
toluene	580
2-octanone	103
1.082	148
1.504	32
“anti” 2-methyl butyraldehyde oxime	49
3-octanone	34
1.107	117
1.509	2
0.806	10
resorcinol	3
1.511	24
“syn” 2-methyl butyraldehyde oxime	27
0.808	14
n-undecane	91
1.519	2
0.810	31
1.122	146
1.528	52
0.472	1
0.817	31
1.126	19
1.537	26
1-octene	25
n-propyl benzene	18
1.127	4
dodecyl aldehyde	5

TABLE 12.1. (Continued).

Name or Identification No.	Relative Amount
0.499	14
hexyl acetate	78
1.133	30
0.502	20
0.829	35
1.139	8
0.510	17
octyl aldehyde	23
1.140	35
butyrolactone	98
0.828	1
1.146	51
0.525	42
0.838	43
1.153	12
1-hexanol	81
0.858	3
1.158	45
isopentyl acetate	15
p-cresol	5
1.162	63
4-heptanone	23
0.871	50
1.170	93
0.561	4
0.879	36
1.171	1
0.580	17
m-cresol	22
1.183	1
2-heptanone	26
0.887	16
1.190	1
0.592	26
ethyl hexanoate	13
1.191	53
0.593a	445
0.918	1
1.197	17
0.593b	600
0.926	71
2-decanone	123
0.605	7
0.928	14
1.210	74
2-heptanol	26
0.932	4
1.216	53
0.615	31
0.936	1
1.221	41

TABLE 12.1. (Continued).

Name or Identification No.	Relative Amount
pentyl acetate	32
0.941	8
1.226	38
0.618	11
1-octanol	3
n-decyl aldehyde	23
0.622	25
0.945	8
1.233	22
hexanoic acid	39
0.946	3
1.237	94

TABLE 12.2. Volatile and semi-volatile compounds found in slow loris (*N. bengalensis*) brachial gland exudate as determined by GC-MS. Compounds are identified by relative area, followed by either the name (if known) or the relative retention time (if the identity has not yet been confirmed).

Name or Identification No.	Relative Amount
methanol	21
0.704	62
1.107	52
ethanol	18
1-heptanol	10
1.141	24
n-butane	4
0.760	6
1.158	9
2-butanol	22
benzaldehyde	16
1.191	3
acetic acid	102
6-Me hepten-2-one	16
1.230	15
0.228	7
0.777	3
1.237	19
2-methyl butyraldehyde	4
3-octanone	3
1.278	13
2-methyl butyronitrile	99
0.787	69
1.297	88
0.314	20
0.822	4

TABLE 12.2. (Continued).

Name or Identification No.	Relative Amount
1.378	34
0.329	944
2-octanol	6
1.395	15
0.357	92
0.844	12
1.409	36
0.496	4
0.856	5
1.465	19
"anti" 2-methyl butyraldehyde oxime	109
0.871	865
1.472	46
0.885	3
1.496	4
"syn" 2-methyl butyraldehyde oxime	30
m-cresol	1503
1.504	13
0.906	26
1.532	4
0.514	187
0.922	9
1.538	2
1-hexanol	23
0.929	29
1.556	5
0.583a	24
0.986	14
dodecyl aldehyde	4
0.583b	12
1.002	5
1.591	3
2-heptanol	9
1.027	97
0.617	58
1.039	30
phenol	86
1.052	117
0.691	25
1.056	4
0.693	29
1.081	24

hydrocarbon, for such a compound should have dominated the volatile profiles in Tables 12.1 and 12.2.

To examine the exudate oil contents by a different approach, samples from both loris species were examined by nanoESI-MS. Although this instrument will not detect neutral molecules, it is sensitive to charge-bearing compounds of a higher

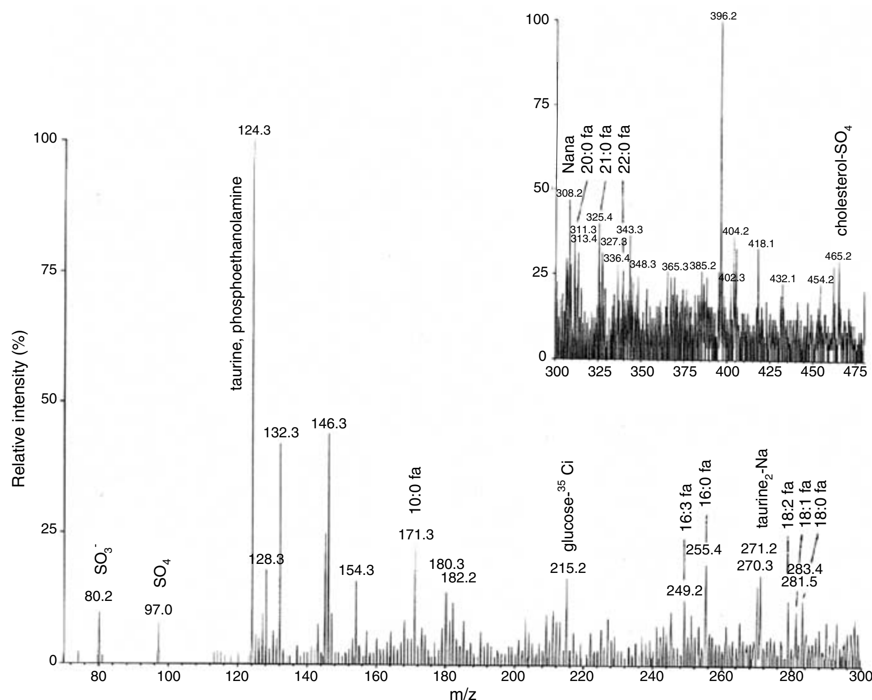

FIGURE 12.3. Negative mode nanoESI-MS profile of pygmy loris (*N. pygmaeus*) exudate. Tentative identifications are listed above the mass per charge value of the peaks. Abbreviations: fa: fatty acids, NANA: N-acetylneuraminic acid

molecular weight than that detectable by GC-MS. The negative (–) mode profile of pygmy loris exudate is shown in Figure 12.3 and tentative identifications of the peaks are labelled. Although the sugars glucose, neuraminic acid, and a variety of fatty acids (fa) were detected, none were present in amounts sufficient to constitute the exudate oil itself. Easily seen by this instrument, but notably absent from the profile were phospholipids.

To detect the presence of higher molecular weight compounds (proteins), SDS-PAGE gels were run on exudates from both species. The gels for both the *N. pygmaeus* and *N. bengalensis* lorises showed single large bands of approximate molecular weight 18,000 daltons. After reduction, the 18 kDa bands, run in identical gel systems, revealed a pair of bands of approximate molecular weight 7 kDa and 8 kDa, as shown in Figure 12.4 (pygmy loris right panel, slow loris left panel). Since the reduction was not 100% complete, some of the original 18 kDa protein can be seen in both gels. The 7 kDa and 8 kDa bands were isolated and used for amino acid sequencing.

LC-MS analysis of the brachial gland secretion from both species also revealed that each contained a single dominant protein component with a molecular weight 17.6 kDa, as shown in Figure 12.5. The pygmy loris contained two isoforms

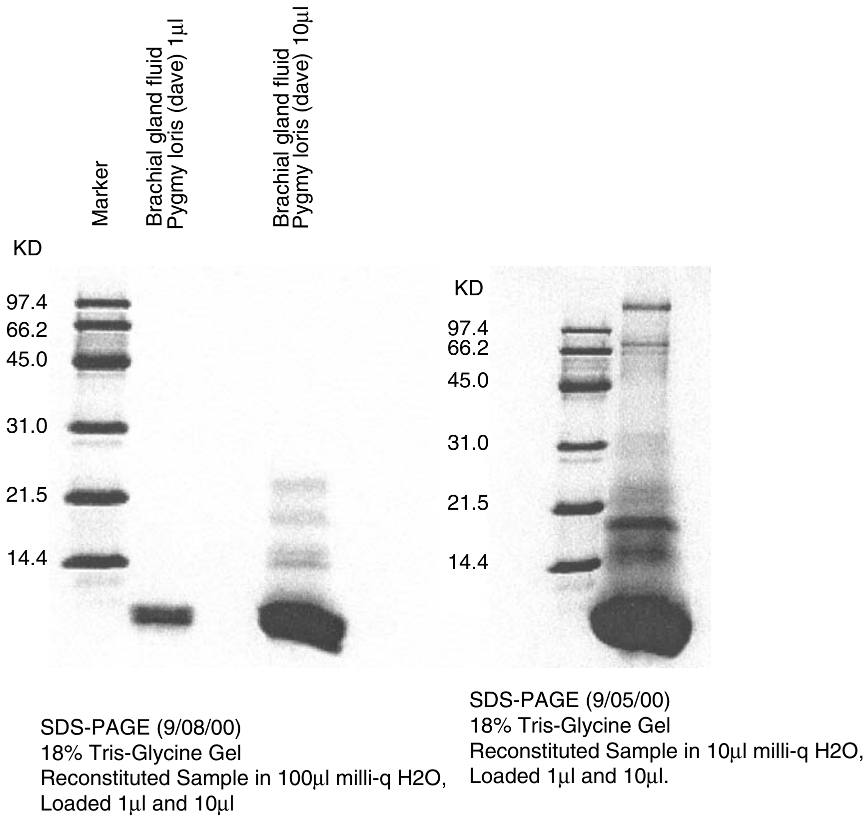

FIGURE 12.4. SDS-PAGE gels of the protein component of *N. pygmaeus* (left panel) and *N. bengalensis* (right panel) loris brachial gland exudate. After reduction with TCEP, a pair of bands of approximate molecular weight α 7.9 kDa and β 9.8 kDa

(17671 and 17601 Da), as did both species (*N. bengalensis*, *N. coucang*) of slow loris (17649 and 17610 Da). Reduction of the disulfide bonds in the 17.6kDa peptide revealed that it was a heterodimer of two smaller peptides, molecular weights 7.8 kDa (α-chain) and 9.8 kDa (β-chain).

The amino terminal sequences of the two chains are shown in Figure 12.6. The initial 35 amino acids of the α-chain sequence reported by Krane et al. (2003) is essentially identical to our sequence, with the single exception in position six, where they found Leu, but we observed Val. At position Val-25 we see some chains with Ala-25 substituted for Val. Krane et al. (2003) also reported the sequence of the first 31 amino acids of the β-chain, which was identical to ours. They reported the presence of Cys in position-1 but we were unable to confirm this. Using the N-terminal sequences of the loris toxin, database searching (http://www.ncbi.nlm.nih.gov/BLAST/) revealed a number of proteins with a high degree of similarity (Figure 12.6). Further, genome mining

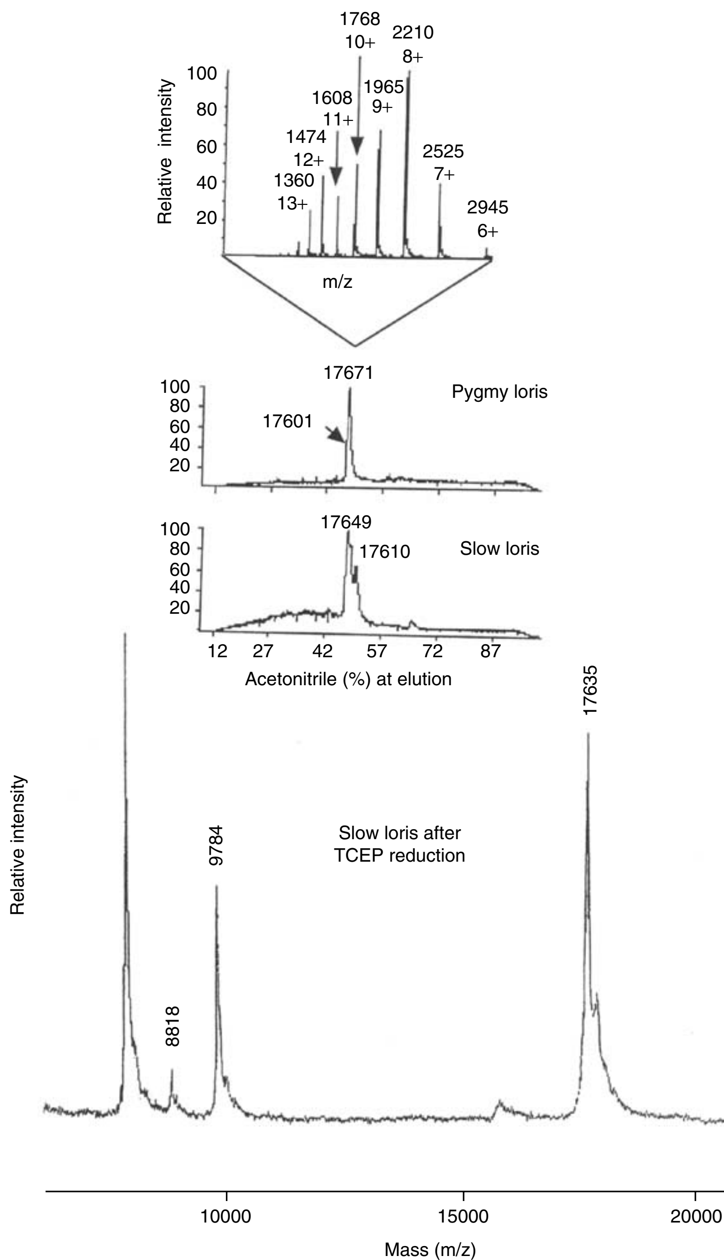

FIGURE 12.5. LC-MS analysis of *N. pygmaeus* and *N. coucang* loris brachial gland secretions. Reconstructed masses are given above each peak

(A)

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1.  SGCKLLEDMVEKTINSDISIPEYKELLQEF-IDSDAAAEAMGKFKQCFLNQSHRTLKNFGL
2.  GLCPALQRKVDLFLNGTT--EEYVQYLKQF-NENRDVLDNAENIKKCSDRTLTEEDKAQAT
3.  GICPAIKEDVCLFLNGTS--EEYVEYVKQY-KNDPEILENTEKIKQCVDSTLTEKDKAHAT
4.  AICPAVEKHANLFLKGTT--DEFLNNAKNF-VKSSEVLEN---------------------
5.  EICPAVKRDVDLFLTGTP--DEYVEQVAQY-NALPVVLENARILKNCVDAKMTEEDKENAL
6.  DICPVVTKDVDLFLVGTP--DEYVDHVAQY*TSS-LILSNARKLKNCFNGKLADEDKRHVL
7.  DICPGFLQVLEALLLGSE--SNYEAALKPF-NPASDLQNAGTQLKRLVD-TLPQETRINIV
8.  EFCPALVSELLDFFFISE--PLFKLSLAKF-DAPPEAVAAKLGVKRCTD-QMSLQKR----

1.  MMHTVYDSIWCN--MKSNNQSHRTLKNFGLMMHTVYDSIWCNMKSN-
2.  SLIR--------------TLTEEDKAQATSLI---------------
3.  AFILSLYMSG-------STLTEKDKAHATAFILSLYMSG--------
4.  -----------------------------------------------
5.  SVLDKIYTSPLC-----AKMTEEDKENALSVLDKIYTSPLC------
6.  S----------------GKLADEDKRHVLS-----------------
7.  KLTEKILTSPLCEQDLRVTLPQETRINIVKLTEKILTSPLCEQDLRV
8.  ------------------SLIAEVLVKILKKCSV-------------
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(B)

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1.  VKMAETCPI-FYD--VFFAVANGNELLLDLSLTKVNATEPERTAMKKIQDCYVENGLISRVLD
2. -------WPLPFSGPSLIG-LIT---NLLNSFSDK-SGISSWFGSITGELA*
3.  -------AP-FVG--AYVKILGGNRLALNAYLSMFQATAAERVAFEKIQDCFNEEPLTTKLKS

1.  GLVMIAIN------EYCMGEAVQNTVEDLKLNTLGR
2.
3.  PQIMMSILFSSECKAYYPEDSVNKMADMFKLDSIN-
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FIGURE 12.6. NH_2-terminal amino acid sequence comparison of the *N.pygmaeus* loris α- and β-chains that make up the 18 kDa major peptide of brachial gland exudate. (a) Comparison between the *N. pygmaeus* loris α-chain sequence and members from each clade of the α-chain superfamily: (1) Secretoglobin AAC79996; (2) Mouse salivary androgen binding protein AAM08259; (3) Mouse putative protein XP_142918; (4) Loris brachial gland secretion; (5) Domestic cat allergen Fel d1-A CAA44344; (6) Human genome putative protein (ensemble, http://www.ensembl.org, accession # AC020910.7.1.203201, location 112772-112948); (7) Uteroglobin NP_037183; and (8) Lipophilin NP_006542. (b) NH_2-terminal amino acid sequence comparison between the *N. pygmaeus* loris β-chain sequence and two members with similar β-chains. (1) Domestic cat allergen Fel d 1-B P30440; (2) Loris brachial gland secretion β-chain; and (3) Mouse salivary androgen binding protein beta AAH24677

(http://www.ensembl.org) revealed a match with an α-chainlike human gene, located on chromosome-19 (accession # AC020910.7.1.203201, location 112772-112948) and a highly similar chimpanzee match (database location AADA0-1344989, genomic location: chromosome 20, 36466963 to 36467142). Both the human and chimpanzee putative proteins have a stop codon in the middle of the sequence, and as a result may be degenerative pseudogenes. Phylogenetic reconstruction of the molecular evolutionary history revealed a strong association between the alpha-chain of the loris protein and the cat allergen alpha-chain as well as the human and chimpanzee genome sequence (Figure 12.7).

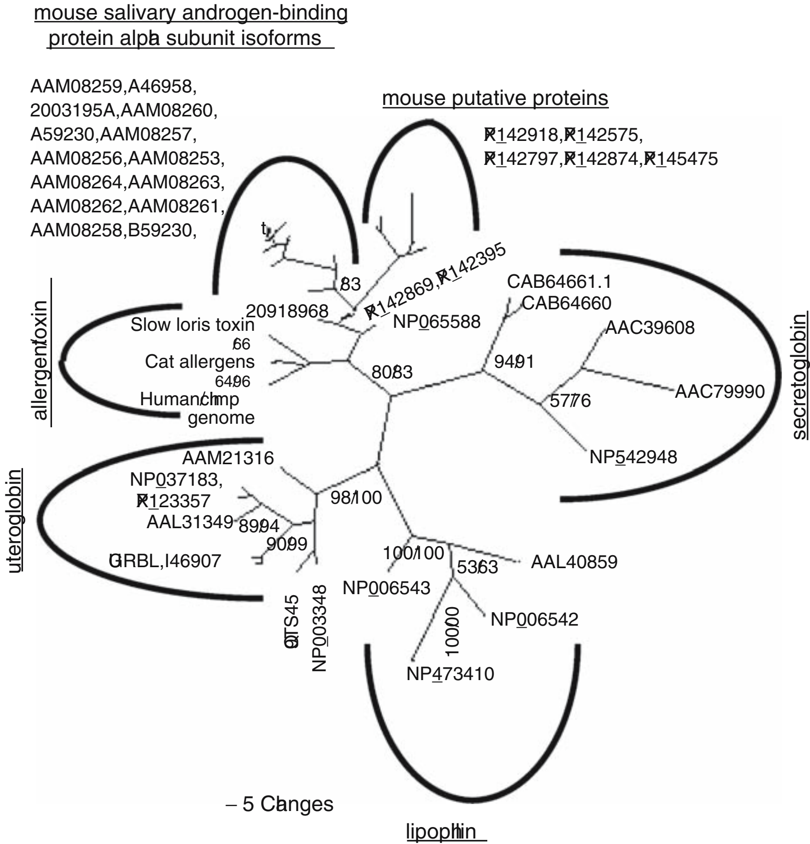

FIGURE 12.7. Phylogenetic analysis of proteins showing similarity to the *N. bengalensis* toxin α-chain. Shown is the maximum parsimony tree. Bootstrap values are the result of 100 replicates (maximum parsimony) or 1000 replicates (neighbor-joining). Accession numbers for representative sequences of the major clades are given (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein&itool=toolbar). Domestic cat allergen is Fel d1-A CAA44344, human genome is from ensemble, http://www.ensembl.org, accession # AC020910.7.1.203201, location 112772-112948 and chimpanzee genome is from ensemble, http://www.ensembl.org, accession # AADA01344989, genomic location: chromosome 20, 36466963 to 36467142

Discussion

Like other nocturnal prosimians occupying individual home ranges or territories with limited social contact, lorises are specialized in olfactory signalling. It has been described that lorises scent mark with copious quantities of urine and that the introduction of lorises to new environments is accompanied by vigorous urine

marking and sniffing behavior (Tenaza & Fitch, 1984). A second site of olfactory-mediated chemical communication is the brachial gland exudate. Once expressed, this secretion dries to amorphous, amber-colored crystals, and the hair on the loris head and neck can become encrusted with the solid. Results from the mass spectrometer (Tables 12.1 and 12.2) show that oil from each species is unique and complex, with more than 68 (*N. bengalensis*) and 200 (*N. pygmaeus*) volatile and semi-volatile components. The qualitative and quantitative differences seen in the scent profiles of the two loris species can code for extensive information, including sex, age, nutritional status, health, and dominance of the sender (Hagey & MacDonald, 2003). The likely recipient (and interpreter) of such a coded message is another loris, as social contact is a clearly recognized source of agitation in these species. Since 48% of the compounds found in slow lorises were not found in pygmy lorises, chemical analysis of the brachial scent may be a useful technique to resolve taxonomy issues among *Nycticebus*.

Brachial gland secretion is not an immediate response to stress or being chased. The characteristic odor is not noticeable to the researcher trying to catch the animal, and lorises only stop to lick this gland after the encounter is completely over (Fitch-Snyder, 1996). It thus seems unlikely that the loris would have time to mount a chemical defense to a stealth attacker. This behavior implies that the BGE functions either to deter a predator or to warn other lorises of the danger, or perhaps both.

Many animal scents show a strong component of bacterial commensalism dominated by bacterial metabolites, particularly low molecular weight short-chain acids (Albone, 1984). Both loris exudates had little or none of these bacterial metabolites, reflecting their origin as a fresh glandular secretion. When dried, metabolic acids form salts, and when embedded in a lipid matrix have fairly long-lasting odors. The short aldehydes, ketones, and aromatics seen in loris exudates (and lack of short-chain acids) are consistent with reports that fresh exudate emits a very distinctive volatile short-range odor that is rapidly lost to evaporation. To the olfaction of a recipient loris, the brachial scent must represent a powerful, but rapidly decaying, burst of detailed information.

The exudate oil in both species contained isoforms of a 17.6 k protein, which was composed of α/β-heterodimeric subunits (α-chain MW 7880, β-chain MW 9784) linked together by two disulfide bridges. Sequencing of the α/β -chains showed that the loris brachial gland peptide is a new member of the secretoglobin (uteroglobin/Clara cell 10 k) family. For a list of 40 peptide members in 11 species, see Klug, 2000). Based on the close sequence homology with domestic cat Fe-dI chain I peptide, loris peptide could be assigned to subfamily 4, using the nomenclature of Klug (2000). The sequences of seven of these family members are listed in Figure 12.6, including the human genome match. The secretoglobin family is characterized by small lipid-loving peptides found as major constituents in a variety of mammalian secretions. These proteins are all α/β-homo- and heterodimers stabilized by two or three intramolecular cystine disulfide bonds (Lehrer et al., 2000). In what is termed the uteroglobin-fold, the α- and β-monomers are formed from grouping four α-helices, and (for the two

monomers) the combined eight α-helix bundle folds to form a pocket for the binding of different hydrophobic molecules (Callebaut et al., 2000). This simple structural motif of the uteroglobin fold stands in sharp contrast to the wide array of biological activities assigned to this group of proteins.

In the loris 17.6 k protein, the smaller β-chain forms a slightly pyramidal shaped lid that is hinged along one edge by the two disulfide bridges to the larger α-chain, forming a unit roughly in the shape of a cigar box. The β-subunit has a shallow hydrophobic center in the lid, which sits over a similar but deeper pocket in the α-chain box, which may act as a molecular snare for a small hydrophobic molecule. A potential for molecular docking of hydrophobic molecules like progesterone, polychlorinated biphenyls, and retinol has been shown using the crystal structure of human uteroglobin (Pattabiraman et al., 2000). Other than the disulfide bridges located together in the "hinge," only these interacting hydrophobic regions hold the lid to the box. The behavior of the loris may add to an understanding of how the molecular snare functions. When the snare is in the hydrophobic environment of the secreted oil on the arm, the lid is free to open, and the box can accept a signaling molecule. One function for the box would be to hold a species-specific message, and the varying compositions of the α/β-chains in different species support this idea. In the case of the mouse salivary binding protein, the signaling molecule is an androgen (Karn & Russell, 1993).

In the loris, the message molecule enters the gland oil through an equilibrium state with blood serum. When the loris licks the gland, the external environment of the box is mixed with water and the lid closes, ensnaring the message molecule. These are not swallowed, but are deposited on the external fur to be conveniently dropped or rubbed off. For related molecules like lipophilins secreted in tears, it has been speculated that the protein could function as a snare to capture pheromones or other lipophilic molecules from the atmosphere, as the tear drains into the nasal cavity (via the nasolacrimal ducts) and conveys them to receptors for further sensory processing (Lehrer et al., 2000). Although the function of domestic cat allergen peptide is not known, cats heavily contaminate their environments with Fe-dl (Morgenstern et al., 1991), using the protein not as a toxic defense, but as a species recognition molecule. Rather than possessing a brachial gland like the loris, small cats directly add salivary allergen proteins to inanimate objects during cheek rubbing, as well as transferring them to their body fur with washing behaviors (Mellen, 1993). The idea that species recognition systems share a close relationship with immune recognition has been investigated by Palumbi & Metz (1991).

The key question here is why invest so much to put a single molecule in a snare. From a communications viewpoint there is not much information in a single molecule. It could easily report on the sex of the originator, but urine could serve the same purpose. Licking of the box will deliver the message to vomeronasal glands inside the oral cavity, and in this hydrophobic environment the box will reopen. Many members of the secretoglobin family are excreted in saliva and urine. The need for the loris to use a brachial gland as its vehicle for secretion may reflect either the need for added olfactory components to accompany the molecular snare

(the complex list of compounds in Tables 12.1 and 12.2) or a uniquely hydrophobic message for the box.

Although the secretoglobin family has been associated with many functions (progesterone binding, transglutaminase substrate, protease inhibitor, phospholipase A_2 inhibitor, calcium binding, anti-inflammatory activity, immunomodulatory activity, prevention of renal disease) (Singh & Katyal, 2000), none of these is assumed to be particularly toxic. However, humans suffer severe effects (and have even died) from loris bites (Wilde, 1972). The cause is not certain, but is likely due to an anaphylactic shock, as loris researchers readily develop allergies to the glandular secretions (Fry, unpubl. obs.). Similar to the dual functionality of cat allergen, loris glandular secretion likely evolved as a communication molecule, and it is a toxin only for certain (incidentally) susceptible species, like humans. Further work will be necessary to elucidate the toxic actions on humans of the secretions from these fascinating animals.

Acknowledgments. H. Fitch-Snyder would like to dedicate this chapter to Mike Minch, who did the initial chemical analysis of loris brachial gland extract and co-authored a manuscript on this topic in 1989. He, unfortunately, died before his contributions were published. We wish to thank Matthew Williams and his staff at the Division of Biological Sciences Protein Sequencing Facility, University of California, San Diego campus, for their assistance in performing the peptide sequence analysis, and Dr. Paolo Martelli at the Singapore Zoo for all of his help. The authors also thank Richard Tenaza and three anonymous reviewers for their insightful comments and helpful suggestions.

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