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Chemical composition of the substances from dorsal patches of males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae)

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In the absence of visual cues, chemical signals are especially important for nocturnal mammals such as bats, because they facilitate individual recognition, communication, and mate selection. In a recent study, it was reported that adult males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae*, develop an odoriferous dorsal patch during the short mating season. It was postulated that dorsal patches signal health condition to females, and that females are preferentially attracted to the odor of males with dorsal patches. The chemical characterization of the dorsal patch is key to understanding its implications in chemical communication in a sexual context. In the present study, organic compounds collected from dorsal patches of males of *L. curasoae* from northwestern Venezuela were extracted using solid-phase micro-extraction (SPME) techniques and tentatively identified using GC-MS (Gas Chromatography-Mass Spectrometry). Nineteen compounds were present in 75% or more of the patches examined: 3-methyl-2-buten-1-thiol, acetic acid, 2'-aminoacetophenone, diacetyl, 2-pentanone, 2,3-dimethylpyrazine, 2-nonanone, acetamide, 2-undecanone, piperidinone, 4-methylquinazoline, 2,4-dimethylquinazoline, 3-methyl-2,5-pyrrolidinedione, 2-butanone, 2-methylfuran, 3-methyl-2-butenal, δ-valerolactone, 3-methyl-2-butenoic acid, and 2-methyl-2-butenoic acid. Although some of these compounds have been reported as important in female attraction among other male mammals during their respective breeding seasons, the actual function of these chemicals in *L. curasoae* remains to be determined. Some of these compounds have also been identified as natural insecticides, and this may be associated with lower ectoparasite loads reported on males with dorsal patches. These results, when considered along with previous observations, suggest that the dorsal patch in males of *L. curasoae* promotes the attraction of females during the mating season, and/or provides protection against ectoparasites.

Key words: chemical communication, dorsal patch, gas chromatography-mass spectrometry, mating, odor, volatiles

INTRODUCTION

Most mammals produce odors that play important roles during attraction, recognition, identification, and mating (Eisenberg and Kleiman, 1972; Blaustein, 1981; Penn and Potts, 1998; Brennan and Kendrick, 2006). For mammals, odors may function as reliable cues to locate, identify, and attract conspecifics (De Fanis and Jones, 1995; Dechmann and Safi, 2005). In part, this may reflect the specificity of chemical signals that can directly and permanently affect recipients, and moreover such signals remain in the environment in the absence of the sender (Eisenberg and Kleiman, 1972; Mykytowycz and Goodrich, 1974; Doty, 1986; Dechmann and Safi, 2005). Chemical signals are particularly important for bats because they often occupy dark roosts where hundreds to thousands of individuals may roost together (Bloss, 1999; Altringham and Fenton, 2003; Dechmann and Safi, 2005). Small mammals such as bats and rodents, which may show little, if any, sexual dimorphism in size, may be sexually dimorphic with respect to skin glands and the unique compounds they produce (Blaustein, 1981; Fernández-Vargas *et al*., 2008). Largely produced by skin glands and other odor-producing structures, chemical compounds have been postulated as important for individual recognition and mate selection in bats (Hood and Smith, 1984; Gustin and McCracken, 1987; Höller and Schmidt, 1993; De Fanis and Jones, 1995; Voigt and von Helversen, 1999; Krutzsch, 2000; Bouchard, 2001; Voigt *et al*., 2001, 2006; Bloss *et al*., 2002; Safi and Kerth, 2003). However, few studies have investigated the chemical composition of secretions from these glands, other odor-producing structures, urine, and feces produced by bats

(Dapson *et al*., 1977; Brooke and Decker, 1996; Bloss *et al*., 2002; Lily and Vanitharani 2005; Wood *et al*., 2005; Nielsen *et al*., 2006; Vanitharani *et al*., 2007; Nassar *et al*., 2008; Caspers *et al*., 2009, 2011). Although hydrocarbons, carboxylic acids, alcohols, aldehydes, ketones, esters, and amides are important compounds possibly involved in chemical communication in bats (Muñoz-Romo, 2008), specific substances have not been evaluated experimentally to assess responses of individuals to these compounds.

A recent study by Nassar *et al*. (2008) described an interscapular patch that develops in adult males of *Leptonycteris curasoae* and *L. yerbabuenae*, which contains odor-producing substances. This interscapular structure develops for several days once each year in November and December (Martino *et al*., 1998), exclusively during the mating season (Nassar *et al*., 2008; Muñoz-Romo and Kunz, 2009). This patch has been postulated as a source of chemical substances involved in courtship and mating in these two species (Nassar *et al*., 2008; Muñoz-Romo and Kunz, 2009; Muñoz-Romo *et al*., 2011*b*). The latter authors demonstrated that males with dorsal patches had fewer ectoparasites than those without them, suggesting that dorsal patches could provide cues to females about the health status (i.e., ectoparasite load) of potential mates. It was also experimentally demonstrated that females prefer odors from a male with a dorsal patch than odor from a male without such a patch (Muñoz-Romo *et al*., 2011*a*). Thus, if females are attracted to the odor from dorsal patches (Muñoz-Romo *et al*., 2011*a*), and dorsal patches signal the body condition of males to females (Muñoz-Romo and Kunz, 2009; Muñoz-Romo *et al*., 2011*b*), then knowledge of the chemical composition of the substance from these patches should be of interest to understanding the function of these structures. Preliminary analyses of the chemicals associated with the dorsal patch in male *L. curasoae* indicated that fatty acids, cholestanes, and cholesterol were present (Nassar *et al*., 2008), but additional studies are needed to more fully characterize the chemical properties that comprise this patch and their possible functions. Moreover, the chemical characterization conducted by Nassar *et al*. (2008) did not include females or males without dorsal patches. Thus, it is important to document whether these chemicals are unique to males with a dorsal patch, or whether they are also present in females and in males without dorsal patches. In addition to their potential role in mate recognition and selection, Muñoz-Romo and Kunz (2009) postulated that some of the substances that impregnate dorsal patches might provide males with protection against ectoparasites since males with dorsal patches had a lower mean intensity of streblid (batfly) infestation than males without dorsal patches and females (Muñoz-Romo and Kunz, 2009; Muñoz-Romo *et al*., 2011*b*).

The primary goals of this study were to: 1) characterize the chemical composition of substances that form dorsal patches in males of *L. curasoae* by tentatively identifying the substances that impregnate hairs in the interscapular region (i.e., dorsal patch), and 2) to determine which chemicals are unique to dorsal patches and absent in females or males without such patches. Finally, we consider the biological functions of the most commonly discovered chemical compounds present in the dorsal patch based on the published literature.

Materials and Methods

Study Species

The Curaçaoan long-nosed bat (*Leptonycteris curasoae*) is a medium-sized, Neotropical, migratory, nectarivorous species that inhabits arid and semiarid regions in northern South America and the Netherlands Antilles (Fleming and Nassar, 2002; Cole and Wilson, 2006). This species is highly gregarious, sometimes forming colonies of thousands of individuals (Cole and Wilson, 2006). In the Paraguaná Peninsula, Venezuela, *L. curasoae* exclusively selects caves during November and December, but subsequently abandons these sites at the end of the mating season (Martino *et al*., 1998; Fleming and Nassar, 2002). Females and males again return to these cave roosts during the maternity period (Martino *et al*., 1998).

Study Site

Bats were studied at Piedra Honda Cave (11º55'1.3''N, 70º01'8.7''W, 154 m a.s.l.), a limestone cavern located in the Paraguaná Peninsula, Falcón State (Venezuela). The landscape surrounding this cave is semiarid, dominated by spiny legumes and several cactus species. Mean annual temperature recorded near the study site is 27ºC. Annual average precipitation is 316 mm, with maximum precipitation occurring between November and December (Martino *et al*., 1998).

Bat Sampling

All sampling protocols were performed following guidelines of the American Society of Mammalogists for capture, handling, and care of mammals (Gannon *et al*., 2007), and were approved by Boston University's Animal Care and Use Committee. Permission to conduct captures was approved by the Venezuelan Ministry of Environment (permission No. 2778). The present study was conducted during the mating season of *L. curasoae* (November–December 2006 and 2007). Bats were captured using 12-m long, 38-mm mesh, 50 denier, four-shelf mist nets — Avinet, Dryden, New York, USA (Kunz *et al*.,

2009) between 18:30 and 20:30 h. Each captured bat was individually placed into a clean cotton cloth bag and transported to a data collection station. We estimated age of bats using the relative ossification of wing bones (Kunz *et al*., 1996; Brunet-Rossini and Wilkinson, 2009), and determined sex and reproductive status following standard criteria (Kunz *et al*., 1996; Racey, 2009). All individuals were released near the cave at dawn immediately after measurements were recorded.

Dorsal Patch Samples

The dorsal patch is an area ranging in size from 0.6 to 3.6 cm^2, that forms in the interscapular region of the upper back of some males, typically with tufts of dry pelage impregnated with odoriferous substances (Fig. 1) (Muñoz-Romo and Kunz, 2009). Hair was sampled from dorsal patches using metal forceps and scissors that were meticulously cleaned with 95% ethanol before collecting each sample. Sufficient time was allowed for total evaporation of ethanol before these instruments were used for collecting samples. These samples consisted of agglutinated tufts of hair from the interscapular region, because no isolated hairs were present in the dorsal patch (Fig. 1). Each hair tuft was immediately placed into an individually labeled 40 ml, clear glass, screw cap vial, fitted with PP Teflon lined septum closures (Fisher, 140-40C/EP), closed tightly, and kept refrigerated for later analyses in the laboratory. During sampling, powder-free nitrile gloves were worn to prevent contact with the skin of the experimenter. This procedure was followed (1) in the field station for each of 19 males that showed evidence of a dorsal patch during the first week of December 2006, and (2) directly from bats in a mist net for each of 10 other bats captured during December 2007. The latter samples were collected because we learned from our preliminary analysis that the cloth bags we used could chemically interfere with sample analysis (see below), because bats captured in 2006 were transported to the lab in cloth bags. The 10 additional samples (obtained on 9 December 2007) included four adult males with dorsal patches, three adult males without dorsal patches, and three adult females to determine whether the compounds were unique to dorsal patches. All hair samples taken in 2007 were removed directly from bats while they were still in the mist net to avoid potential chemical contamination from cloth holding bags.

A portable SPME (solid phase microextraction) fiber (Supelco, 75 µm Carboxen/PDMS) was exposed to the environment each time that samples were collected for 120 min to obtain background volatiles, allowing us to determine whether the chemicals present in the dorsal patches were also present in the surrounding environment (four fibers total). Four unused (clean) cloth bags were also analyzed, making it possible to distinguish potential contaminants from each of the samples collected in 2006. These samples were corrected by ignoring chemical interferences from the surrounding environment and cloth bags (in which individual bats were transported from the field to the field station in 2006), whereas samples from 2007 were only corrected by ignoring chemical interferences from the surrounding environment.

Chemical Analysis

Vials containing hair samples were shipped to the laboratory at Microanalytics (Round Rock, Texas) and analyzed using solid-phase microextraction (SPME, the SPME fiber used was Carboxen™/PDMS StableFlex™ 85 µm, Supelco PN 57334-U),

FIG. 1. Dorsal view of a male *L. curasoae* showing a dorsal patch

a solvent-free technique that uses polymer-coated fibers to capture organic compounds when exposed to samples for 24 hours at 25°C (Pawliszyn, 1997; Vas and Vékey, 2004). Fibers used for chemical sampling were inserted directly into the inlet of the gas chromatograph-mass spectrometer (GC-MS) at 250°C. At this temperature the volatile compounds were desorbed into the GC column for separation followed by mass spectrometric analysis (Pawliszyn, 1997; Vas and Vékey, 2004).

Chemical analysis of samples was performed on a Microanalytics AromaTrax system consisting of an Agilent model 6890 gas chromatograph (GC) configured for dual column multidimensional component separation, and coupled to an Agilent 5973 mass spectral detector (MSD). The system's Agilent split/splitless GC inlet was equipped with a Merlin Microseal septum and operated in split mode with a flow of 5 ml/min. The first GC column in the multidimensional GC system was a BP5 of 12 m × 0.53 mm ID with 1.0 micron film thickness (SGE, Austin, Texas). The second (tandem) column was a BP20 of 25 m × 0.53 mm ID with a 1.0 micron film thickness (SGE, Austin, Texas). Helium was the carrier gas at a flow of 10 ml/min. Column switching was achieved by a Dean pressure switch between the two columns. The GC oven temperature was programmed with an initial hold of 3 minutes at 40°C, then a heating rate of 7°C/min to 220°C with a final hold of 25 min.

An MSD utilizing electron impact ionization detection (70 eV) was arranged in parallel, by means of a split of the carrier flow from the second column, with an olfactory detector (sniff port). Compounds were detected simultaneously from the second column by mass spectrometry and odor characteristics. Mass spectra were compared with those in the Wiley mass spectra library (John Wiley & Sons, Ltd.) and an in-house library of known spectra for their identification. Spectral search of the Wiley library was conducted using the Benchtop/PBM Software Library Search Program (Palisade Corporation, NY). Sniff port olfactory response was recorded using AromaTrax odor tracking software obtained simultaneously with the mass spectra.

RESULTS

From 40 chemical compounds present in hair samples from 2006, 31 were tentatively identified

using spectral matching with the Benchtop/PBM software package (Appendix I). Trimethylamine, 3-methyl-2-buten-1-thiol, acetic acid, 2-methyl-3-isopropylpyrazine, 2'-aminoacetophenone, and γ-undecalactone were present in samples of hairs from the dorsal patch of male *L. curasoae* in 2006, although only three compounds (3-methyl-2-buten-1-thiol, acetic acid, and 2'-aminoacetophenone) were present in more than 75% of these samples (Table 1).

From 136 chemical compounds present in hair samples collected in 2007, 120 were tentatively identified from dorsal regions of males with dorsal patches, males without dorsal patches, and females (Appendix II). From these 120 compounds, 63 (52.5%) were found only in males with dorsal patches, 5 (4.2%) were found only in males without dorsal patches, and 24 (20.0%) were found only in females (Fig. 2). Males with dorsal patches had an average of 50 ± 14 (n = 4) chemical compounds, whereas males without dorsal patches had 16 ± 6 (n = 3) compounds. By contrast, females had 19 ± 12 (n = 3) compounds (Fig. 2).

From the 63 compounds tentatively identified and present only in samples from males with dorsal patches, 10 were present in all of the males, and six were found in 75% of them (Table 1).

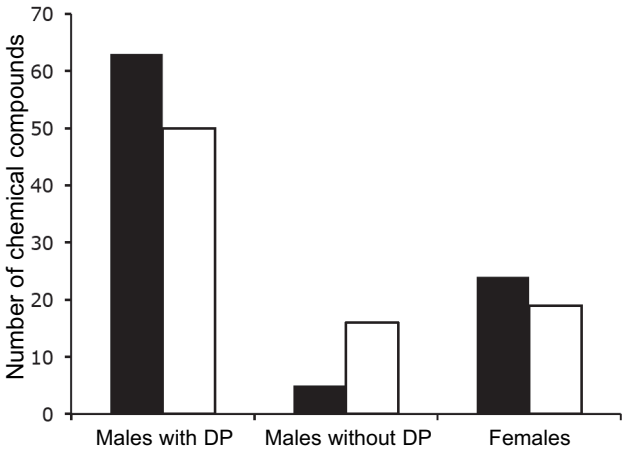

FIG. 2. Total number of chemical compounds found exclusively in males with dorsal patches (DP), in males without DP or in females (black bars), and average of chemical compounds for males and females (white bars)

DISCUSSION

Analysis of substances sampled from dorsal patches of male *Leptonycteris curasoae* suggest a complex chemical profile, characterized by the presence of many compounds unique to these structures. This condition and the fact that the dorsal patch only appears during the mating season in this species (Nassar *et al*., 2008; Muñoz-Romo and Kunz, 2009) suggests that at least some of these compounds are involved in female attraction, courtship and mating. The fact that males with dorsal patches had more compounds than males without dorsal patches and than females, and that these males also had an exceptionally diverse set of unique chemical compounds indicates that the dorsal patch is rich in specific chemicals. However, the specific concentrations of these compounds, and whether some specific combination of them is actively involved in chemical communication in *L. curasoae* remains to be determined.

Some differences were observed between samples from 2006 and 2007. Those analyzed from 2006 contained fewer compounds than samples collected in 2007. These differences may reflect several undetermined factors, such as time from sample collection to analysis and different temperatures and humidity levels at the times of collection. Notwithstanding, both data sets are complementary and

TABLE 1. Chemical compounds exclusively found in 75% or more samples of hair from dorsal patches of males *L. curasoae*. Years are shown to indicate sampling date

Present in		
80–100% of samples (2006)	100% of samples (2007)	75% of samples (2007)
3-methyl-2-buten-1-thiol	Diacetyl	2-butanone
Acetic acid	2-pentanone	2-methylfuran
2'-aminoacetophenone	2,3-dimethylpyrazine	3-methyl-2-butenal
	2-nonanone	δ-valerolactone
	Acetamide (guanadine?)	3-methyl-2-butenoic acid
	2-undecanone	2-methyl-2-butenoic acid
	Piperidinone (valerolactam)	
	4-methylquinazoline	
	2,4-dimethylquinazoline	
	3-methyl-2,5-pyrrolidinedione	

provide a list of tentative compounds that can be confirmed in future analyses and also tested experimentally.

Specific attention should focus on the unique compounds present in dorsal patches, especially on those present in the majority (i.e., >75%) of samples. From the 10 chemical compounds present in all males with dorsal patches, 2-undecanone is an organic compound used in insect and animal repellents owing its particularly strong odor. Small concentrations of 1 to 2% of this compound are used in dog and cat repellents (Dean, 1992). Moreover, 2-undecanone and their analogues have been bioassayed for their repellency against the maize weevil, *Sitophilus zeamais*, in olfactometric assays (Lwande *et al*., 1992). This compound also has activity comparable to a synthetic commercial insect repellent (Lwande *et al*., 1992). Interestingly, 2-undecanone and 2-nonanone (both present and unique to dorsal patches) are products of bacterial activity, and are also considered highly effective as nematicides (Gu *et al*., 2007). These findings support our hypothesis that dorsal patches may provide males of *L. curasoae* with protection against ectoparasites (Muñoz-Romo and Kunz, 2009), based on our observations that males with dorsal patches had significantly lower intensity of streblid (batfly) infestation than males without dorsal patches and females. A similar function was suggested by Wood and Szewczak (2007) in Brazilian free-tailed bats, *Tadarida brasiliensis*. These authors identified three aldehydes (nonanal, heptanal, and octanal), present in the pelage of *T. brasiliensis* and attribute antimicrobial properties against some surface-dwelling cutaneous bacteria, fungi, and ectoparasites in this species.

4-Methylquinazoline was another compound exclusively present in all males with dorsal patches. This compound was also reported in urine and anal glands from the domesticated European ferret, *Mustela furo*, where it was significantly more abundant in males than in females (Zhang *et al*., 2005). Ruther *et al*. (2008) reported this compound as a minor component of the male sex pheromone in the jewel wasp, *Nasonia vitripennis*, but was primarily associated the characteristic male odor of this parasitoid species. Moreover, the mixture of this compound and 2,4-dimethylquinazoline (also present and unique to dorsal patches in *L. curasoae*) has been confirmed experimentally as an important sex attractant for females of the kissing bug, *Triatoma infestants* (Alzogaray *et al*., 2005). Although the effect of these compounds in insects may be completely different in mammals, the association of these and other compounds with sexual attraction in some taxa leads us to suggest that future experiments could involve these substances to test responses, if any, of individuals of *L. curasoae* in a sexual context.

The compound 2'-aminoacetophenone has been reported as significantly more abundant in males than in females of the domesticated European ferret (Zhang *et al*., 2005). These authors suggested that males and females of this species use urine for individual recognition because it may convey specific signals. 2-Aminoacetophenone was also found in the wing sac of male *Saccopteryx bilineata* and *S. leptura* (Caspers *et al*., 2009), which was, in fact, a male-specific substance. 2'-Aminoacetophenone was present in all samples from males of *L. curasoae* with dorsal patches collected in 2006 (n = 19) and 2007 (n = 4), but also in a single sample from a male (from 2007) without a dorsal patch. Both 4-methylquinazoline and 2,4-dimethylquinazoline can be synthesized from 2'-aminoacetophenone (Alzogaray *et al*., 2005; Bailey *et al*., 2006), compounds that are present in the dorsal patches of male *L. curasoae*. Recent analysis also suggests that 2'-aminoacetophenone is an important volatile compound in the Brazilian free-tailed bat, *T. brasiliensis* (Nielsen *et al*., 2006), and in the big brown bat, *Eptesicus fuscus* (Bloss *et al*., 2002).

The compound 3-methyl-2-buten-1-thiol was present in more than 75% (n = 16) of the samples taken from males of *L. curasoae* with a dorsal patch in 2006, and from a single male with dorsal patch in 2007. This compound is also present in shoulder glands of males of the Indian fruit bat, *Pteropus giganteus* (Wood *et al*., 2005). A similar compound (3-methylbutan-1-thiol) was reported from facial glands in male ringed seals (*Phoca hispida*) during the breeding season (Ryg *et al*., 1992).

Trimethylamine and acetic acid both have been reported from urine in other bat species (Nielsen *et al*., 2006). Acetic acid, heptanal and 2-butanone were also found in the urine of male and female lions, *Panthera leo* (Andersen and Vulpius, 1999). The latter two compounds were present and exclusive to males of *L. curasoae* with dorsal patches. 2'-aminoacetophenone also was present in the urine of *T. brasiliensis* (Nielsen *et al*., 2006), and the body surface of *E. fuscus* (Bloss *et al*., 2002). The presence of these compounds in dorsal patches of males in *L. curasoae* suggest that, assuming they are also present in the urine and/or urogenital secretion of this species, they are most likely be transferred from

the urogenital region to the dorsal patch with their feet during development and maintenance of the dorsal patch (Muñoz-Romo and Kunz, 2009). Some chemical compounds (indole, trimethylamine, 2'-aminoacetophenone) are also found in the feces of mammals (Wright *et al*., 2005). The fact that these compounds were found in dorsal patches supports the hypothesis that this and other substances are readily transferred to the dorsal patch (Muñoz-Romo and Kunz, 2009). Based on these behavioral observations, urine and/or other urogenital secretions, feces, and saliva are smeared onto the dorsal patch with the feet and thus are fundamental to the development and maintenance of this structure during the courtship and mating period, presumably conferring a unique odor to individual bats based on specific combinations of these body fluids (Muñoz-Romo and Kunz, 2009).

Some compounds found in dorsal patches could be products of the microbiological breakdown of proteins, carbohydrates, and cholesterol, owing to the presence and activity of microorganisms in this region, as has been reported for other odor-producing structures in bats (Mykytowycz and Goodrich, 1974; Dapson *et al*., 1977; Studier and Lavoie, 1984; Scully *et al*., 2000), such as the wing sacs of male *S. bilineata* (Voigt *et al*., 2005). The compound 2-nonanone, present in all samples from dorsal patches (also found in the interdigital secretions of the bontebok, *Damaliscus dorcas dorcas*, and the blesbok, *D. d. phillipsi*), could be a product of microbial activity, as has been synthesized in vitro by *Bacillus brevis* (Burger *et al*., 1999). Studier and Lavoie (1984) suggested that microbes in inguinal pockets of the greater bulldog bat (*Noctilio leporinus*) and lesser bulldog bat (*N. albiventris*) were involved in odor production. Dapson *et al*. (1977) proposed a similar suggestion for the big brown bat (*E. fuscus*) and two free-tailed bat species (*Molossus bondae* and *T. brasiliensis*). Some substances must first be bacterially decomposed, and experience biochemical changes, before they acquire detectable odor characteristics and before they become meaningful signals (Mykytowycz and Goodrich, 1974). Voigt *et al*. (2005) showed that males of the greater sac-winged bat (*S. bilineata*) have unique odor profiles in which bacteria are involved. As suggested by Nassar *et al*. (2008), Muñoz-Romo (2008), and Muñoz-Romo and Kunz (2009), some compounds found in dorsal patches could be products of bacterial activity.

The physiological or behavioral functions, if any, of other common compounds present in dorsal patches (diacetyl, 2-pentanone, 2,3-dimethylpyrazine, acetamide, piperidinone, 2,4-dimethylquinazoline, and 3-methyl-2,5-pyrrolidinedione) remain unknown. However, it is known that the compound 2,3-dimethylpyrazine is a common bacterial volatile (Schulz and Dickschat, 2007).

Odors produced by bats may play an important, although often-underestimated role in mating strategies of males and females (Voigt and von Helversen, 1999). Specific odors of males may be important for female choice (Voigt and von Helversen, 1999; Voigt, 2002) and in addition, chemical signals may provide information on male parasite load (Kavaliers and Colwell, 1993, 1995; Ehman and Scott, 2002; Kavaliers *et al*., 2003), and perhaps provide a signal of the animal's MHC (Major Histocompatibility Complex) genotype (Penn and Potts, 1998). It is unknown whether the combination of chemical substances found in dorsal patches of males of *L. curasoae* are involved in female choice, provide information on health status or the individual's MHC genotype, or provide evidence of a unique identity (Muñoz-Romo, 2008). However, the fact that no two individuals had an identical mixture of compounds suggests that a combination of chemical compounds for each male would provide a chemically unique odor. Alternatively, a specific group of compounds or even an isolated substance could signal unique identities, but this hypothesis remains to be tested. A goal of future research should be to establish the relative importance of each compound in chemical communication in *L. curasoae*, and whether variation in the relative potency of each compound changes during different periods of reproduction or among individuals with different body conditions.

The tentative chemical characterization provided in this study likely does not represent all of the compounds present in dorsal patches. Other compounds will most likely be identified and confirmed. Notwithstanding, this study provides a tentative list of compounds that could be tested separately or as mixtures in experimental trials designed to determine whether and how females respond to these substances in courtship and mating behavior. We also suggest that some compounds present in dorsal patches could provide protection against ectoparasites, and thus are worthy of additional study.

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Appendix I

Tentative identification of chemical compounds in hair samples from dorsal patches of males *L. curasoae* obtained in 2006

Retention time (RT, in min)	Tentative identification	Number of males with compound
1.88	trimethylamine	1
2.06	trimethylamine	9
2.99	1,2-dichloroethene	1
4.03	3-methylbutanal	2
5.55	ethyl N,N-dimethylcarbamate	1
7.83	3-methyl-2-buten-1-thiol	16
8.90	unidentified	1
9.29	3-methyl-3-buten-1-ol	3
10.01	3-hydroxy-2-butanone	3
10.40	2-methylpyrazine	2
12.28	1,8-cineole	4
12.60	2-butoxyethanol	3
12.89	acetic acid	17
13.41	2-ethyl-6-methylpyrazine	1
14.08	2-methyl-3-isopropylpyrazine	14
15.16	isobutyric acid	8
15.30	1-methyl-4-(1-methyl-ethyl)benzene	2
16.63	trimethylethylpyrazine	4
17.75	2-hydroxybenazldehyde	7
17.88	unidentified	1
18.43	2-methylpropyl-acetamide	6
19.28	4-methyl-pentanoic acid	5
19.36	α-cedrol	1
19.47	unidentified	1
19.80	carvone	2
21.47	1-phenylethylethanol	4
22.11	tridecanone	1
22.12	2-bromophenol	1
22.25	2-methylnaphthalene	1
22.35	unidentified	1
22.69	benzyl isovalerate	1
23.58	para-cresol	1
25.41	unknown w/ MW 186	8
25.85	2'-aminoacetophenone	19
27.84	unknown w/ MW 158	1
28.67	an unidentified lactone	2
29.27	indole	2
29.39	γ-undecalactone	9
30.56	2'-aminoacetophenone analog	1
31.32	unknown w/ MW 224	1

Appendix II

Tentative identification of chemical compounds in hair samples from males with dorsal patches (DP), males without dorsal patches (M), and females (F) of *L. curasoae* in 2007. 'X' indicates that the compound is present in the sample; unique compounds for each group are marked in bold

RT (min)	Tentative identification	DP	DP	DP	DP	M	M	M	F	F	F
1.86	methyl mercaptan			**X**	**X**						
1.88	trimethylamine	X	X		X		X				
2.06	trimethylamine	X	X	X	X					X	
2.10	2-methylpentane			X		X	X		X	X	
3.33	2-butanone	**X**	**X**	**X**							
3.51	2-methylfuran	**X**	**X**	**X**							
3.65	2-ethyl-1,3-butadiene				**X**						
4.03	3-buten-2-one						**X**				
4.03	3-methylbutanal				X					X	
4.17	diacetyl	**X**	**X**	**X**	**X**						
4.90	2,5-dimethylhexane					**X**					
4.94	2-pentanone	**X**	**X**	**X**	**X**						
5.55	unidentified		**X**								
6.11	3-methyl-2-pentanone			X	X	X					
6.84	2-methy-2-butenal							**X**			
6.87	dimethyl disulfide			**X**	**X**						
6.85	3-hexanone			**X**							
7.15	4-methyl-2,3-dihydrofuran	**X**									
7.18	2-hexanone	**X**		**X**							
7.41	3-penten-2-one			**X**							
7.70	2-propen-1-ol	**X**	**X**								
7.78	unidentified		**X**	**X**							
7.83	3-methyl-2-butene-1-thiol				**X**						
8.00	unidentified	**X**		**X**							
8.18	pyridine			**X**							
8.69	unidentified			X							X
8.81	5-methylhexanone			**X**							
8.85	3-methyl-2-butenal	**X**		**X**	**X**						
8.90	2,3-dihydro-4-methylfuran		**X**								
8.91	unidentified			X		X		X			
9.30	methylpyrazine				**X**						
9.40	alpha-pinene		X					X		X	
9.45	prenyl formate							**X**			
9.55	1-pentanol	**X**									
9.74	n-heptanal					X	X	X	X	X	X
9.85	3-hydroxy-2-butanone	X	X	X			X				
10.31	ethyl sorbate								X		
10.39	2-methylpyrazine	**X**									
10.60	β-pinene									**X**	
10.95	N,N-dimethylformamide			**X**	**X**						
11.15	β-myrcene		X	X						X	
11.45	2-methyl-3-(methylthio)butane			**X**							
11.71	2,5-dimethylpyrazine	X	X	X	X			X			
11.72	2,3-dimethylpyrazine	**X**	**X**	**X**	**X**						
11.82	3-octanone			**X**							
11.90	2,6-dimethylpyrazine	X		X						X	
11.92	2-octanone			**X**							
12.31	1,8-cineole	X	X		X	X	X	X		X	X
12.89	acetic acid	X	X	X	X	X	X	X	X	X	X
13.02	undecane	X	X	X	X	X	X	X	X	X	X
13.40	furfural		X		X		X				
13.70	trimethylpyrazine	X	X	X	X	X					
14.08	2-methyl-3-isopropylpyrazine	**X**		**X**							
14.25	2-nonanone	**X**	**X**	**X**	**X**						
14.28	2-(methylthio)ethanol	**X**		**X**							

APPENDIX II. Continued

RT (min)	Tentative identification	DP	DP	DP	DP	M	M	M	F	F	F
14.29	ethyl octanoate								X		
14.59	propionic acid	X	X		X	X	X	X	X	X	X
14.70	unidentified	X							X		
15.16	isobutyric acid	X	X	X	X		X			X	X
15.53	benzalmethylamine			**X**							
15.80	3,5-octadien-2-one			**X**							
15.90	dihydro-3-methyl-2(3H)-furan	**X**	**X**								
16.06	a pyrazine?				X			X			X
16.06	menthone		X							X	
16.21	alpha-methylbutyrolactone			**X**	**X**						
16.31	2-piperidinone	**X**		**X**							
16.45	5-methyl-2-(1-methylethyl)cyclohexanone			**X**							
16.51	δ-valerolactone		**X**	**X**	**X**						
16.63	trimethylethylpyrazine			X		X					
16.80	dihydro-3-methyl-2(3H)-furanone isomer	**X**									
17.27	2-butenoic acid							X	X		
17.69	unidentified										**X**
17.80	alpha-terpineol								**X**		
18.09	dihydrocarvone		**X**								
18.10	γ-heptalactone?	**X**									
18.16	valeric acid	X			X		X	X			
18.43	unidentified			**X**							
18.48	acetamide (guanadine?)	**X**	**X**	**X**	**X**						
19.11	2-undecanone	**X**	**X**	**X**	**X**						
19.19	undecanal								**X**		
19.36	alpha-cedrol								**X**		
19.60	3-methyl-2-butenoic acid	**X**	**X**		**X**						
19.70	4-methylpentanoic acid	X	X	X	X		X				
19.55	an aliphatic alcohol	X									
19.69	unidentified				**X**						
19.82	1-decanol								**X**		
20.01	2-methyl-2-butenoic acid	**X**	**X**	**X**							
20.06	1-decanol			**X**							
20.29	an unidentified sesquiterpene								**X**		
20.42	ethyl decanoate								**X**		
20.50	ethylbenzaldehyde				X		X				
20.61	N-(3-methylbutyl)acetamide			**X**							
20.80	an amide?				**X**						
20.82	caryophyllene oxide?								**X**		
21.21	4-methyl-5H-furan-2-one			X			X				
21.30	3-methylbutanamide			**X**	**X**						
21.35	geraniol	X							X		
21.51	dodecanal								**X**		**X**
21.61	3-methylbutyl octanoate								**X**		
21.71	safrol								**X**		
21.88	2-methylnaphthalene	X		X			X				
21.90	unidentified			X				X			
22.09	alpha-neoclovene								**X**		
22.12	unidentified			X			X				
22.31	unidentified								**X**		
22.45	γ-octalactone	X									
22.51	methylnaphthalene	X		X			X				
23.00	valencene								**X**		
23.05	β-bisabolene								**X**		
23.11	ethyl (E),(Z)-2,4-decadienoate								**X**		
23.40	2-tridecanone			**X**							
23.41	β-cadinene								**X**		
23.51	4-methylpentanamide			**X**							
23.79	γ-iso-methylionone			**X**							
23.94	γ-nonalactone	X							X		

APPENDIX II. Continued

RT (min)	Tentative identification	DP	DP	DP	DP	M	M	M	F	F	F
24.15	β-ionone			**X**	**X**						
24.21	ethyl (Z),(Z)-2,4-decadienoate								**X**		
24.51	piperidinone (valerolactam)	**X**	**X**	**X**	**X**						
24.70	ethyl dodecanoate								**X**		
24.71	4-methylquinazoline	**X**	**X**	**X**	**X**						
24.74	2,4-dimethylquinazoline	**X**	**X**	**X**	**X**						
25.03	unidentified								**X**		
25.32	unidentified						**X**				
25.41	unidentified			**X**							
25.98	ethyl (Z)-2-(E)-6-dodecadienoate								**X**		
26.33	2'-aminoacetophenone	X	X	X	X		X				
26.55	ethyl 4-ethoxybenzoate			X	X		X				
26.65	5-dodecenol acetate?			**X**							
26.79	ethyl p-hydroxybenzoate	**X**									
26.80	2-pentadecanone			**X**							
26.90	decanoic acid								**X**		
27.00	4-isobutyl-2-methylquinazoline				**X**						
27.50	3-methyl-2,5-pyrrolidinedione	**X**	**X**	**X**	**X**						
27.53	N,N-diethyl-3-methylbenzamide			X	X		X				
32.60	hydroquinone									**X**	
Total compounds in each individual:		49	37	69	44	11	22	14	32	15	10