Seasonal sebaceous patch in the nectar-feeding bats Leptonycteris curasoae and L. yerbabuena (Phyllostomidae: Glossophaginae): phenological, histological, and preliminary chemical characterization

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Abstract

Many species of bats secrete a wide variety of substances, frequently associated with olfactory communication. We characterized a seasonal phenomenon of dorsal sebaceous secretion in the Curacaoan long-nosed bat, Leptonycteris curasoae, in Venezuela, and the lesser long-nosed bat, Leptonycteris yerbabuena, in Mexico. The phenology of the sebaceous patch was determined, a histological analysis of the affected area was conducted using specimens of L. curasoae from Venezuela, and finally, a preliminary chemical characterization of the substance secreted was performed combining histochemical techniques with gas chromatography and mass spectrometry analyses. The sebaceous patch was detected exclusively in male adult specimens. Individuals presenting it had a variable area of fur covered with a fatty and odoriferous substance at the level of the interscapular zone. Occurrence of the sebaceous patch was cyclical and coincided with the mating season in Venezuela and Mexico. The following histological changes associated with occurrence of the patch were observed: increase of epidermis thickness and decrease of dermis and hypodermis thicknesses, increase in density of sebaceous glands, increase of percentage of skin covered by sebaceous glands, increase of size of sebaceous glands previous to secretion followed, and increase of the sebum volume within sebaceous glands previous to secretion. Several compounds tentatively identified as fatty acids, cholestanes and cholesterol were present in the sebaceous secretion. Based on the evidence obtained, we hypothesize that the sebaceous patch could be involved in olfactory communication, possibly related to mating behavior in these bats.

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Introduction

Olfactory communication is one of the poorly explored areas of study in bat biology (Bradbury 1977; Bloss 1999; Dechmann and Safi 2005). Lack of knowledge on this subject is reflected in our limited understanding of social behavior, mating conduct, and related topics in the Chiroptera. For most mammals, olfaction is the dominant sense and behavior is primarily influenced by chemical signals produced by individual conspecifics (Wyatt 2003). As research on bat biology progresses, an increasing number of investigations indicate that many species produce and secrete a wide variety of chemical substances, frequently identified as scents. Although their precise function is still poorly understood, these substances have been frequently associated with different forms of olfactory communication in bats: mate recognition (De Fanis and Jones 1995; Bouchardeau 2001; Bloss et al. 2002; Safi and Kerth 2003), territory marking (French and Lollar 1998), offspring recognition (Nelson 1965; Gustin and McCracken 1987; McCracken and Gustin 1991; De Fanis and Jones 1996), and sexual selection (Voigt and von Helversen 1999; Voigt et al. 2001).

Considerable variation and complexity in the structure of scent glands have been described in bats of different families (Dalquest and Werner 1954; Werner and Lay 1963; Valdivieso and Tamsitt 1964; Quay 1970; Fenton 1985; Gustin and McCracken 1987; Brooke and Decker 1996; French and Lollar 1998; Haffner 1998, 2000; Krutzsch 2000; Scully et al. 2000). These structures may be located on different parts of a bat’s body, including face, ears, neck, chest, shoulders, subaxillary region, and genital area. Most are normally composed of sebaceous and sudoriferous glands that synthesize different odoriferous molecules (Scully et al. 2000). Scent secretions originating in these glands can be ultimately affected by bacterial fermentation, which has been shown to be involved in odor production in several species of bats (Scully et al. 2000; Voigt et al. 2005). Odor molecules can be held in the sebum produced in the sebaceous glands and released slowly to the environment (Regnier and Goodwin, 1977). Besides forming specialized glandular organs, most areas of a bat’s body containing sebaceous and sudoriferous glands can be potentially involved in production of odors (Haffner 1998).

The process of marking with scent glands is often a sexually dimorphic behavior in mammals (Eisenberg and Kleiman 1972). Many species of bats are sexually dimorphic in the chemical compounds (odors) and the organs that produce and release them. As in most mammals, male bats show a more pronounced development of glandular scent organs and produce stronger odors than females (Dalquest and Werner 1954; Davis and Clyde 1960; Valdivieso and Tamsitt 1964; Quay 1970; Hood and Smith 1984; Gustin and McCracken 1987; Hickey and Fenton 1987; Brooke and Decker 1996; Scully et al. 2000). These sexual differences suggest that odors and the structures involved in their production, storage and display have an important role in the sexual behavior of bats. Correspondence between seasonal peaks of activity of scent organs and mating season in several species of bats helps support this hypothesis (Davis and Clyde 1960; Herreid 1960; Nelson 1965; Heideman et al. 1990). But despite this body of evidence, use of olfactory cues for mate choice and sexual selection in bats has only been studied in detail in one species, the greater sac-winged bat, Saccopteryx bilineata (Voigt and von Helversen 1999; Voigt et al. 2001; Voigt 2002; Voigt et al. 2006). In this species, male quality associated with olfactory cues has been demonstrated. More research is needed to explore how extended is the use of scents in male–male competition and female mate choice among bats.

Phyllostomid bats are good candidates as study objects in the search for new examples of species in which reproductive behavior could be mediated by olfactory cues. This family is rich in species and possesses the highest degree of adaptive radiation among bats (Simmons and Conway 2003). Despite this, only a limited number of studies have examined the presence of scent glands in this group (Dalquest et al. 1952; Valdivieso and Tamsitt 1964; Krutzsch 2000; Scully et al. 2000). The Curaçaoan long-nosed bat, Leptonycteris curasoae Miller 1900 (Phyllostomidae: Glossophaginae) in Venezuela and the lesser long-nosed bat, Leptonycteris yerbabuenae Martínez and Villa-R. 1940 in Mexico, are large nectar-feeding bats associated with dry and xeric habitats in the Neotropics (Fleming and Nassar 2002; Cole and Wilson 2006a, b). These bats have gregarious habits year-round, sometimes forming colonies larger than 100,000 individuals (Cole and Wilson 2006a, b). Up to the present time, we do not know what type of signals these bats use within a colony to communicate with conspecifics and to engage in social and reproductive activities. The mating season of L. curasoae in Venezuela and of L. yerbabuenae in central Mexico (northern birth schedule) occurs between November and early December (Martino et al. 1998; Fleming and Nassar 2002; Stoner et al. 2003). Any type of communication these bats could use in relation to mating should be expressed during the mating season in the form of elaborated displays, vocalizations or production of chemical signals. In this study, we characterize the formation of a sebaceous patch on the dorsum of some individuals of both species and explore its possible relationship with the reproductive process in these bats.

We determined the phenology of the sebaceous patch in one colony of each bat species. In addition, we conducted a histological study of the affected area on
the back of the bats, using specimens of *L. curasoae* from the Paraguaná Peninsula, Venezuela. Finally, a preliminary chemical characterization of the secretion was performed combining histochemical techniques with gas chromatography and mass spectrometry analyses.

**Materials and methods**

**Study sites**

Specimens used for histological and chemical analyses were collected at Guano Cave (11°53′50″N, 69°56′44″W), Buena Vista, Paraguaná Peninsula, Falcón State, Venezuela. This is a limestone cave located in a nearly flat region of 120 m elevation above sea level. Up to five bat species roost in this refuge during the day: *Mormoops megalophylla*, *Pteronotus parnellii*, and *Pteronotus davyi* (Mormoopidae), *L. curasoae* (Phyllostomidae), and *Natalus tumidirostris* (Natalidae) (Matson 1974; Genoud et al. 1990; Bonaccorso et al. 1992; Arends et al. 1995). *L. curasoae* is the only species that abandons the cave and probably the Paraguaná Peninsula during part of the year. Phenological information gathered in this study was obtained from two sites, Guano Cave in Venezuela, and a sea cave on Don Panchito Island (19°30′N, 105°03′W), Bahía Chamela, Jalisco, Mexico. This cave is humid and its floor is covered with seawater daily as the tide changes. During daytime hours, up to five bat species roost in this refuge: *L. yerbabuenae*, *P. parnellii*, *P. davyi*, *P. personatus*, and *M. megalophylla*. The greatest occupancy of the cave occurs from October to December and it has been estimated that more than 50,000 individuals of *L. yerbabuenae* can inhabit the cave (Stoner et al. 2003).

**Animals**

Forty adult specimens of *L. curasoae* (25 males and 15 females) were captured from Guano Cave between September 2003 and April 2004. Bats were captured inside the cave with the help of a hand net with a 5 m long handle and with a 2.6 m × 6.0 m polyester mist net (38.0 mm mesh, Avinet, Dryden, NY, USA) placed at the entrance of the cave. Eight specimens, five males, and three females were obtained on each of the following sampling dates: September 15, October 15, and November 15 during 2003, and February 15 and April 15 during 2004. This time span allowed us to obtain information before, during, and after the development of the sebaceous patch. We were unable to capture specimens in December and January, because the colony moved out of the Paraguaná Peninsula during those 2 months. Bats were transported to the Light Microscopy Service, Centre of Biophysics and Biochemistry, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas. Animals were rendered unconscious with chloroform and euthanized by cervical dislocation in accordance with the American Society of Mammalogists’ guidelines for the capture, handling, and care of mammals. This study is in accordance with all legal requirements established by the Venezuelan Ministry of Environment (MARN) for collection of wildlife for scientific purposes (Permit no. 01-03-03-2415).

**Phenology of the sebaceous patch**

Occurrence of the sebaceous patch was monitored by periodically capturing bats with hand nets and mist nets in diurnal roosts or their proximity at each study site. At Guano Cave, bat captures were conducted according to the following schedule: once per month in May, September, October, and December 2001; once per month from September 2003 to April 2004, and once in December 2004. At Don Panchito Island, bats were captured once monthly, from November 1999 to October 2000. The total number of individuals captured per monitoring period varied from 37 to 79 bats in Guano Cave and from 28 to 120 bats on Don Panchito Island. Information recorded from captured specimens included: sex, relative age (subadult, adult) determined by examining the area of fusion between epiphysis and diaphysis of the fourth metacarpal (Anthony 1988), reproductive condition (non-reproductive, lactating, pregnant) determined by examination of the abdominal region and breasts of females and testicle size in males (Racey 1988), and presence/absence of the sebaceous patch. We considered as sebaceous patch the interscapular area on the back of the animals if it was covered with matted hair and secretion. To estimate the area of the sebaceous patch we took pictures of the back of the animals, and using the program ImageJ, version 1.28 (public domain Java image processing package, National Institute of Health, Bethesda, MA, USA) we calculated the area covered with the secretion. As a reference value we used the distance between the base of the ears of each animal. Except for individuals collected for histological analysis (see section below), bats were released unharmed at the capture site.

**Histological procedures**

After sacrifice, the specimens were initially fixed by cardiac perfusion using 4% weight/volume formaldehyde, buffered with 0.067 mol L⁻¹ phosphate at pH 7.6. For skin analysis two areas on the back of male specimens were defined: the ‘sebaceous area’ (SA) in the interscapular zone where the sebaceous patch appears; and the ‘control area’ (CA) in the dorsal area above the pelvis where no visible alterations were
observed during the year. In female specimens the area corresponding to the sebaceous patch in males was termed the ‘interscapular area’ (IA). Skin portions of the SA, CA, and IA were immersed in either 4% weight/volume formaldehyde or Bouin’s fluid for 24 h at room temperature. The samples were dehydrated with ethanol, clarified with xylene, and embedded in Paraplast (Sigma-Aldrich, St. Louis, MO, USA) following Schöhn et al. (2004). We obtained sections between 4 and 8 μm in thickness using a microtome (Leitz 1212, Germany). Tissue sections were stained using the periodic acid-Schiff (PAS) staining protocol and viewed using bright field illumination on a Nikon E600 light microscope (Nippon Kogahu KK, Tokyo, Japan). The 2560 × 1920 pixel images were saved as 24-bit color TIFF files. ImageJ (version 1.28) was used to calculate areas and other measurements. The software was calibrated in μm and μm² for length and area measurements, respectively (Gao et al. 2002).

Skin sections of the SA, CA, and IA were dewaxed in xylene and rehydrated with distilled water. Part of these sections were stained with the following methods: (a) hematoxylin and eosin; (b) Masson’s and Gomori’s trichrome (Bradbury and Gordon 1990); (c) periodic acid-Schiff to detect neutral polysaccharides, alcian blue at pH 2.50 and 1.00 to detect sulphated and carboxylated mucopolysaccharides, respectively (Cook 1990). Skin sections of the three areas were also processed for lipid histochemistry. Samples were finally fixed in Baker’s calcium formalin at 4°C (Baysliss 1990). To cryoprotect tissues before freezing, tissues were incubated in 10% sucrose in PBS for 60 min, transferred to 20% sucrose in PBS for 60 min, and finally, to 30% sucrose in PBS overnight. Tissues were embedded in OCT compound (Tissue-Tek; Sakura Finetek Inc., Torrance, CA), frozen in liquid nitrogen, and then stored at −80°C until use (Bhatti et al. 2003). Sections (thickness 8 μm) were cut with a cryostat microtome at −20°C and mounted on glass slides coated with poly-L-lysine (Zhu et al. 2000). The sections were air dried and treated with the following histochemical tests: Sudan Black B as general staining for lipids (Baysliss 1990) and differential extraction for polar lipids, non-polar lipids, and phospholipids after Elleeder et al. (1983); PAS and alcian blue at pH 2.50 and 1.00 as indicated above.

Ten histological parameters were determined in transversal skin sections: epidermis thickness (μm), dermis thickness (μm), hypodermis thickness (μm), skin’s epidermis percentage (%), skin’s dermis percentage (%), skin’s hypodermis percentage (%), area of one sebaceous gland (μm²), sebaceous gland density (number of SG/mm²), percentage of skin covered with sebaceous glands (%), and percentage of space in a sebaceous gland filled with sebum (%). In addition, we examined the size relationship between sebaceous glands and hair follicles (modified from Haffner 1998). In transversal skin sections, we determined the area of the pilosebaceous complex and the proportion covered by sebaceous glands. According to Haffner (1998), sebaceous glands comparatively enlarged with respect to the hair in a pilosebaceous complex can be indicative of sebum functions other than hair lubricant.

Hair tufts were collected from the SA, CA, and IA, one from each, from a total of 15 males and 10 females. Hairs of each tuft were fixed in 4% formaldehyde, observed in a contrast phase microscope, and digitally photographed. From each tuft, we chose five unbroken hairs; from each hair, five diameter measurements were obtained from the medial zone and averaged. These averages were used to get a mean across the five hairs examined per tuft. Hair diameters were measured using the program ImageJ.

Following the same fixation and dehydration procedures as used for skin samples, we prepared the genital organs of the male specimens. Testis and epididymides were extracted from the scrotum, separated, and cut transversally with a razor blade. Tissue sections of 4 μm in thickness were obtained. One subset of these sections was stained with hematoxylin and eosin. Another subset was stained with PAS, using Orange G as counterstain. Groat’s hematoxylin was used to stain the nuclei (Gabe 1976).

For morphometry, a random sample of at least five tissue sections stained with PAS per specimen was obtained. The samples were viewed at 200× and digitally registered to quantify the proportion of tubular and interstitial compartments in the testis and epididymal tubules following Schöhn et al. (2004). In each field we determined wall thickness, lumen diameter, and total diameter. At least 20 tubules with spherical shape per specimen were considered for these analyses. These variables have been used as indicators of spermatogenesis and sexual maturity in bats (Entwistle et al. 1998).

Chemical composition of the sebaceous secretion

On December 1, 2006, fresh secretion samples were obtained from 13 adult males of L. curasoea leaving Piedra Honda Cave (11°55′01.3″N, 70°01′08.7″W) which is 3 km northeast of Guano Cave. Bats were trapped with a mist net at the exit of the cave between 2000 and 2200 h. The animals were removed from the net and checked for the presence of the sebaceous patch. Hair tufts covered with secretion were removed from the back of the specimens with the help of surgery scissors and forceps. Before each specimen was processed, these instruments were washed in hexane (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA) and dried with tissue paper. Sebum samples were stored in hexane
(HPLC grade) in sterilized glass vials with Teflon caps. During sampling, latex gloves prevented contamination of the samples and instruments through contact with the skin of the researcher. Vials with secretion samples were stored at −20 °C until the time of analysis.

Hexane extracts were concentrated to approximately 50 µl by blowing a gentle flow of pure nitrogen over the samples. A 1 µl sample was injected into a gas chromatograph (GC) coupled to a mass spectrometer (MS). The GC used was a Varian Star 3400 CX (30 m x 0.25 mm ID, coating CP-SIL CB Low Bleed/MS DF = 0.25, SpectraLab Scientific Inc., Canada). Injector temperature was 250 °C. High-purity helium was used at 1 ml/min. The oven was programmed at an initial temperature of 80 °C for 5 min and then heated at a rate of 10 °C/min to 280 °C, then temperature increased at a rate of 2 °C/min to 290 °C. The mass spectrometer coupled to the GC was an MS Varian Saturno 2000 (electronic impact) with an ion trap analyzer at 250 °C and an electronic voltage multiplier of 1800 V, 295 °C transfer line, ionization energy 70 eV, 15–600 m/z scan and 10 µA emission energy. Compounds were tentatively identified using the GC–MS library by interpreting mass spectra and by comparing their retention indices with a database from the literature.

Data analysis

For each skin variable in males, we fitted a general linear model (GLM) with two fixed effects and one interaction: time, skin zone, and time-by-skin zone (SAS Institute 2001). In addition, we tested for differences in skin variables between SA and IA of male and female specimens, respectively, by fitting a GLM with two fixed effects and one interaction: time, gender, and time-by-gender. Hair diameter was compared between SA and CA in males using a Student t-test for dependent variables and between male and female individuals in the SA and IA, respectively, using a Student t-test for independent samples. Morphometric variables measured in the testis were tested for changes over time by fitting a GLM with time as the fixed effect. Prior to statistical tests, all variables in percent were transformed using the arcsine of the square root of the value. All statistical analyses were conducted using the software STATISTICA (99 Edition, StatSoft Inc., Tulsa, OK; StatSoft, 1999). Where appropriate, means are presented with ±1 standard error.

Results

Sebaceous patch phenology

The sebaceous patch was observed exclusively in adult males from Venezuela (N = 703 total individuals) and Mexico (N = 1118 total individuals). When present, the sebaceous patch was found on the back, at the level of the interscapular region. It consisted of an irregular area of variable size (215.42 ± 27.71 mm², range: 155.20–384.88, N = 8; Fig. 1) covered by a sebaceous secretion. Hairs in this region were clumped as a consequence of the sticky nature of the secretion.

At Guano Cave, Venezuela, the sebaceous patch was observed in 58.3% (N = 24) of the males examined in December 2001, in 38.5% (N = 26) of males in November 2003, and in 47.8% (N = 23) of males in December 2004. In some of the bats captured during these time periods the sebaceous area was hairless, suggesting that a local molt of skin had occurred after formation of the sebaceous patch. In February 2004, 93% of the males captured (N = 30) in the cave presented a circle of new hair in the interscapular

Fig. 1. Dorsal view of Leptonycteris curasoae adult male specimens captured in September (A) and November (B) 2003 in Guano Cave, Paraguana Peninsula, Venezuela. The sebaceous patch covers the interscapular area (sebaceous area) of the specimen captured in November.
region, suggesting that most of these bats had the sebaceous patch in November 2003. At Don Panchito Cave, Mexico, the sebaceous patch was observed in 24.2% (N = 66) and in 35.1% (N = 77) of the males examined in November and December 2002, respectively. The following year, the sebaceous secretion appeared again in 35.5% (N = 62) and 50% (N = 48) of males captured in the same cave in September and October, respectively.

**Histology**

The epidermis, dermis, and hypodermis varied in thickness over time in both the SA and CA of male individuals (epidermis: $F_{4,40} = 21.06$, $P < 0.0001$; dermis: $F_{4,40} = 4.23$, $P < 0.01$; hypodermis: $F_{4,39} = 3.19$, $P < 0.05$), while the dermis and hypodermis varied in the IA of females (epidermis: $F_{1,10} = 0.22$, $P = 0.92$; dermis: $F_{4,10} = 15.93$, $P < 0.001$; hypodermis: $F_{4,10} = 9.29$, $P < 0.01$; Table 1). The IA of female specimens had a thicker dermis than the SA in males (dermis: $F_{1,30} = 13.72$, $P < 0.001$). Overall, the three skin layers in the IA maintained a relatively stable proportionality over time.

In the SA of male specimens, the three layers of skin were thicker than in the CA (epidermis: $F_{1,40} = 19.47$, $P < 0.0001$; dermis: $F_{1,40} = 7.84$, $P < 0.01$; hypodermis: $F_{1,39} = 20.0$, $P < 0.001$), with the exception of November, when the pattern reverted for dermis and hypodermis. In November, the SA reached the greatest thickness for epidermis and the lowest thickness for dermis and hypodermis. During this period, the skin showed a regeneration process, with engrossment of the epidermis, leukocyte accumulation in the old epidermis, and few hairs. During November, the epidermis of the SA reached the greatest percentage (14.0 ± 0.2%) with respect to the other months monitored. Noticeable changes in relative percentage occurred in the CA area of males for dermis ($F_{4,40} = 5.88$, $P < 0.001$) and hypodermis ($F_{4,40} = 3.33$, $P < 0.05$) during February. While the dermis increased from 46.0% to 68.0%, the hypodermis decreased from 47.0% to 25.0%.

The density of sebaceous glands varied significantly over time in both SA and CA of male individuals ($F_{4,40} = 4.86$, $P < 0.01$; Fig. 2); however, no overall difference with respect to this parameter was detected between these regions. In the SA, the highest densities of sebaceous glands were recorded between September (104 units/mm²) and November (129 units/mm²). During this time period, the density of sebaceous glands in CA and IA was comparatively lower, although this difference was not significant. The lowest densities of sebaceous glands in males were recorded in February for both SA and CA. In transversal sections of skin, the area containing sebaceous glands varied significantly over time in SA, CA, and IA (SA vs. CA: $F_{4,40} = 15.45$, $P < 0.0001$; SA vs. IA: $F_{4,39} = 21.99$, $P < 0.0001$; Fig. 2). The maximum percentage of skin covered with sebaceous glands was observed in the SA in October 2003 (38.0%), just before the occurrence of the sebaceous patch. In November, the proportion of skin area

**Table 1.** Epidermis, dermis, and hypodermis parameters estimated for male and female specimens of *Leptonycteris curasoae*

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<tbody>
<tr>
<td>Epidermis thickness (μm)</td>
<td>Male (SA)</td>
<td>16.98 (2.01)</td>
<td>15.14 (0.69)</td>
<td>21.75 (1.41)</td>
<td>12.22 (0.15)</td>
<td>14.76 (0.89)</td>
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<td></td>
<td>Male (CA)</td>
<td>14.82 (0.58)</td>
<td>15.44 (0.32)</td>
<td>15.76 (0.38)</td>
<td>9.01 (0.47)</td>
<td>13.02 (0.53)</td>
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<td>Female (IA)</td>
<td>15.41 (0.36)</td>
<td>16.68 (2.71)</td>
<td>14.62 (0.71)</td>
<td>15.16 (0.49)</td>
<td>15.06 (0.18)</td>
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<td>Dermis thickness (μm)</td>
<td>Male (SA)</td>
<td>182.82 (19.97)</td>
<td>179.97 (13.70)</td>
<td>131.69 (5.84)</td>
<td>146.68 (9.15)</td>
<td>189.65 (14.58)</td>
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<td>Male (CA)</td>
<td>179.93 (9.18)</td>
<td>145.45 (9.83)</td>
<td>151.37 (2.07)</td>
<td>148.01 (9.52)</td>
<td>109.06 (2.63)</td>
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<td>Female (IA)</td>
<td>252.54 (5.6)</td>
<td>209.68 (13.31)</td>
<td>155.72 (5.37)</td>
<td>164.79 (4.50)</td>
<td>208.95 (5.52)</td>
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<td>Hypodermis thickness (μm)</td>
<td>Male (SA)</td>
<td>170.85 (20.83)</td>
<td>224.78 (2.46)</td>
<td>146.70 (19.54)</td>
<td>205.60 (44.69)</td>
<td>184.51 (13.52)</td>
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<td>Male (CA)</td>
<td>135.42 (9.46)</td>
<td>171.26 (10.52)</td>
<td>184.11 (16.09)</td>
<td>78.25 (4.26)</td>
<td>90.34 (8.41)</td>
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<td>Female (IA)</td>
<td>290.89 (8.23)</td>
<td>146.89 (29.33)</td>
<td>136.68 (3.97)</td>
<td>144.69 (15.23)</td>
<td>199.87 (13.55)</td>
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<td>Percentage of epidermis in skin (%)</td>
<td>Male (SA)</td>
<td>10.0 (1.4)</td>
<td>9.0 (1.5)</td>
<td>14.1 (0.2)</td>
<td>5.9 (1.1)</td>
<td>7.9 (1.5)</td>
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<td>Male (CA)</td>
<td>11.4 (1.6)</td>
<td>10.9 (0.3)</td>
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<td>Female (IA)</td>
<td>9.3 (0.2)</td>
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<td>Percentage of dermis in skin (%)</td>
<td>Male (SA)</td>
<td>51.0 (2.0)</td>
<td>43.0 (3.0)</td>
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<td>42.0 (5.0)</td>
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<td>Male (CA)</td>
<td>54.0 (2.0)</td>
<td>46.0 (3.0)</td>
<td>46.0 (4.0)</td>
<td>68.0 (1.0)</td>
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<td>Female (IA)</td>
<td>40.0 (0.0)</td>
<td>51.0 (5.0)</td>
<td>50.0 (1.0)</td>
<td>47.0 (3.0)</td>
<td>47.0 (1.0)</td>
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<td>Percentage of hypodermis in skin (%)</td>
<td>Male (SA)</td>
<td>39.0 (2.0)</td>
<td>48.0 (3.0)</td>
<td>46.0 (3.0)</td>
<td>52.0 (6.0)</td>
<td>44.0 (4.0)</td>
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<td></td>
<td>Male (CA)</td>
<td>34.0 (1.0)</td>
<td>43.0 (3.0)</td>
<td>47.0 (5.0)</td>
<td>25.0 (1.0)</td>
<td>47.0 (3.0)</td>
</tr>
<tr>
<td></td>
<td>Female (IA)</td>
<td>51.0 (1.0)</td>
<td>38.0 (6.0)</td>
<td>40.0 (0.0)</td>
<td>40.0 (3.0)</td>
<td>42.0 (2.0)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (1 standard error). SA: sebum area in males, CA: control area in males, IA: interscapular area in females.
The area covered by one sebaceous gland varied over time in male specimens ($F_{4,40} = 3.03, P < 0.05$; Figs. 2 and 3). The largest sebaceous glands were observed in the SA in October (9091.3 $\mu$m$^2$), and in November the glands reached the smallest size (2316.3 $\mu$m$^2$) recorded during the entire period of observation. No significant temporal changes in area were observed in the CA. In the IA in females, the largest sebaceous glands were observed between November and February. Sebaceous glands in the SA had the same histochemical properties as observed in the secretion covering the hair tufts in that region (see below), which suggests that this substance originates in the sebaceous glands (Fig. 4). Results obtained from the histochemical tests conducted for lipids suggest that sebum production in the sebaceous glands of the SA was intensive during October. At that time, the sebaceous portion of the glands showed PAS-positive areas, indicating the presence of polysaccharides (Fig. 4a). This is in accordance with the detection of alcian blue-positive zones in the sebaceous and cellular zones in sebaceous glands. In the CA of males, sebaceous glands did not have the histochemical properties observed in the SA, and lipids present in the sebaceous portions of the glands were non-polar, as was the case in the SA in males during the rest of the months monitored and in the IA of females all the time. The percentage of sebum area within a sebaceous gland also varied over time in both males ($F_{4,40} = 7.91, P < 0.001$; Fig. 2) and females ($F_{4,10} = 4.34, P < 0.05$). In the SA, the sebum occupied up to 32% of a sebaceous gland in October. In the IA, the highest sebum production per gland also occurred in October. The lowest percentages of sebum within sebaceous glands were observed for SA, CA, and IA between February and April.

In males, the area covered by sebaceous glands in the pilosebaceous complex varied over time ($F_{4,39} = 41.19, P < 0.0001$; Fig. 2). In the SA, sebaceous glands represented between 75% and 81% of the pilosebaceous complex during September and October, respectively. The area covered by sebaceous glands decreased abruptly in November (29%), reaching the minimum value in February (19%). By April, sebaceous glands again occupied a substantial proportion (65%) of the pilosebaceous complex. In CA and IA, no major differences in the estimates of this parameter were observed between September and February, never surpassing 60% of the pilosebaceous complex area.

Hairs from the SA in male bats did not differ in diameter (18.47 ± 0.32 $\mu$m) with respect to hairs from the CA (17.90 ± 0.43 $\mu$m; t = 1.12, d.f. = 14, P = 0.28) and hairs from the IA in female subjects (17.39 ± 0.47 $\mu$m; t = 1.79, d.f. = 23, P = 0.09).

Structural characterization of both testis and epididymides allowed us to distinguish two stages in the process of sperm production: spermatogenesis and...
spermiogenesis. Spermatogenesis occurred from April to October, and was characterized by an increase in thickness of seminiferous tubules (Fig. 5). Between September and October we observed spermatids in the walls and lumen of the tubules. Thickness of the seminiferous tubules decreased in November, with few cells in the walls and numerous spermatids in the lumen. The lowest thickness of the seminiferous tubules was observed in February, with very few germinal cells in the walls and an empty lumen. Spermiogenesis occurred between October and November, when epididymis tubules increased in thickness (Fig. 5). Starting in September, the epithelium of the epididymis tubules increased in height, with few stereocilia and spermatids in the lumen. The epithelium reached maximum height in October, when numerous stereocilia and spermatids were observed in the lumen. During November, the epithelium height decreased, although numerous spermatids remained in the lumen. In February, the epithelium reached the minimum height recorded and the lumen contained residual bodies. From February to April, the epididymis tubules increased again due to connective tissue growth, but without any epithelium growth.

Chemical composition of the sebaceous secretion

The secretion produced in the SA of some males was an amorphous and sticky substance that adhered to the hairs (Figs. 1 and 4). In fresh condition on the animal, it had an intense rotten smell. The histochemical phospholipid test indicated that the sebum in the sebaceous glands of the SA did not contain phospholipids. The differential delipidation test indicated that the secretion contains polar lipids different from phospholipids (Fig. 4b and c). The secretion had a positive reaction to eosin and acid fuchsine, indicating the presence of proteins (Fig. 4d). It also had a positive reaction to PAS, which indicates the presence of polysaccharides (Fig. 4e). The test with alcian blue (pH 2.5) was positive, suggesting occurrence of carboxylated mucopolysaccharides (Fig. 4f). The delipidation treatment reduced the effect observed in the PAS and alcian blue reactions; however, these reactions were not abolished. This suggests the presence of carboxylated mucopolysaccharides not associated with lipids.

Fourteen compounds were tentatively detected in the 13 male secretion samples analyzed with the GC–MS system (Table 2). On average, each secretion sample...
contained 9.0 (±0.45; range: 6–11) compounds. Several compounds were found in the majority (N≥9) of specimens examined: four esters of fatty acids (tetradecanoic acid ester, hexadecanoic acid ester, octadecanoic acid ester -R₁, and -R₂, R₁ being an alkyl group), adipic acid ester, three cholestanes, and cholesterol. Other molecules identified in some individuals included two additional cholestanes, dodecanoic acid ester, pentacosane, and a long-chain fatty acid. Fit and reverse fit search values obtained for compounds

Fig. 4. Photographs of sebaceous glands, secretion, and hairs from the sebaceous area (SA) obtained from males captured in October 2003. (A) Sebaceous gland in the SA stained by PAS reaction; arrows indicate PAS-positive zones; (B) sebaceous gland stained by Sudan Black B, demonstrating positive reaction for lipids in the sebaceous area of the gland, which occupies a great portion of the glandular volume; (C) sebaceous gland from the same section as in the previous picture, but pre-treated with acetones before staining with Sudan Black B to demonstrate the presence of polar lipids in the secretion; (D) secretion in hair tuft stained with eosin; arrow indicates an eosinophilic zone of secretion adhering to hairs, which demonstrates the presence of proteins; (E) secretion in hair tuft stained by PAS reaction; arrow indicates a PAS-positive zone of secretion adhering to hairs; this reaction demonstrates presence of polysaccharides; (F) secretion in hair tuft stained with alcian blue; arrow indicates alcian blue-positive zone; this reaction demonstrates the presence of carboxylated polysaccharides. Scale bars: 10.0 μm.
identified with the library indicated variable accuracy in the assignment of the compounds’ identities. Because of their similar mass spectra, we were unable to determine the identity of the five cholestanes.

Discussion

*L. curasoae* and *L. yerbabuenae* share a common seasonal phenomenon not previously documented; a sebaceous patch is formed on the interscapular area of the dorsum. The fact that this patch is produced exclusively by males, that it appears cyclically during the period of spermiogenesis and mating, and that it has a strong odor easily perceived by humans, suggest that this patch could be involved in olfactory communication, possibly related to mating behavior in these bats.

Secondary sexual characteristics are expressed through a broad spectrum of morphological, acoustic, and behavioral traits in the Chiroptera (Krustzsch 2000). Integumentary glands that produce scents to which conspecifics respond could be considered one type of secondary sexual trait. Individual males of a number of bat species tend to exhibit a more marked development of their scent glands and produce stronger scents than females (Dalquest and Werner 1954; Davis and Clyde 1960; Quay 1970; Hood and Smith 1984; Gustin and McCracken 1987; Brooke and Decker 1996; Scully et al. 2000). Examples of sexually dimorphic scent glands among phyllostomid bats include those located on the throat of *Phyllostomus discolor* (Goodwin and Greenhall 1961), on the chest of male *Phyllostomus hastatus* (Valdivieso and Tamsitt 1964), and on the shoulders and neck region of male *Sturnira lilium* and *Sturnira mordax* (Goodwin and Greenhall 1961; Scully et al. 2000). The sebaceous patch described in *L. curasoae* and *L. yerbabuenae* fits the typical...
characteristics of secondary sexual traits in bats. Sebaceous glandular tissues seem to be involved in the appearance and development of this patch, the scent produced is clearly distinctive and pronounced, and both species are sexually dimorphic for this character. In addition to this, the sebaceous patch develops during the mating season (November and December) of *L. curasoeae* (Martino et al. 1998) and *L. yerbabuenae* (northern birth schedule) (Fleming and Nassar 2002). Several studies on the reproductive biology of bats have demonstrated a correspondence between glandular activity and the reproductive cycle of males (Davis and Clyde 1960; Herreid 1960; Davis et al. 1962; Nelson 1965; Heideman et al. 1990).

Most records of glandular secretions associated with odor production in bats correspond to specialized glandular structures, accessory organs, and specialized hairs (osmetrichia) easily identified on the bat’s body (Werner and Lay 1963; Valdivieso and Tamsitt 1964; Fenton 1985; Brooke and Decker 1996; Voigt and von Helversen 1999; Scully et al. 2000). In the case of the study species, however, neither specialized glands nor modified hairs were identified on their bodies at any particular time during the year. Results of the histological and histochemical analyses suggest that the sebaceous glands present in the interscapular area of males could be involved in the development of the sebaceous patch. We identified a sequence of histological changes that occurred in the SA between October and November when the sebaceous patch first appeared: (1) increase of epidermis thickness and decrease of dermis and hypodermis thicknesses, (2) increase in density of sebaceous glands, (3) increase of percentage of skin covered by sebaceous glands, (4) increase of size of sebaceous glands prior to secretion, followed by a marked decrease after secretion, and (5) increase of the sebum volume within sebaceous glands previous to secretion. Furthermore, the sebaceous glands in the SA had the same histochemical properties as the sebaceous secretion covering the hair tufts in that zone.

Our knowledge of the chemical composition of scent gland secretions in bats is limited to a few species, and the number of compounds identified in a sample varies from a few to hundreds (Dapson et al. 1977; Brooke and Decker 1996; Bloss et al. 2002; Wood et al. 2005). The chemical analysis of the secretion conducted in this study should be considered preliminary, because *n*-hexane as a solvent is mainly effective in revealing straight-chain hydrocarbons and other non-polar compounds (Decker et al. 1992) and also because we did not compare the compounds present in the samples with standards.

The sticky and fatty nature of the secretion may reflect the combined effect of carboxylated mucopolysaccharides and glycoproteins in association with polar lipids on the one hand, and apolar lipids on the other hand. Many of these lipophilic compounds could have a structural function in the secretion, retaining volatile molecules that can be released slowly into the air. Cholesterol, presumably found in most samples examined, is considered an unreactive apolar matrix that can deliver compounds acting as semiochemicals (Escobar et al. 2003). Most of the carboxylic acids detected in the samples in the form of esters have been identified in exocrine secretions of other mammals (Burger 2005) and reptiles (López and Martin 2005a, b). Despite their potential function as a matrix for other compounds, fatty acids could be directly involved in odor production in bats. Dapson et al. (1977) suggested that unsaturated fatty acids present in secretions produced by integumentary glands in *Tadarida brasiliensis*, *Molossus bondae*, and *Eptesicus fuscus* were responsible for their characteristic odors. The other compounds detected in the sebaceous patch that could be potentially associated with olfactory signals are the cholestanes. Several cholestanes have been identified in the anogenital gland secretions of the giant panda, *Ailuropoda melanoleuca* (Yuan et al. 2004). Females of two species of European lizards show a preference for areas marked by males with femoral secretions containing high concentrations of two cholestanes (López and Martin 2005a, b; Martín and López 2006a, b).

Even though our results suggest that the substance present on the sebaceous patch seems to originate, at least partially, in the sebaceous glands of that region, we do not discard the possibility that the chemical composition of that substance could be the result of a combination of multiple body secretions coupled with the action of bacterial fermentation. Among mammals, chemical signals indicating individuality are highly complex mixtures (Brennan and Kendrick 2006). As an example in bats, males of the sac-winged bat, *S. bilineata*, store odoriferous secretions in their propatagial sacs that contain products from the genital region, gular gland, urine, saliva, and some volatiles of microbial origin (Voigt and von Helversen 1999; Voigt et al. 2005). Preliminary observations of male *L. yerbabuenae* from Don Panchito Island during the breeding season (November and December) by one of us (KES) showed that males repeatedly lick their claws on their hind feet, scratch their genital area and then scratch their back in the area where the sebaceous patch is formed. Thus, the sebaceous patch could contain saliva and secretions produced by the genital organs. A detailed behavioral study of the two species in natural colonies would help determine if the combination of these displays contributes to the formation of the sebaceous patch. In addition, a study of the microflora associated with the sebaceous patch and their fermentation products would help determine if some of the volatiles present in the secretion are of bacterial origin.
More than two decades ago, Blaustein (1980) called the attention of mammalogists to the fact that ignoring scents in models incorporating mammalian sexual dimorphism and sexual selection was a mistake, because olfaction is the dominant sense for most mammals, and it is particularly important in their social lives (Ralls 1971; Eisenberg and Kleiman 1972; Seboek 1977; Wyatt 2003). He also emphasized that sexual selection in small mammals is mostly mediated through olfactory signals, and not through visual signals such as body size. Despite numerous examples of bat species that are sexually dimorphic in odor and the organs that produce and release it, the use of olfactory cues for mate choice and sexual selection in bats has only been studied in detail in one species, the greater sac-winged bat, S. bilineata (Voigt and von Helversen 1999; Voigt et al. 2001; Voigt 2002; Voigt et al. 2006). Males of this species display odors stored in their wing sacs and perform energetically costly hovering flights in front of females to release their scent and presumably encourage them to choose mates (Voigt and von Helversen 1999).

L. curasoae and L. yerbabuenae do not present sexual dimorphism at the morphological level (Arita 1999). Although mating behavior has not been described for these species, preliminary observations suggest a polynogynous mating system (Stoner, unpubl. data). If some form of sexual selection occurs in these species, it should be mediated through olfactory cues. The phenomenon of sebaceous secretion described for these bats includes several of the traits and conditions that should be present in a model of sexual dimorphism and sexual selection mediated through olfactory signals. The secretion appears cyclically during the mating season of both species, it is produced only in males and its chemical composition is apparently complex. Our findings should be considered as the starting point for chemical and ethological studies aimed to analyze in detail the composition of the sebaceous patch and to test the hypothesis that the sebaceous secretion produced in these bats is involved in a mechanism of sexual selection.

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