Journal of Chemical Ecology (2026) 52:74
https://doi.org/10.1007/s10886-026-01744-0

RESEARCH

Individual Variation in the Chemical Profiles of Odorous Male Bat Crusts

Mariana Muñoz-Romo[1,2,5] · Candelario Rodriguez[3,4,5] · Gregg Cohen[1] · Hermógenes Fernández-Marín[1,4,5] · Rachel A. Page[1]

Received: 20 February 2026 / Revised: 15 July 2026 / Accepted: 17 July 2026 / Published online: 12 August 2026
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2026

Abstract
Visually conspicuous sexually dimorphic traits such as body size, coloration, weapons, and ornaments are less effective in nocturnal mammals, favoring the evolution of signals in alternative sensory modalities. Acoustic and chemical signals, in contrast, function well in low-light environments, and chemical cues in particular may act as honest indicators of individual quality, as physiological condition and immune responses can alter odor profiles. Reproductively active male fringe-lipped bats, *Trachops cirrhosus*, exhibit a sexually dimorphic forearm crust characterized by a strong odor and seasonal enlargement coincident with female fertility. Crust size is positively correlated with testosterone levels, and males with larger crusts are more frequently found roosting with multiple females, suggesting a role in sexual signaling. Previous work identified six major chemical components of the crust. Here, we evaluated inter-individual variation in both chemical composition and compound concentrations of the crust. Gas chromatography – mass spectrometry (GC–MS) analyses revealed twelve volatile organic compounds detected in crust samples, with individual males containing an average of 7 ± 3 chemical compounds. Chemical composition and chemical concentrations varied substantially among individuals, indicating that male crusts are not composed by a determined set or standardized ratios of compounds. Instead, chemical profiles were highly individualized. Crust size was positively associated with both chemical diversity and compound abundance. Larger crusts (>10 mg) contained more compounds and higher overall concentrations than smaller crusts. Cholesterol and Cholest-7-en-3-ol were the most consistently detected compounds across males. Five of six previously reported compounds were confirmed. Concentrations of individual compounds differed by several orders of magnitude among males, and some compounds were absent in certain individuals. These results indicate pronounced individual chemical variability in male forearm crusts, supporting the hypothesis that this trait functions as a complex and individualized chemical signal potentially relevant to mate choice and sexual selection.

Keywords Chiroptera · Mate attraction · Odors · Scent · Sexual dimorphism · *Trachops cirrhosus* · Volatile organic compounds

Mariana Muñoz-Romo and Candelario Rodriguez contributed equally to this work.

Hermógenes Fernández-Marín and Rachel A. Page contributed equally to this work.

✉ Mariana Muñoz-Romo
munozromoml@si.edu

[1] Center for Animal Behavior, Smithsonian Tropical Research Institute, Balboa, Ancón, República de Panamá

[2] Departamento de Biología, Universidad de Los Andes, Mérida, Venezuela

[3] Estación Científica Coiba, Ciudad del Saber, 0816-02852 Ancón, Panamá, República de Panamá

[4] División de Biodiversidad, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología INDICASAT-AIP, Ciudad del Saber, Clayton 0843-01103, República de Panamá

[5] Sistema Nacional de Investigación, Ciudad del Saber, Ancón, República de Panamá

Introduction

The study of mammalian sexual dimorphism has been traditionally focused on a set of characters such as body size, coloration, and canine length (Andersson 1994). While critically important to sexual selection in many taxa, in nocturnal mammals the absence of light often selects for an entirely different suite of sexual dimorphic traits. Visually conspicuous sexually dimorphic characteristics such as body size, color, weapons, and ornaments lose their effectiveness under dark conditions (Altringham and Fenton 2003; Dechmann and Safi 2005) to the point of becoming useless in total darkness. Both sounds and odors, however, function well in the absence of light. Odor especially can be an honest indicator of health condition and overall quality (Andersson 1994; Stevens 2013), because disease can trigger immunological responses that alter an individual's odor and chemical profile (Schmidt 1985; Penn and Potts 1998; Bloss 1999; Johansson and Jones 2007). Elaborated chemical traits are used by a wide range of mammals as sexual advertisement signals, but the use of such signals to date has received less attention (Buchinger and Li 2023).

It has been shown that females of many mammal species use chemical cues for intraspecific and interindividual recognition or discrimination as these vertebrates possess well-developed olfaction (Eisenberg and Kleiman 1972; Blaustein 1981; Bloss 1999; Altringham and Fenton 2003; Dechmann and Safi 2005; Brennan and Kendrick 2006). Female mammals have also been shown to be able to use male odors to detect sickness (Penn and Potts 1998). For example, Zala et al. (2004) experimentally demonstrated that scent marks of the house mouse, *Mus musculus*, function as sexual courtship displays that provide honest indicators of health and disease. Females of this species were able to discriminate the scent marks (containing urine and saliva) of males infected with *Salmonella enterica* versus uninfected males. Males exposed to female odors (i.e., sexually stimulated) marked more frequently which enabled females to assess males' health more effectively (Zala et al. 2004). Consistent results were shown by González-Quiñonez et al. (2011) in a study where female *M. musculus* avoided urine odors from males infected with the blood parasite, *Trypanosoma cruzi*. Across taxonomic groups, studies have shown that a wide range of mammals are able to detect information related to age, social status, sex, sexual receptiveness, group membership, and identity through odors (Blaustein 1981; Müller-Schwarze 1983; Schmidt 1985; Penn and Potts 1998; Bloss 1999).

As nocturnal mammals, bats are excellent candidates for the study of chemical signaling, and indeed, numerous aspects of reproduction in bats are mediated by chemical signals produced, stored, and released by skin glands involved in sexual communication (Quay 1970; Mykytowycz and Goodrich 1974; Muñoz-Romo et al. 2021). The mechanisms underlying these chemical signals and its divergence, however, are still rather poorly understood (Voigt 2013; Zheng et al. 2026). In bats and most mammals, odors and other chemical signals are usually sexually dimorphic, with stronger or distinctive odors found in males (Eisenberg and Kleiman 1972; Blaustein 1981; Müller-Schwarze 1983; Andersson 1994; Andersen and Vulpius 1999; Brennan and Kendrick 2006; Stowers and Logan 2010; Muñoz-Romo et al. 2021).

Although bat odors are still poorly studied in this extraordinarily diverse group of mammals, the Neotropical greater sac-winged bat *Saccopteryx bilineata*, has been extensively investigated in this context. Males *S. bilineata* perform wing displays towards females involving enlarged odoriferous wing sacs filled with different fluids (Voigt and von Helversen 1999) that become active signals after bacterial decomposition (Voigt et al. 2005). Variation in male scent profiles was observed between seasons and between individuals in the relative amount of chemical substances, indicating the potential of their scent to convey multiple information (Caspers et al. 2008), potentially including age as age-specific differences in male-specific substances were also found (Schneeberger et al. 2016). Likewise, harem male profiles also differed significantly from those of bachelor males roosting without females in the greater spear-nosed bat, *Phyllostomus hastatus*, and profiles differed significantly among individuals as well, which would suggest that both mating status and individual identity is informed through their scent (Adams et al. 2018). Individual identity, dominance rank, and physiological quality is also informed through olfactory signals in the great Himalayan leaf-nosed bat, *Hipposideros armiger,* as significant differences in type of compounds and concentrations were found between males (Sun et al. 2025). Bacteria seem to be critical for some bat species as microbiome can shape the host's olfactory phenotype, and thus potentially influence sexual selection (Maraci et al. 2024; Voigt et al. 2005). Males of the greater sac-winged bat, *Saccopteryx bilineata,* were found less diverse than females in terms of microbial richness, but males had more bacteria that might potentially be involved in odor production (Maraci et al. 2024). Besides microbiome, changes in temperature, humidity, diet composition, and/or pathogen activity can explain annual differences in chemical profiles (Schneeberger et al. 2016; Zala et al. 2004).

Some bat species have been shown to create and display non-glandular, odorous structures that appear as the outcome of complex stereotyped behaviors (Muñoz-Romo et al. 2021). A few years ago, a novel odorous crust was discovered on the forearms of male fringe-lipped bats,

Trachops cirrhosus (Flores and Page 2017). This unique dimorphic trait is found only in adult males, and is created by the individual following a complex stereotypical behavior of smearing both forearms with a thick, yellow, odorous liquid that appears to come out of the mouth. The thick substance completely dries and becomes an amber-like yellow odorous forearm crust (Fig. 1) whose size is positively correlated with testosterone levels (Muñoz-Romo et al. 2020), an important sexual hormone for reproduction, generally associated to the development of conspicuous dimorphic male traits in many vertebrates (Andersson 1994).

Because crusts appear to be larger during the mating season (Flores and Page 2017) and show a positive relationship with testosterone levels when females are fertile (Muñoz-Romo et al. 2020), it has been speculated that crusts play a critical role in reproduction. The odor of the crusts is so strong during mating season that it can be perceived by humans from a distance of several meters away in the forest, allowing researchers to locate places where adult males roost. Experiments using y-maze tests in captivity did not show female preference for crust odor (Flores et al. 2019), but most adult males with enlarged crusts (67%) have been observed roosting with several females (Flores et al. 2020). This combined evidence (i.e., dimorphic male trait, intense odor, and the association of groups of females with males displaying enlarged crusts) led us to consider that this trait has a relevant reproductive function.

Chemical assays indicated six important chemical components in the crust: 2-aminoacetophenone, 4-methylquinazoline, Cholesterol, 5,6-dihydro-6-propyl-2 H-pyran-2-one, 2-methylquinoline, and Squalene (Flores et al. 2019). This analysis involved samples from five males, but the results were pooled; the study did not investigate the potential for individual differences in chemical composition of the crust. It is unknown whether all males consistently display the same chemicals in equivalent concentrations and/or proportions in their crusts, or whether males differ in their individual chemical profiles. Examining precise chemical components and their associated concentrations in the crusts of different individuals is key to achieving a better understanding of the expression of this intriguing secondary sexual trait.

During the mating season the crust not only reaches larger sizes, but also it is easier to notice from long distances

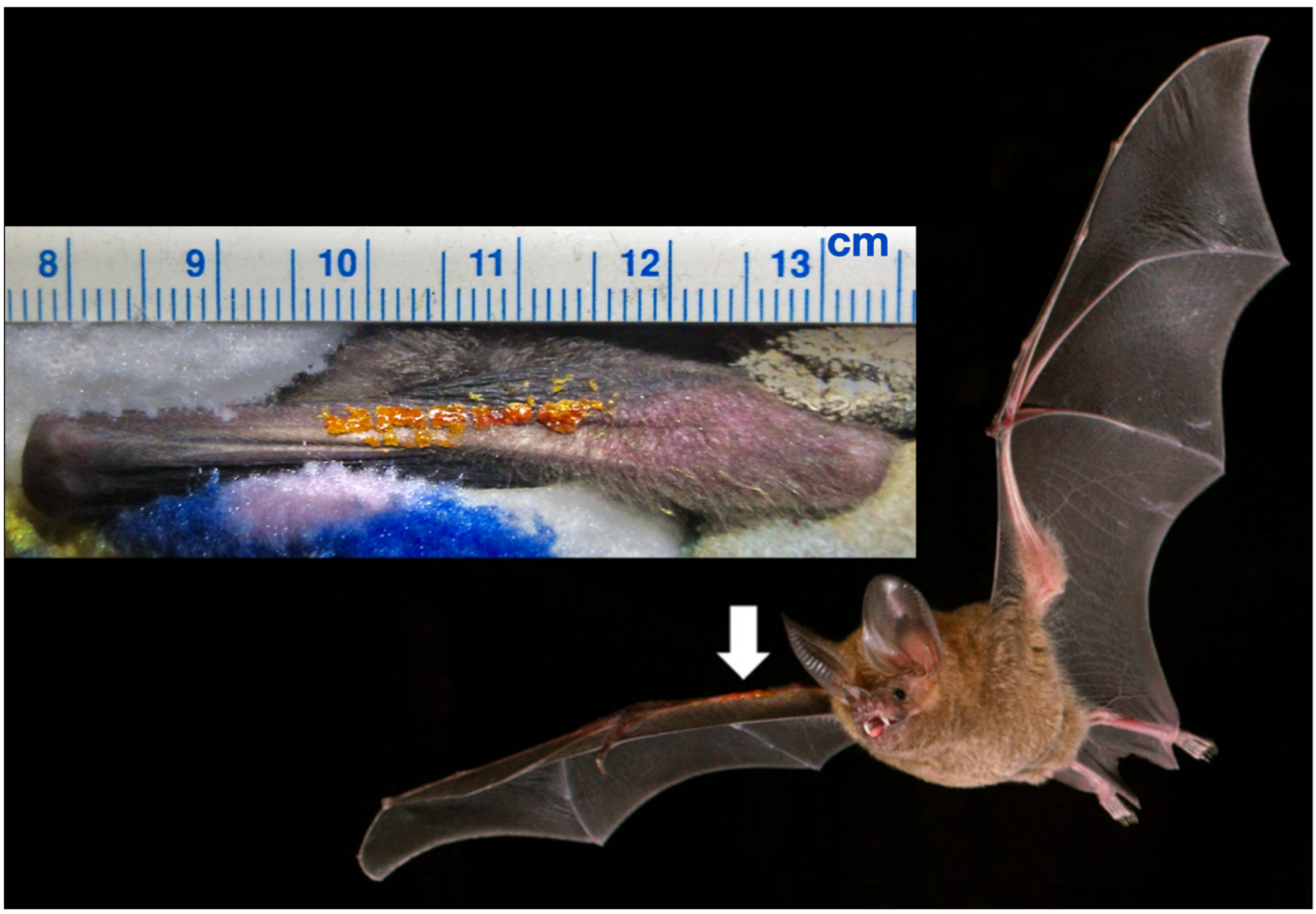

Fig. 1 An enlarged crust on the forearm of an adult male fringe-lipped bat, *Trachops cirrhosus* (photo: Marko König, http://www.koenig-naturfotografie.com), and detail of the crust with scale bar in millimeters (photo: this study)

by a more intense odor during this period. Thus, we hypothesized that the crust in *T. cirrhosus* is a secondary sexual characteristic produced by males and used by females in mate choice. Our goals were to: (1) assess the variability of crusts' chemical composition/concentration between males, (2) determine whether males are chemically different from each other, but individually consistent through time if captured repeatedly, including during mating and non-mating seasons, and (3) explore the relationship between crust size and chemical composition/concentrations. We predict that crust size would show a positive relationship with chemical compounds concentration. We further expected that this positive relationship between chemical compound concentration and crust size would differ between males. Since males show individual variation in biological traits, crusts were also expected to vary in their chemical composition and concentrations, making males unique despite shared chemical compounds. We also predicted that concentrations would increase during the mating season because crust odor is much more evident during the reproductive season. Finally, we expected larger crusts to contain not only more chemicals, but higher concentrations as well.

Methods

Study Species

T. cirrhosus, the fringe-lipped bat, is widespread in tropical dry and moist forests of Central and South America, from southern Costa Rica to Brazil, typically found at elevations of 500 m to 1,400 m near ponds and streams (Cramer et al. 2001; Da Silva Fonseca et al. 2024). Fringe-lipped bats roost in small mixed-sex groups usually of 1 to 13 individuals consistent with a polygynous mating system (Flores et al. 2020) in hollow trees, culverts, buildings, and caves (Cramer et al. 2001). Long-term observations (2012–2017) indicate that groups often switch roosts, but have preferred roosting partners (Flores et al. 2020). The mating season occurs from mid-October to December, and most offspring are born at the end of the dry season (approximately May in Panama; Flores and Page 2017), which suggests that individuals reproduce just once per year. Gestation length is unknown, but a five-month period is expected (Flores and Page 2017). Flores and Page (2017) described the stereotypical forearm licking performed by males to create the crust that show a positive association with testosterone concentration (Muñoz-Romo et al. 2020). Male bats scratched their whole body with one of the hind claws, repeatedly put this same claw into the mouth to lick and nibble it, and then licked one of the forearms several times.

Captures and Sampling

In our study, bats were captured at their roosts between 11:00 and 13:00 h from October to December 2018 (9 samples), March (1 sample) and October (1 sample) 2019, September 2021 (1 sample), and June-October 2022 (6 samples). All sampling sites were located near and/or in Soberanía National Park, Panama (9°07'42.6"N 79°42'55.2"W). Several roosts were visited and mistnets were placed to capture *T. cirrhosus*, in accordance with permits from the STRI Institutional Animal Care and Use Committee (IACUC: 2018-0901-2021) and the Panamanian Ministry of the Environment (SE/A-78-18).

Following capture and crust sample collection (see below), bats were held separately in soft cloth bags until processed. Individuals were examined to determine additional details such as reproductive status (i.e., enlarged testes; Racey 2009), body mass (Ohaus Cs 200.0 g x 0.1 g), and forearm length (Fowler Sylvac Ultra-Cal Mark IV/0–150 mm). Age was confirmed by examining the relative ossification of the joint bones of the fourth finger of the wing (Brunet-Rossini and Wilkinson 2009). Bats were marked with passive integrated transponder tags (Biomark, Boise, ID) to allow for identification of recaptured bats in successive capture events. Some bats were already marked and those marked males with crusts were sampled again when possible to compare chemical profiles. After obtaining all measurements and samples (see below), bats were released at their site of capture.

Samples were taken immediately after capturing the bats and before processing them, and the whole crust sample (Fig. 1) of each male was stored in a separate vial and immediately frozen, until their analysis were conducted. The size of crust in this study was taken as the amount in mg of the deposited dry crust, obtained using an analytic balance (readability 0.00001 g).

Chemical Analysis

Crust samples were macerated with 1.5 mL of dichloromethane and sonicated by 10 min in glass vials. Supernatants were filtrated through a glass fiber employing Pasteur pipettes. The macerated was then dried under a nitrogen beam, resuspended in 50 µL of dichloromethane (HPLC spectra grade) and transferred to inserts of 250 µL in ALS vials.

Samples were homogenized under infrared light and 2 µL were injected by splitless mode into a 6890 N gas chromatograph (GC) coupled to a 5973 N mass spectrometer (MS) (Agilent technologies, Palo Alto, CA, USA). For chromatography analysis, we employed automatic injections by the

automatic liquid sampler and all samples were run in batch mode. Injection port was set at 250 ˚C, helium at 1 mL/minute was used as the gas carrier, a capillary column HP-5MS (Agilent, 0.25 mm x 30 m x 0.25 µm) was employed and elution of the volatile compounds was achieved with the next temperature program: 60 °C for 3 min, a ramp of 5 °C/minute until 280 °C and kept isothermally by 5 min (52 min of total running time). GC-MS transfer line was set at 280 °C and molecules were ionized by electron impact at 70 eV of energy. Before recording the compounds, we employed a solvent delay of 3 min and then ions were analyzed in scanning mode from 30 to 550 *m/z* with the MS source at 230 ˚C and quadrupole set at 150 °C.

GC-MS chromatograms were analyzed by the software Agilent Mass Hunter (version 7.0) and compounds were identified employing the standard mass spectra library of the National Institute of Standards and Technology (NIST, version 2.0). Chromatographic peaks were analyzed individually and considered as a hit as those with more than 75% of match with the library's mass spectra. Linear retention index was calculated experimentally by comparing retention times with those from an alkane series (C8-C36). Absolute concentration of the compounds was determined by peak area comparison employing methyl-salicylate as internal standard added to all samples at 50 ng/mL. Zero (0) was assigned for undetected chemicals.

Metabolomic Analysis

To investigate the metabolome occurring in the bats´ crust we used a metabolomic approach based on mass spectrometry. Global Natural Products Social Molecular Networking (GNPS) is an open-source tool which can identify chemicals in complex mixtures. Metabolites appear as circles (nodes) and compounds can be grouped since their chemical similarity in molecular families. In addition, quantitation of chemicals can be done by displaying the relative abundance in terms of size of the node. We used the GNPS to conduct all the metabolomic analysis. In brief, GC-EIMS raw data files (.d) were transformed to the mzxml format employing the vendor converter from GNPS. To deconvolute chromatograms and mass spectra, we employed the free software mzmine (version 2.53). The resulting files (.csv and .mgf) were uploaded to the GNPS workflow. The precursor ion mass tolerance was set to 20,000 Da and the MS/MS fragment ion tolerance to 0.5 Da. A molecular network was then created, where edges were filtered to have a cosine score above 0.9 and more than 7 matched peaks. All matches kept between network spectra and library spectra were required to have a score above 0.50 and at least 7 matched peaks. The molecular networks were visualized using the Cytoscape software (version 3.9.1) (Wang et al. 2016).

Statistical tests

Analyses included only the concentrations of identified compounds. A Shapiro-Wilk test (Daniel and Cross 2013) was used to assess normality of the variables. Due to the lack of normality of crust size and chemical compound concentrations, we used Spearman's correlation coefficients to assess significant relationships between them, visualized through scatterplots (Daniel and Cross 2013). Cluster analysis (PAST; Hammer et al. 2001) was performed with the Jaccard similarity index (Real and Vargas 1996), a binary index (1: compound present; 0: compound absent) to confirm composition similarities between samples, and with Ward's method, which groups the samples by minimizing the dispersion (concentration) in each group (Murtagh and Legendre 2014). A principal component analysis (PCA) was conducted on the concentrations of the 12 chemical compounds found. Variables were standardized prior to analysis (mean = 0, standard deviation = 1). To characterize the relationship between each chemical compound and the extracted components, variable-PC correlations were computed as the product of the eigenvector coefficients and the square root of the corresponding eigenvalue (aij = vij x sqrt(lambda_j), where vij is the i-th component of the j-th eigenvector and lambda_j is the j-th eigenvalue. This yields the Pearson correlation between each original variable and each principal component score. To assess the relationship between the chemical profile summarized by PC1 and the crust, Pearson correlation was computed between individual PC1 scores and crust size. Descriptive analysis and correlations were performed with JASP (JASP Team, 2023).

Results

Variability of Crust Chemical Composition Between Males

A total of 18 crust samples from adult males of *T. cirrhosus* were analyzed from 14 individuals. Crust samples (Fig. 1) varied between 0.3 and 22 mg of weight. It was possible to obtain samples from one male three times on different dates (1 A, 1B, 1 C), and from two males twice on different dates (5 A, 5B and 12 A, 12B). These samples from the same individuals were used to assess/understand the variation of chemical profile of the same individual.

Chemical analysis by GC-MS allowed for the identification of twelve volatile organic compounds detected in crust samples from males *T. cirrhosus* that included six classes of compounds: acids, alcohols, alkenes, amines, esters and ketones (Table 1). According to the abundance, alcohol corresponded to the major class followed

Table 1 List of volatile organic compounds tentatively identified in CH_2Cl_2-extracts of crusts from *T. cirrhosus*

No.	Compound Name	Class	Retention Time (min)	LRI*	Molecular Formula	Molecular Mass (Da)	Major Ions (m/z)	Mean Concentration (ng/mL)
1	4-Nonanone, 7-ethyl-	ketone	5.3	1030	$C_{11}H_{22}O$	170.29	43, 84, 86	11.30
2	3-Nonen-2-one	ketone	7.1	1113	$C_9H_{16}O$	140.22	55, 84, 125	8.94
3	2 H-Pyran-2-one, 5,6-dihydro-6-pentyl-	ester	9.6	1217	$C_{10}H_{16}O_2$	168.23	97, 68, 69	61.70
4	Quinoline, 2-methyl-	amine	10.9	1271	$C_{10}H_9N$	143.18	143, 115, 128	63.21
5	Quinazoline, 4-methyl-	amine	11.8	1304	$C_9H_8N_2$	144.17	144, 129, 103	166.86
6	Formamide, (2-acetylphenyl)-	ketone	16.0	1476	$C_9H_9NO_2$	163.17	120, 135, 92	152.54
7	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	ester	24.6	1875	$C_{17}H_{24}O_3$	276.40	205, 57, 175	3.52
8	11-Eicosenoic acid	acid	31.7	2273	$C_{20}H_{38}O_2$	310.50	55, 69, 83	24.90
9	Squalene	hydrocarbon	39.4	2785	$C_{30}H_50$	410.70	69, 81, 41	25.61
10	Cholesterol	alcohol	42.5	3027	$C_{27}H_{46}O$	386.70	386, 275, 301	414.68
11	Cholesta-5,7-dien-3-ol, (3β)-	alcohol	43.0	3065	$C_{27}H_{44}O$	384.63	351, 384, 325	133.73
12	Cholest-7-en-3-ol, (3β,5α)-	alcohol	43.1	3071	$C_{27}H_{46}O$	386.65	386, 255, 371	200.76

*Lineal Retention Index is determined experimentally

by amines, ketones and esters. Acids and alkenes were found at lower levels in proportional concentrations. Other chemical compounds identified were also present in single individuals (Table 2).

From the list of twelve identified substances, crust samples from males had 7±3 chemical compounds (Fig. 2). Only Cholesterol was present in all samples, followed by Cholest-7-en-3-ol, (3β,5α)- present in all samples except one. The chemical compound 11-Eicosenoic acid was only present in three males (Fig. 2).

Chemical Compounds Concentration

Chemical compounds concentrations varied between a minimum of 2.6 and a maximum of 1616.74 ng/mL ($n=18$, Fig. 2).

Table 2 List of organic compounds tentatively identified in CH_2Cl_2-extracts of crust from single individuals

Individual	Compound Name	Class	Retention Time (min)	LRI	Molecular Formula	Concentration (ng/mL)
1 A	Ethanone, 1-(2-aminophenyl)-	Ketone	10.8	1264	C_8H_9NO	10.5
1 A	Hexadecane, 1-(ethenyloxy)-	hydrocarbon	28.4	2077	$C_{18}H_{36}O$	7.5
1 A	Erucic acid	Acid	34.9	2471	$C_{22}H_{42}O_2$	29.3
1 A	α-Amyrin	Alcohol	45.1	3233	$C_{30}H_{50}O$	9.0
4	Cholest-4-ene, 3β-(methoxymethoxy)-	hydrocarbon	43.9	3133	$C_{29}H_{50}O_2$	6.1
5B	7-Quinolinol	Alcohol	10.1	1236	C_9H_7NO	6.0
6	trans-2-Undecenoic acid	Acid	11.5	1292	$C_{11}H_{20}O_2$	0.7
6	Gephyrotoxin 195b	Amine	19.0	1605	$C_{13}H_{25}N$	3.4
6	Gephyrotoxin 181b	Amine	19.4	1623	$C_{12}H_{23}N$	0.7
6	Tetradecanoic acid, ethyl ester	Ester	22.6	1774	$C_{16}H_{32}O_2$	0.6
6	Hexadecanoic acid, ethyl ester	Ester	26.5	1974	$C_{18}H_{36}O_2$	1.9
6	15-methyl-hexadecanoic acid, ethyl ester	Ester	27.6	2033	$C_{19}H_{38}O_2$	0.7
6	Heptadecanoic acid, ethyl ester	Ester	28.3	2071	$C_{19}H_{38}O_2$	0.8
6	Oleic acid, ethyl ester	Ester	29.5	2138	$C_{20}H_{38}O_2$	1.7
6	Octadecanoic acid, ethyl ester	Ester	30.0	2167	$C_{20}H_{40}O_2$	3.0
6	Campesterol	Alcohol	43.9	3133	$C_{28}H_{48}O$	1.2
6	Lanost-8-en-3-ol, (3β)-	Alcohol	45.1	3233	$C_{30}H_{52}O$	1.9
9	4-Nonanol, 4-methyl-	alcohol	8.4	1168	$C_{10}H_{22}O$	5.3

*Linear Retention Index calculated experimentally

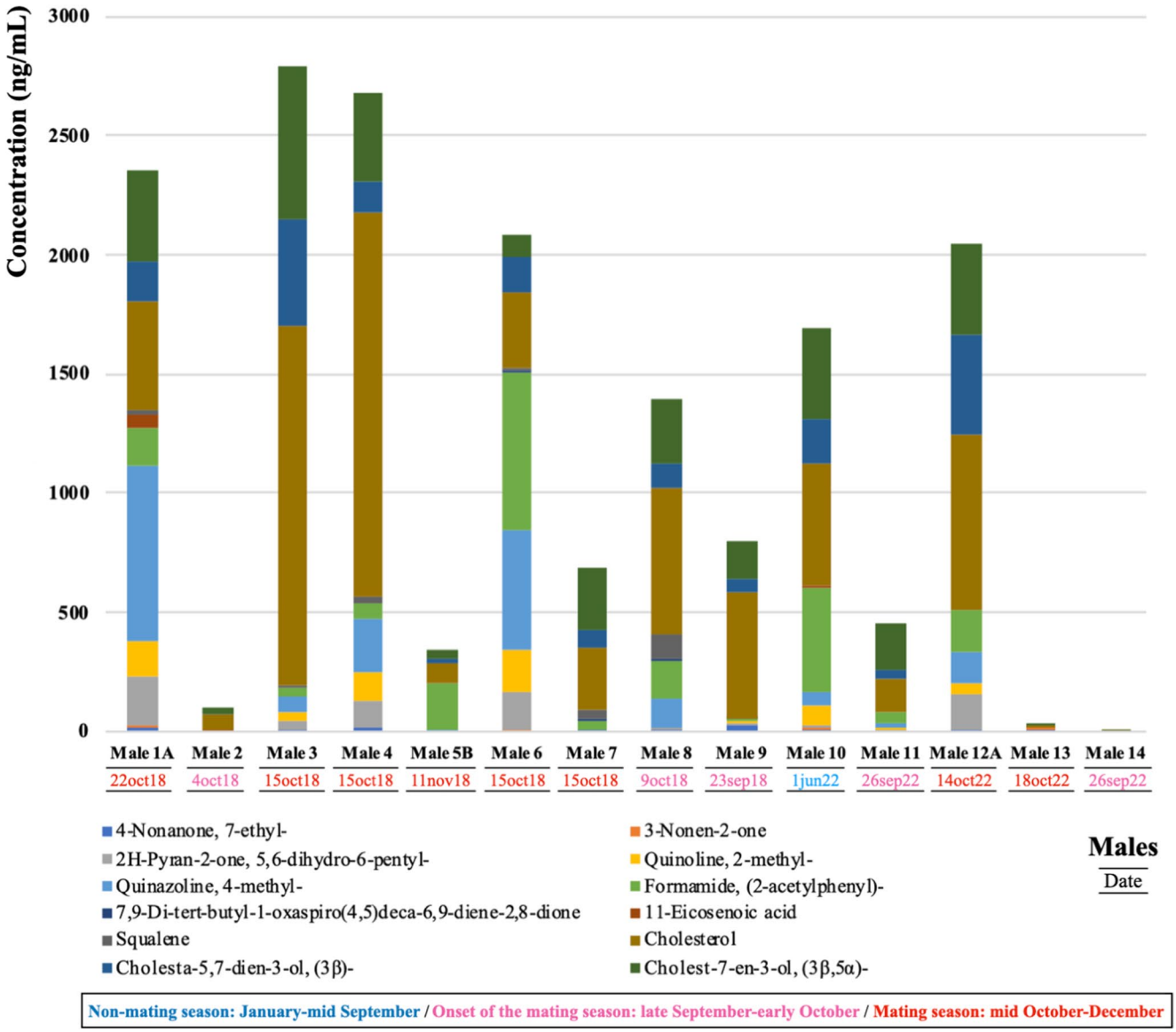

Fig. 2 Variability in crust chemical composition and concentration from 14 individual males sampled during this study. All males showed varied concentrations of chemical compounds representing unique profiles, despite common chemical compounds

Similarity Between Male Crust Samples

Samples from the same males (male 1: A, B, C; male 5: A, B; male 12: A, B) captured in different dates were not similar in chemical composition in none of all three cases (three samples of male 1: 1A, 1B and 1C; two samples of male 5: 5A and 5B; two samples of male 12: 12A and 12B) (Fig. 3A). Larger crust samples tend to be more similar in composition.

Two pairs of different males (male 5A and male14; male 3 and male 4) showed identical composition (Fig. 3A), but no identical samples existed in terms of chemical concentration (Fig. 3B). Some samples from males with samples from different dates (i.e., 1, 5, or 12) are closer (Fig. 3B) in terms of concentrations (5A and 5B; 1B and 1C) but not in composition (Fig. 3A). Smaller crust samples tend to be more similar in terms of concentrations.

Variation in Chemical Compounds Over Time

Concentrations of identified substances increased towards the mating season (Fig. 4). In fact, the same individual (Male 1) had the lowest chemical compound concentrations observed during the non-mating season. Males showed increased concentrations during the mating season compared to the onset of the mating season (Fig. 4), indicating changes in chemical compound concentrations several times larger than before in a few weeks. Of all identified substances present in these

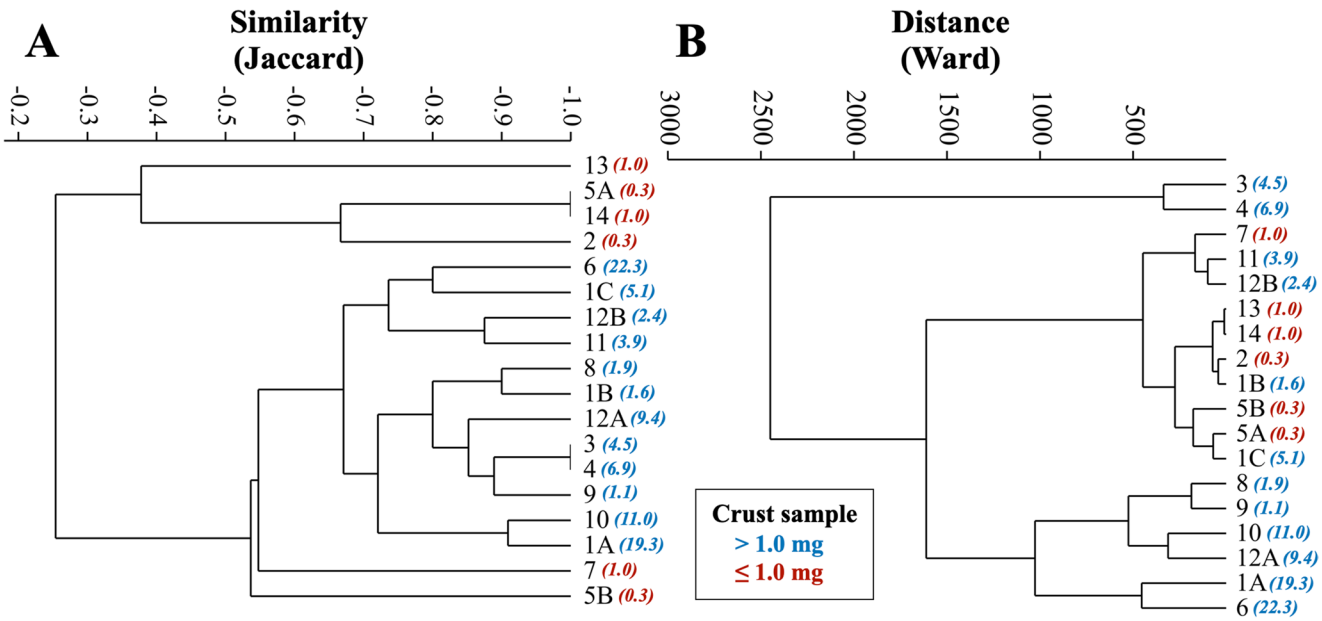

Fig. 3 Cluster analyses showing similarity between crust chemical composition (A), and compound concentrations (B). Note that one male was sampled three times in different dates (1A, 1B, 1C), and two males were sampled twice in different dates (5A, 5B and 12A, 12B), but they are not grouped together except once (see 5A and 5B in B) when forming a group with more similar concentrations, but also including a different individual (1C) with them. Sample size (mg) is listed in parenthesis

samples, only concentrations of Formamide, (2-acetylphenyl)- and Cholest-7-en-3-ol, (3β,5α)- were similar in both males during the mating season.

Type of Identified Compounds

Molecular families detected among males correspond to acids, alcohols, amines, carbonyl (aldehydes and ketones), esters, ethers, and hydrocarbons. Chemical compounds that do not fix with the GNPS´s data bases are displayed as unknowns (Fig. 5). Males 1 and 12 (same males in Fig. 4) showed different chemical profiles during the mating season (Figs. 1A and 5 A), the onset of the mating season (Figs. 5 and 12B) and the non-mating season (Figs. 1B and 5). An increase of alcohols and carboxylic compounds was identified in individual 1, while a reduction followed by an increase was detected for amines. Likewise, individual 12 (12 A) showed five nodes of the amine family during the mating season, but it was not detected any amine previously in the onset of the mating season (12B). Thus, amines were present in both males (male 1, male 12) during the mating season.

Crust Size and Chemical Composition

We found a positive relationship ($R^2 = 0.64$) between crust size and number of identified chemical compounds (Fig. 6). Samples of less than 1 mg contained up to five chemical compounds, whereas samples near 20 mg showed eleven chemical compounds. Crusts over 10 mg always contained 10 or more chemical compounds.

Crust size and Chemical Compounds Concentrations

The first two principal components explained 61.4% of the total variance (PC1: 39.6%; PC2: 21.8%). The positive correlation of each compound with PC1 was strongest for 2 H-Pyran-2-one, 5,6-dihydro-6-pentyl- ($r = 0.893$), Quinoline, 2-methyl- ($r = 0.891$) and Quinazoline, 4-methyl- ($r = 0.859$), all significant at $p < 0.001$. Moderate positive correlations were found for Cholest-7-en-3-ol, (3β ,5α)- ($r = 0.698$), Cholesta-5,7-dien-3-ol, (3β)- ($r = 0.675$), Cholesterol ($r = 0.609$), cis-11-Eicosenoic acid ($r = 0.594$), Formamide, (2-acetylphenyl)- ($r = 0.535$) and 4-Nonanone, 7-ethyl- ($r = 0.497$), with $p < 0.05$ for all except 3-Nonen-2-one ($r = 0.417$, $p = 0.085$), which did not reach statistical significance. The compounds 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (r = -0.186, $p = 0.459$) and Squalene ($r = 0.142$, $p = 0.574$) showed no association with PC1. PC1 scores correlated strongly with crust size ($r = 0.87$, $p < 0.001$), indicating that the chemical axis defined primarily by 2 H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Quinoline, 2-methyl- and Quinazoline, 4-methyl- is closely associated with crust size.

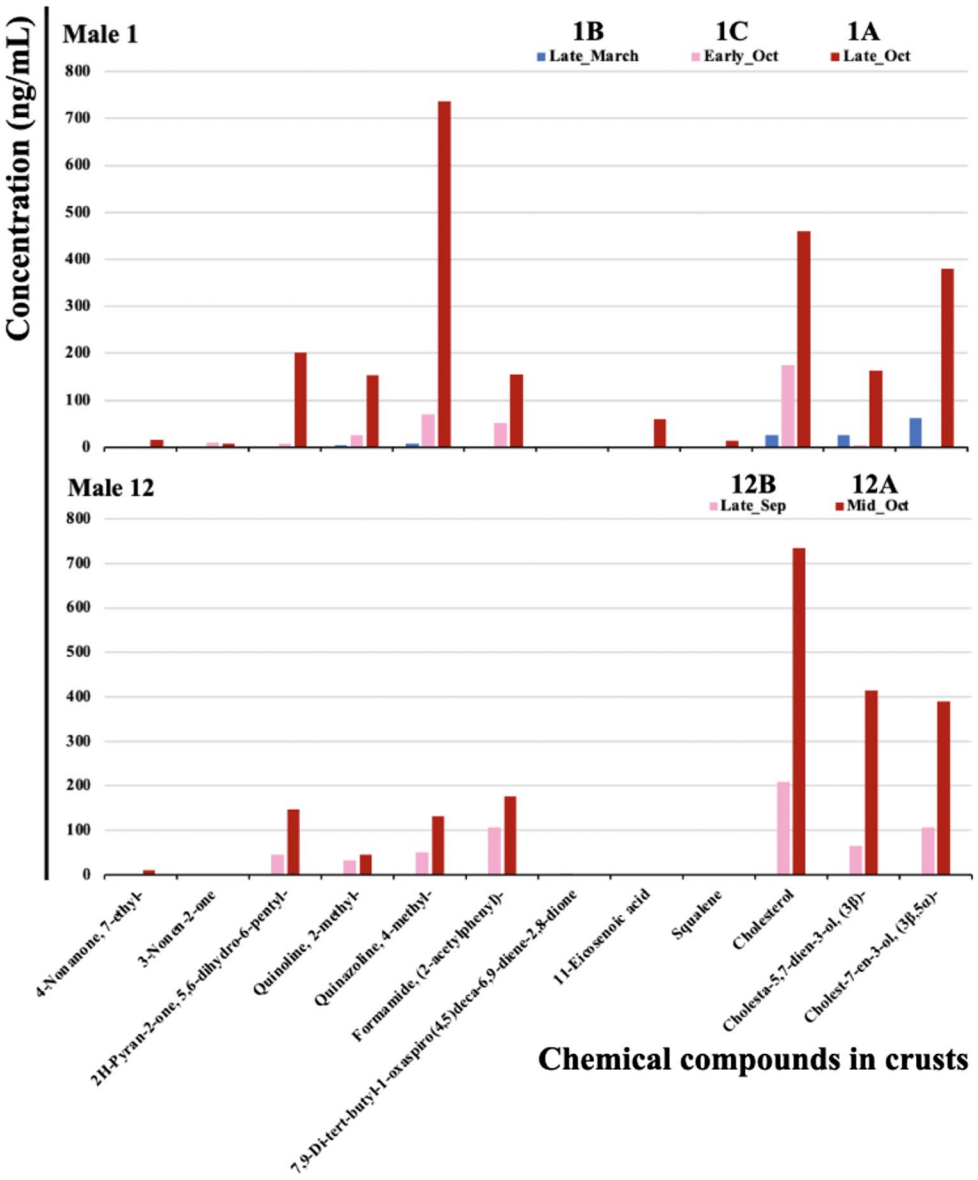

Fig. 4 Variability in crust chemical composition and concentrations in males 1 and 12 (male 5 not included due to very low compound concentrations) during the onset of the mating season (pink; late September - early October), the mating season (red; mid October - December), and the non-mating season (blue; January-mid September)

Discussion

Results from this study confirmed the striking variability in size of odorous forearm crusts of adult male fringe-lipped bats as showed in previous studies (Flores and Page 2017; Muñoz-Romo et al. 2020). This pronounced variability was also consistent with both chemical composition and chemical concentrations. Our results indicate that there are no identical crusts in composition and concentrations, and even for the same individual male, the crust seems to be a 'different' chemical trait every time a male creates it. If our hypothesis is supported, this means that a male crust is not composed by the same list of compounds in specific, consistent concentrations. An average of seven substances from the list of identified chemical compounds in highly variable concentrations is present in crusts of male fringe-lipped bats, in which Cholesterol and Cholest-7-en-3-ol, (3β,5α)- are usually present. Samples taken years ago (i.e., 2018) still have as many chemicals as fresh ones (i.e., 2022) which proved an effective storage method for crust samples. A critical component of crust chemical variability seems to be its size: small crusts contain fewer chemicals than large crusts, and those enlarged ones (i.e., >10 mg) likely contain not only the largest amount of chemicals, but also higher concentrations of them, in particular 2 H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Quinoline, 2-methyl-, and Quinazoline, 4-methyl-. Two of these chemical compounds have also been reported in reproductive males of other bat and mammal species (McLean et al. 2021; Muñoz-Romo et al. 2012) suggesting a relationship with reproduction. The compound Quinazoline, 4-methyl- was present in all samples from sexually dimorphic dorsal patches of

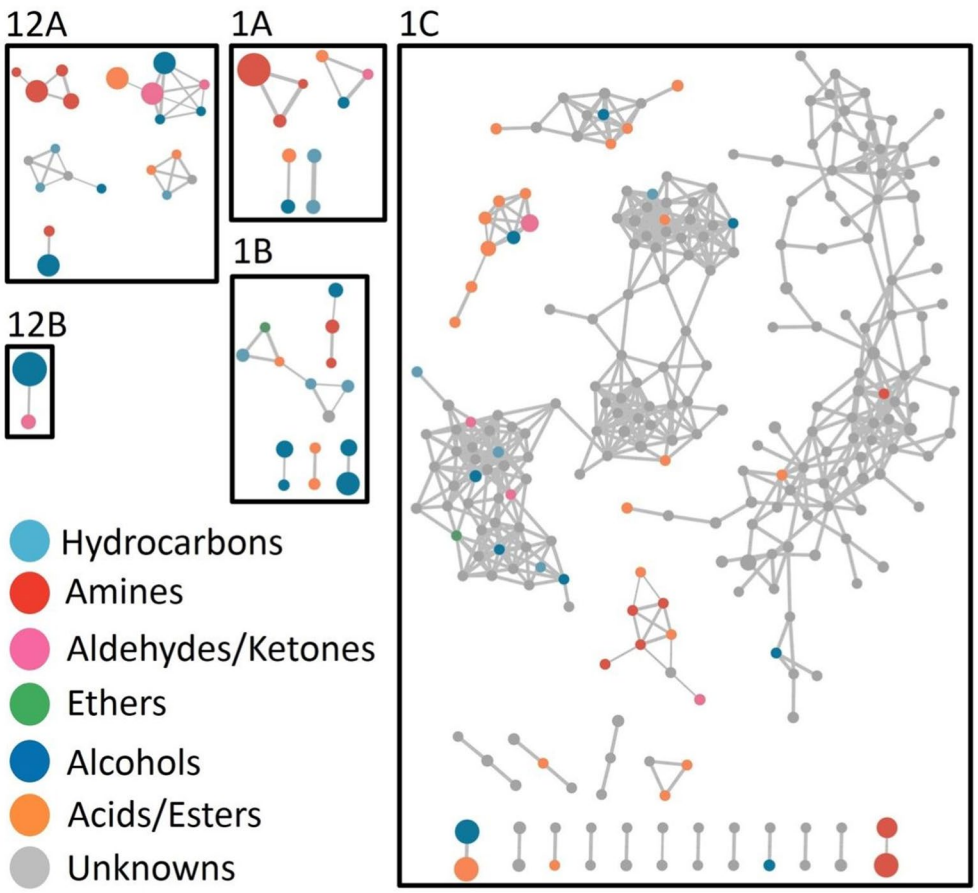

Fig. 5 Molecular networks showing volatile organic compounds of crust extracts from male *T. cirrhosus*. Intensity is proportional to spectral counts (number of mass spectra registered). Samples 12A and 12B corresponds to male 12 captured during the mating season (12A) and on the onset of the mating season (12B). Samples 1A, 1B, and 1C corresponds to male 1 captured during the mating season (1A), the non-mating season (1B), and on the onset of the mating season (1C). Note these are the same males in Fig. 4

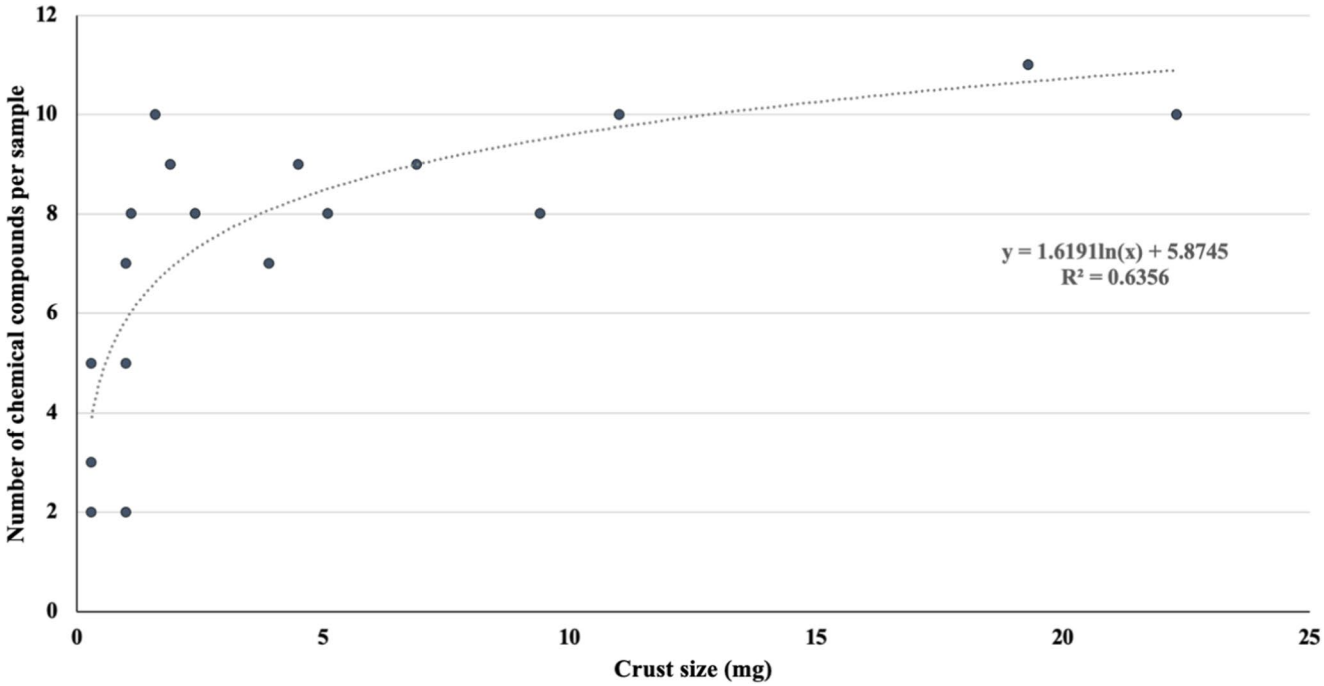

Fig. 6 Relationship between crust size and number of chemical compounds. Larger crusts showed more chemicals compounds. The curve tends to stabilize between 5 and 10 mg and approaches the horizontal asymptote after crusts weighing 10 mg

reproductive males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae,* obtained during the mating season of 2007 (Muñoz-Romo et al. 2012). Similarly, the compound Quinoline, 2-methyl- was reported in urine of the red fox, *Vulpes vulpes* (McLean et al. 2021) and the ferret, *Mustela furo* (Zhang et al. 2005), and also in anal sacs of the skunk, *Mephitis macroura* (Wood et al. 2002).

Five of the six chemical compounds of crusts tentatively identified in a previous contribution (Flores et al. 2019) were confirmed in our study. These chemical compounds are: 2 H-Pyran-2-one, 5,6-dihydro-6-pentyl-; Quinoline, 2-methyl-; Quinazoline, 4-methyl-; Squalene; and Cholesterol. According to mass spectra analysis by NIST, Formamide, (2-acetylphenyl-) has a molecular weight of 163 Da, but Flores et al. (2019) found a compound with the same molecular weight as this (163), but instead they reported it as 2-aminoacetophenone which has a molecular weight of 135 Da. Concentrations of many identified chemical compounds increased towards the mating season reaching the highest values during this period, a consistent observation with the higher concentrations observed in some fatty acids in male greater sac-winged bats (*S. bilineata;* Caspers et al. 2008) or fatty aldehydes in male ring-tailed lemurs (*L. catta*; Shirasu et al. 2020) during the mating season than during the non-mating season. Cholesta-5,7-dien-3-ol, (3β)- is one of the identified chemical compounds that reaches its highest concentration during the mating season, and it is positively correlated with crust size. Experiments involving Iberian rock lizards, *Lacerta monticola*, confirmed that females were able to discriminate cholesta-5,7-dien-3-ol from similar steroids also found in secretions of males, and were more attracted to higher concentrations of this chemical compound, suggesting that secretions with higher proportions of cholesta-5,7-dien-3-ol might be reliable advertisements of male quality, which females could use to select mates (Martín and López 2006). Similarly, in male Iberian wall lizards, *Podarcis hispanica,* only males with a good immune system may allocate higher amounts of cholesta-5,7-dien-3-ol in femoral secretions to signaling, and females selected scents of males with higher proportions of cholesta-5,7-dien-3-ol. Producing this compound is energetically costly, providing females with an honest indicator of a stronger T-cell-mediated immune system (López and Martín 2005). The increase of concentrations of identified chemical compounds during the mating season in fringe-lipped bats should be further confirmed, validated and monitored based on the analysis of larger samples of males, also assessing the presence and role of amines in odor profiles during this period.

Variability in chemical composition between samples was remarkable as some males had several compounds while others only two or three –a difference likely related to the size of their crust. Some individuals were grouped together likely because these were the largest samples, which would be consistent with the observation that the larger the crust, the richer the chemical profile, but also the higher the concentrations. Variability in chemical compound concentrations showed differences of several orders of magnitude between samples, and even some of them were undetected. High variability in chemical profiles has been observed in other bat species and could be involved in signaling individual identity (Caspers et al. 2008; Adams et al. 2018; Sun et al. 2025), which could also increase variability of mating options. Chemical profiles differed significantly among males of the greater sac-winged bat, *Saccopteryx bilineata* (Caspers et al. 2008), involving variation between seasons and between individuals suggesting that the wing-sac substance represents a composite trait conveying multiple information. Male profiles also differed significantly among harem males of the greater spear-nosed bat, *Phyllostomus hastatus,* that defend and roost with females, and bachelor males roosting in all male groups, but also found that profiles differed significantly among individuals, suggesting that both mating status and individual identity is conveyed through their chemical signal (Adams et al. 2018). Recently, significant differences in the concentrations and type of compounds were found between males of the great Himalayan leaf-nosed bat, *Hipposideros armiger*, indicating that olfactory signals could transmit information about individual identity, dominance rank and physiological quality (Sun et al. 2025). Although our data indicate high variability of chemical profiles, we found no evidence for individual repeatability of chemical profiles, in those few males with repeated samples. Lack of evidence for individual repeatability of chemical profiles was observed across breeding stages in preen oil of females of the pied flycatcher, *Ficedula hypoleuca*, a wild European passerine (Gilles et al. 2024). As in *F. hypoleuca*, the lack of individual chemical profiles repeatability would be more consistent with sexual signaling as one of the main functions of the crust, although other functions should not be excluded until confirming trends with a larger sample size. Annual differences in chemical profiles may also be the result of changes in temperature, humidity, diet composition, and/or physiological condition (Schneeberger et al. 2016; Zala et al. 2004). Our study does not allow us to firmly conclude on repeatability partially due to samples correspond to different seasons and/or different moments in the same season. For example, comparing chemical profiles of the same individual males obtained in similar dates of the mating seasons from longer periods (several years) would allow us to appropriately explore variability in chemical signals.

Female responses to each one of the identified compounds in *T. cirrhosus* remain to be studied, and also which

concentrations -combined or isolated compounds- could elicit female responses is still unknown. Moreover, which of the identified chemical compounds produce the distinctive conspicuous odor, how age could affect chemical and odor profiles, and how each chemical compound contributes to reproduction still requires further research. Male greater sac-winged bats, *Saccopteryx bilineata,* showed age-specific differences in male-specific substances (Schneeberger et al. 2016) indicating age could affect male chemical profiles. This is consistent with observations in other organisms as scents of younger adults in male orchid bees, *Euglossa imperialis,* were more diverse and had higher concentration of compounds than older males (Henske and Eltz 2024). Age determination in bats is challenging as generally used methods can differentiate juveniles from adults, but are limited in further age determination, which is problematic as bats tend to be extremely long-lived for their body size. This challenge can be overcome in bat populations where individuals have been PIT-tagged when young.

Another unexplored approach that should be addressed in future crust studies is the potential role of associated bacteria in odor production as microbiome can shape the host's olfactory phenotype, and thus potentially influence sexual selection (Voigt et al. 2005; Maraci et al. 2024; Zheng et al. 2026). Although males of the of greater sac-winged bat, *Saccopteryx bilineata,* had lower microbial richness than females, males had more bacteria that might potentially be involved in odor production and female attraction (Maraci et al. 2024). Additionally, it would be appropriate to identify compounds with organoleptic significance by olfactometry, as well as isolate chemicals by liquid chromatography techniques, and present them to females to assess their preferences in experiments (see below).

Sexual signaling as the potential function of the crust is also consistent with the unexpected previous observation of a group of six reproductive males forming a unisexual group, captured at the beginning of the mating season back in 2018 (Muñoz-Romo et al. 2020). Similar chemical compositions and concentrations in the crusts of those males could have been caused by shared environmental conditions and/or microbiomes. Unisexual grouping behavior of males with enlarged crusts could respond to sexual signaling as joining could maximize not only the diversity of chemical compounds, but also the intensity of scent: larger groups emanate more diverse and intense scents, increasing female attraction. This observation would be consistent with the observation of 14 males and a female captured in a chamber in Veracruz in 1949 (Hall and Dalquest 1963), and also 18 all-male groups observed over a six-year period in Panama (Flores et al. 2020). Confirming whether males with crusts join together to maximize variability and concentration of chemical compounds as a lekking mechanism for mate attraction requires further research.

Experiments are paramount to confirm this hypothesis (i.e., odor intensification) and further investigate the role of the crust in male and female behavior. Flores et al. (2019) placed a cotton bag in which a male displaying crusts had been held for one hour, but did not observe female attraction to male odor. They also observed that males with forearm crusts were preferentially attracted to the odor of unfamiliar males without forearm crust over the odor of unfamiliar males with a forearm crust. Allowing males and females choose between the odor of an isolated crust from a single male and the combined odors of crusts from several males would be a revealing experiment to investigate the odor intensification hypothesis, but it would be essential to conduct these tests when individuals are reproductively active: at the onset of and during the mating season.

Experiments are the critical next step for future research, and must include testing a stronger signal, possibly achieved by mixing crusts from different males, coupled with chemical analyses that provide information on diversity and concentration of the stimulating chemical signal. Future experiments should also include testing each isolated chemical in different concentrations to see whether specific concentrations of each individual component elicit a response, and to determine if specific concentrations of isolated chemicals are the key components to producing conspecific reactions. The crust is composed of several chemical compounds, and it remains unknown which are critical to reproductive success.

This study constitutes a novel, focused contribution expanding our understanding of an unusual, complex, and still enigmatic secondary sexual trait which may be critical for the reproduction of an important Neotropical bat species. There are still several behavioral and chemical aspects that remain to be studied to understand the significance of the crust as an odor signal, the underlying mechanisms for the expression of this fascinating male strategy, and its likely role in attracting females. Future efforts should address female assessment of male crust odor, and whether testosterone impacts both the odor intensity and the specific chemical composition of the crust. Our study helps in understanding the role of odorous crusts in the social life of fringe-lipped bats, a Neotropical bat that for years raised questions regarding an aromatic golden substance covering both forearms (Flores and Page 2017), and which has now become an excellent candidate for studying mate choice and sexual selection.

Ethics

The research with bats was carried out in accordance with permits from the STRI Institutional Animal Care and Use Committee (IACUC: 2018-0901-2021) and the Panamanian

Ministry of the Environment (Ministerio del Ambiente: SE/A-78-18). Procedures were also consistent with the guidelines of the American Society of Mammalogists for the capture, management and care of mammals (Sikes et al. 2016), and the Association for the Study of Animal Behaviour.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-026-01744-0.

Acknowledgements For invaluable field assistance we thank Nikolaj Meyer, Ram Mohan, Marie Guggenberger, Marjorie Dixon, Amanda Savage, Brian Leavell, Amber Litterer, Dineilys Aparicio, Nair Cabezón, and Inga Geipel. MMR is grateful to Lourdes Suárez-Villasmil for her help and advice with statistical analyses. We are deeply grateful to two anonymous reviewers and editor for their meticulous reading and their helpful, insightful comments to improve this manuscript. We are grateful for the invaluable support of the Smithsonian Tropical Research Institute (STRI), in particular the Smithsonian Bat Lab. We thank Callum Kingwell, Paola Galgani, and Bill Wcislo for technical support with the GC-MS. We are very grateful to the National Geographic Society for support of MMR through a National Geographic Explorer Grant (46617 NGS).

Author contributions All authors jointly conceived the study. MMR, GC and RAP captured the bats and collected the crust samples. HFM and CR planned the chemical analysis. CR conducted the GCMS work, analyzed its results, and prepared Figure 5. MMR wrote the main document, analyzed results, and prepared all figures, except Figure 5. All authors contributed to the writing and final editing of manuscript.

Funding This research was supported by a Smithsonian Tropical Research Institute (STRI) COVID fellowship to MMR, with additional STRI support to cover materials, and by a National Geographic Explorer grant (46617 NGS) to MMR. Additional funding from INDICASAT and from the Smithsonian Bat Lab supported CR.

Data Availability Data is available as 'related files' (uploaded).

Declarations

Competing interests The authors declare no competing interests.

References

Adams DM, Li Y, Wilkinson GS (2018) Male scent gland signals mating status in greater spear-nosed bats, *Phyllostomus hastatus*. J Chem Ecol 44:975–986. https://doi.org/10.1007/s10886-018-1003-8

Altringham JD, Fenton MB (2003) Sensory ecology and communication in the Chiroptera. In: Kunz TH, Fenton MB (eds) Bat ecology. Chicago University Press, Chicago, pp 90–127

Andersen KF, Vulpius T (1999) Urinary volatile constituents of the lion, *Panthera leo*. Chem Senses 24:179–189. https://doi.org/10.1093/chemse/24.2.179

Andersson MB (1994) Sexual selection. Princeton University Press, Princeton, New Jersey

Blaustein A (1981) Sexual selection and mammalian olfaction. Am Nat 117:1006–1010

Bloss J (1999) Olfaction and the use of chemical signals in bats. Acta Chiropterol 1:31–45

Brennan PA, Kendrick KM (2006) Mammalian social odours: attraction and individual recognition. Philos Trans R Soc (Lond) 361:2061–2078

Brunet-Rossini AK, Wilkinson GS (2009) Methods for estimating age in bats. In: Kunz TH, Parsons S (eds) Ecological and behavioral methods for the study of bats. Johns Hopkins University, Baltimore, pp 315–326

Buchinger TJ, Li W (2023) Chemical communication and its role in sexual selection across Animalia. Commun Biol 6:1178. https://doi.org/10.1038/s42003-023-05572-w

Caspers B, Franke S, Voigt CC (2008) The wing-sac odour of male greater sac-winged bats *Saccopteryx bilineata* (Emballonuridae) as a composite trait: seasonal and individual differences. In: Hurst JL, Beynon RJ, Roberts SC, Wyatt TD (eds) Chemical signals in vertebrates 11. Springer, New York, NY, pp 151–160

Cramer MJ, Willig MR, Jones C (2001) *Trachops cirrhosus*. Mamm Spec 656:1–6

Da Silva Fonseca B, Soto-Centeno JA, Simmons NB, Ditchfield AD, Leite YLR (2024) A species complex in the iconic frog-eating bat *Trachops cirrhosus* (Chiroptera, Phyllostomidae) with high variation in the heart of the Neotropics. Am Mus Novit 1–28. https://doi.org/10.1206/4021.1

Daniel W, Cross CL (2013) Biostatistics: A Foundation for Analysis in the Health Sciences (10a ed.). Wiley

Dechmann DKN, Safi K (2005) Studying communication in bats. Cognitie, Creier, Comportament IX, pp 479–496

Eisenberg JF, Kleiman DG (1972) Olfactory communication in mammals. Annu Rev Ecol Syst 3:1–32

Flores V, Page RA (2017) Novel odorous crust on the forearm of reproductive male fringe-lipped bats (*Trachops cirrhosus*). J Mammal 98:1568–1577

Flores V, Mateo JM, Page RA (2019) The role of male forearm crust odor in fringe-lipped bats (*Trachops cirrhosus*). Behaviour 156:1–24

Flores V, Carter GG, Halczok TK, Kerth G, Page RA (2020) Social structure and relatedness in the fringe-lipped bat *(Trachops cirrhosus)*. Royal Soc Open Sci 7:192256

Gilles M, Fokkema RW, Korsten P, Caspers BA, Schmoll T (2024) Preen oil composition of pied flycatchers is similar between partners but differs between sexes and breeding stages. Ibis 166:171–186

González-Quiñonez N, Pirela A, de Durán R, Muñoz-Romo M (2011) Female mice preference: does *Trypanosoma cruzi* affect males'. scent? Interciencia 36:764–767

Hall ER, Dalquest WW (1963) The mammals of Veracruz. University of Kansas Publications. Museum of Natural History. Lawrence Kans 14:165–362

Hammer O, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4:9

Henske J, Eltz T (2024) Age-dependent perfume development in male orchid bees, *Euglossa imperialis*. J Exp Biol 227:jeb246995. https://doi.org/10.1242/jeb.246995 PMID: 38511547; PMCID: PMC11006377

JASP Team (0.18.1) (2023) [Software]. University of Amsterdam. https://jasp-stats.org/

Johansson BG, Jones T (2007) The role of chemical communication in mate choice. Biol Rev Camb Philos Soc 82:265–289

López P, Martín J (2005) Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response. Biol Lett 1:404–406. https://doi.org/10.1098/rsbl.2005.0360

Maraci Ö, Antonatou–Papaioannou A, Jünemannn S, Schneeberger K, Schulze M, Scheffler I, Caspers BA (2024) Bats, bacteria, and bat smell V.2.0: Repeatable sex–specific differences in scent organ microbiota. Microb Ecol 87:55. https://doi.org/10.1007/s00248-024-02368-1

Martín J, López P (2006) Links between male quality, male chemical signals, and female mate choice in Iberian Rock Lizards. Funct Ecol 20:1087–1096

McLean S, Nichols DS, Davies NW (2021) Volatile scent chemicals in the urine of the red fox, *Vulpes vulpes*. PLoS ONE 16:e0248961. https://doi.org/10.1371/journal.pone.0248961

Müller-Schwarze D (1983) Scent glands in mammals and their functions. Pp. 150–197, In Advances in the study of mammalian behavior (J. F. Eisenberg and D. G. Kleiman, Eds.). Special Publication 7, The American Society of Mammalogists, Lawrence, Kansas

Muñoz-Romo M, Nielsen LT, Nassar JM, Kunz TH (2012) Chemical composition of the substances from dorsal patches of males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae). Acta Chiropterol 14:213–224. https://doi.org/10.3161/150811012X654411

Muñoz-Romo M, Flores V, Page RA (2020) The crust of a male: does size matter when females are fertile? Behav Ecol Sociobiol 74:151. https://doi.org/10.1007/s00265-020-02914-0

Muñoz-Romo M, Page RA, Kunz TH (2021) Redefining the study of sexual dimorphism in bats: following the odour trail. Mammal Rev 51:55–177. https://doi.org/10.1111/mam.12232

Murtagh F, Legendre P (2014) Ward's hierarchical agglomerative clustering method: Which Algorithms Implement Ward's Criterion? J Classif 31(3):274–295. https://doi.org/10.1007/s00357-014-9161-z

Mykytowycz R, Goodrich BS (1974) Skin glands as organs of communication in mammals. J Invest Dermatol 62:124–131

Penn D, Potts WK (1998) Chemical signals and parasite-mediated sexual selection. Trends Ecol Evol 13:391–396

Quay WB (1970) Integument and derivatives. In: Wimsatt W (ed) Biology of bats, vol II. Academic, New York, pp 1–56

Racey PA (2009) Reproductive assessment of bats. In: Kunz TH, Parsons S (eds) Ecological and behavioral methods for the study of bats. Johns Hopkins University, Baltimore, pp 249–263

Real R, Vargas JM (1996) The Probabilistic Basis of Jaccard's Index of Similarity. Sys Biol 45:380–385. https://doi.org/10.1093/sysbio/45.3.380

Schmidt U (1985) The bats: order Chiroptera. In: Brown RE, MacDonanld DW (eds) Social odours in mammals. Clarendon, Oxford, pp 217–234

Schneeberger K, Voigt CC, Müller C, Caspers BA (2016) Multidimensionality of chemical information in male greater sac-winged bats *(Saccopteryx bilineata)*. Front Ecol Evol 4:83. https://doi.org/10.3389/fevo.2016.00083

Shirasu M, Ito S, Itoigawa A, Hayakawa T, Kinoshita K, Munechika I, Imai H, Touhara K (2020) Key male glandular odorants attracting female ring-tailed lemurs. Curr Biol 30:2131–2138e4. https://doi.org/10.1016/j.cub.2020.03.037

Sikes RS, the Animal Care and Use Committee of the American Society of Mammalogists (2016) Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J Mammal 97:663–688. https://doi.org/10.1093/jmammal/gyw078

Stevens M (2013) Sensory Ecology, Behaviour, and Evolution. Oxford University Press

Stowers L, Logan DW (2010) Sexual dimorphism in olfactory signaling. Curr Opin Neurobiol 20:770–775. https://doi.org/10.1016/j.conb.2010.08.015

Sun C, Wang Y, Wang L, Lucas JR, Zhuang C, Li J, Zhang C, Li D (2025) Male forehead gland scent may encode multiple information in the great Himalayan leaf-nosed bat. Anim Behav 225:123227. https://doi.org/10.1016/j.anbehav.2025.123227

Voigt CC (2013) Sexual selection in neotropical bats. In: Macedo RH, Machado G (eds) Sexual selection – Perspectives and models from the Neotropics. Elsevier, Amsterdam, pp 409–432

Voigt CC, von Helversen O (1999) Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). Behav Ecol Sociobiol 47:29–40. https://doi.org/10.1007/s002650050646

Voigt CC, Caspers B, Speck S (2005) Bats, bacteria, and bat smell: sex-specific diversity of microbes in a sexually selected scent organ. J Mammal 86:745–749. https://doi.org/10.1644/1545-1542(2005)086[0745:BBABSS]2.0.CO;2

Wang M, Carver J, Phelan V, Sanchez L et al (2016) Sharing and comunnity curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat Biotechnol 34:828–837

Wood WF, Sollers BG, Dragoo GA, Dragoo JW (2002) Volatile components in defensive spray of the hooded skunk, *Mephitis macroura*. J Chem Ecol 28:1865–1870. https://doi.org/10.1023/a:1020573404341

Zala SM, Potts WK, Penn DJ (2004) Scent-marking displays provide honest signals of health and infection. Behav Ecol 15:338–344. https://doi.org/10.1093/beheco/arh022

Zhang JX, Soini HA, Bruce KE, Wiesler D, Woodley SK, Baum MJ, Novotny MV (2005) Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. Chem Senses 30:727–737. https://doi.org/10.1093/chemse/bji065

Zheng Z, Lucas JR, Zhang C, Sun C (2026) Do symbiotic microbes drive chemical divergence between colonies in the Pratt's leaf-nosed bat. *Hipposideros pratti*? Biology 15:114. https://doi.org/10.3390/biology15020114

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.