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Research

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Head Louse Feces: Chemical Analysis and Behavioral Activity

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Abstract

Human head lice *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) are insect parasites closely associated with humans, feeding on the blood of their hosts and causing them skin irritation and probable secondary infections. Despite being a severe nuisance, very few studies have reported on intraspecific chemical communication in head lice. Here, we evaluated the attractive response of head lice to the volatile compounds and solvent extracts from their feces. We also chemically analyzed the main volatile components of these feces and those of the feces' extracts. Head lice were attracted to the methanol extract of their feces but not to the hexane or dichloromethane extracts, suggesting the polar nature of bioactive chemicals present in head louse feces. Follow-up chemical identifications, in fact, showed the presence of hypoxanthine, uric acid, and another purine tentatively identified as either guanine or iso-guanine. Additionally, head lice were significantly attracted by volatiles emitted from samples containing feces. The volatiles emanated from feces alone contained 19 identified substances: 2-pentanone, hexanal, heptanal, 3-methyl-3-buten-1-ol, octanal, sulcatone, nonanal, acetic acid, 2-ethyl-1-hexanol, decanal, 1-octanol, butyric acid, 1-nonanol, hexanoic acid, octanoic acid, 2,6-dimethyl-7-octen-2-ol, 2-undecanone, geranylacetone, and hexadecane. The major compounds found were decanal, nonanal, hexanal, and acetic acid, together representing approximately 60% of the identified compounds. This work represents the first chemical evidence of intraspecies communication among head lice. The results support the existence of active substances present in the feces of *P. humanus capitis* that may be involved in its aggregation behavior.

Keywords: *Pediculus humanus capitis*, feces, behavioral activity, chemical analysis

Head lice, *Pediculus humanus capitis* (Phthiraptera: Pediculidae), are insect parasites that feed on the blood of their host and are closely associated with humans. Head louse infestation (pediculosis) is a major widespread condition causing skin irritation and probable secondary infections (Toloza et al. 2009). Although head lice are considered a severe nuisance and are potential vectors of *Rickettsia prowazekii* (Robinson et al. 2003), few studies have investigate head louse biology and even fewer report on intraspecific chemical communication, probably due to the impossibility of rearing them in the laboratory and their short survival time outside the human head environment (Gallardo et al. 2009).

Insects typically communicate with each other using odors called pheromones, such as those that attract males to females as well as those termed aggregation pheromones. These pheromones act as recognition signals allowing proximity between individuals of the same group (Jaffe 1987). Different arthropod species produce various aggregation substances that are excreted in their feces. This behavior has been well studied in different species of ticks, where it was found that two components of the excreta (xanthine and hypoxanthine) were components of the arrestment pheromone (Dusbábek et al. 1991a). Also, the sheep tick *Ixodes scapularis* (Say) (Ixodida: Ixodidae) responded strongly to fecal exudates primarily containing guanine, xanthine, and hematin (Sonenshine et al. 2003).

Regarding the kissing bug, *Triatoma infestans* (Stal) (Hemiptera: Reduvidae), its aggregation behavior was mediated by both airborne attractants and chemotactile arresting pheromones released from its feces (Lorenzo Figueiras et al. 1994, Lorenzo and Lazzari 1996, Lorenzo Figueiras and Lazzari 1998). In another triatomine insect, *Triatoma dimidiata* (Latreille) (Hemiptera: Reduvidae), the chemical composition of its feces showed nonanal as the main component (Galvez-Marroquin et al. 2018). Initial research on the aggregation pheromone in the feces and exuvia of the bed bug *Cimex lecturiarus* (Linnaeus) (Hemiptera: Cimicidae) has identified 10 compounds: nonanal, decanal, (*E*)-2-hexenal, (*E*)-2-octenal, (2*E*, 4*E*)-octadienal, benzaldehyde, (+) and (−) limonene, sulcatone, and benzyl alcohol. These compounds were essential for attracting adult males, virgin adult females, and juvenile bed bugs (Siljander et al. 2008).

For human body lice *Pediculus humanus humanus* (Linneus) (Phthiraptera: Pediculidae), Wigglesworth (1941) long time ago reported that these insects gravitate toward papers soiled with their excreta. Later, Mumcuoglu et al. (1986) validated the aggregation response of body lice to their excretory products and identified

chemical compounds in the louse feces (e.g., hemoglobin, xanthine, hypoxanthine, uric acid, and ammonium salts). Considering that head lice are very closely related to body lice (Kittler et al. 2003, Yong et al. 2003), we hypothesized that head lice are capable of communicating with each other via their feces. In this study, we evaluated the response of head lice to their feces and we isolated volatile compounds and solvent extracts of feces. Further, we conducted a chemical analysis of the main volatile components found in head louse feces and those in the feces extracts.

Materials and Methods

Collection of Head Lice

Head lice were collected from 6- to 12-year-old children in schools of Buenos Aires, Argentina. For louse removal, we used a commercial metallic comb (Assistance OTC Products, Argentina) and followed the protocol developed by CIPEIN (Buenos Aires, Argentina) archived in our laboratory (#BA20061995ARG, June 1995). Collected insects were placed in plastic trays inside an environmental chamber at 60–70% relative humidity (RH) and a temperature of 17–19°C, since these conditions were reportedly as optimal for head louse survival at the laboratory (Picollo et al.1998). The collected insects were sexed and only adult females were selected for bioassays in order to standardize the biological material. All individuals were tested within 3 h after removal.

Collection of Feces Samples

The collected head lice were transported to the laboratory and fed to satiety on the clean arm of a human volunteer. Groups of 170 insects already fed were used to collect feces. A 25-mm diameter bottle was placed inverted on the 30-mm diameter filter paper to allow the lice to walk on the paper and deposit feces. After 2 h (kept at 30°C, 70% RH, and low light), the bottle was removed and the paper with feces was used to obtain the fecal extract.

Preparation of Fecal Extracts

The filter papers with feces (as was described above) were used to obtain fecal extracts. Independent papers with feces were extracted with each of the solvents. This extraction was done by washing each paper with 500 µl of solvent in a 2-ml vial. All vials were kept for 20 min at 30°C in a digital ultrasonic cleaner (Arcano Model PS-10A, Shandong, China) to enhance the components' extraction. Three organic solvents of increasing polarity were selected to obtain the different extracts from feces: hexane, dichloromethane, and methanol. These solvents differ in their ability to remove different compounds that might trigger different behavioral responses. The samples were stored in an ultrafreezer (JAPAN-SANYO-MDF-U33V-PE) at –75°C until used in the experiments.

Collection of Fecal Volatiles

Fed lice, starved lice, and filter papers with feces (in small pieces) were independently placed in different 10-ml vials and kept in an environmental chamber at 30°C for 2 h. Then the volatiles of each vial were adsorbed on a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 µm) solid phase micro-extraction (SPME) fiber (Sigma–Aldrich, St. Louis, MO). The fiber was punctured through the vial cap to expose the active phase to the headspace for 20 min at 30°C.

Behavioral Response to Fecal Extracts

The response of female head lice to the different fecal extracts (methanol, dichloromethane, and hexane) was assessed in an experimental arena that consisted of filter paper discs (Whatman #1, 42.5-mm diameter) divided into two equal areas (6.9 cm^2 each). One area was treated with 150 µl of the test solution, while the other area received same amount of pure solvent (control). All experimental papers were allowed to dry for 30 s (at this time the papers were dry).

Each treated paper was then carefully placed at the bottom of a glass Petri dish (60-mm diameter), and above a glass ring (50-mm diameter, 40-mm high) was set to prevent lice from escaping. The treated and untreated areas of the arena in each experiment were chosen at random to avoid location bias. We also tested solvent in the two halves of the experimental arena to check possible solvent effects. Each louse was individually placed in the arena's center and its movements were recorded for 180 s, for which the response variable of interest was average time of permanence in each area (control or treated) of the arena.

The glass surfaces were always cleaned with ethanol and oven-dried before each test. The experimental arenas were installed in an environmental chamber at 30°C and 70% RH under low-light intensity (21 lux) (Ortega-Insaurralde et al. 2015). Insect movements were recorded with a weather-resistant infrared camera (KIR-J639CE20, Sony, China) connected to a monitor (LG, China, Hangzhou) and a digital video recorder (DVR5104HE, Dajua Technology Co. Ltd., Hangzhou, China). Twenty replicates were performed for testing the individual insect response to each solvent extract of the feces' compounds.

Behavioral Response to Fecal Volatiles

The response of head lice to volatiles released by the excreta was evaluated in an adapted T-tube olfactometer, following Galassi et al. (2018). Briefly, each Whatman #1 filter paper circle with feces was cut into two equal parts, with each half used for one biological assay. One half of treated filter paper was placed inside a 2-ml vial connected to one arm of the olfactometer. Similarly, an untreated filter paper was connected to the other arm of the olfactometer. The treated and untreated papers were randomly placed in the right or left arm of the olfactometer. Airflow through the olfactometer was 5 cm/s, as measured by an anemometer, and the air was filtered by activated charcoal and humidified by a washer flask. Prior to each measurement, the olfactometer was thoroughly cleaned with pure ethanol. After each test, the vials were cleaned with ethanol and after every five successive tests the entire device was thoroughly cleaned with ethanol.

For each measurement, a head louse was placed in the olfactometer via its central opening and allowed to crawl for 180 s. At that elapsed time, the location of the louse in each arm of the olfactometer was recorded by assigning a binary value (1: proximity to the treated paper or 0: proximity to the untreated paper). After this, the tested insect was discarded. To exclude possible bias towards one side of the olfactometer, a control experiment was conducted in which one clean filter paper was placed into each arm. In all, 30 replicates were used to evaluate the response of lice to papers with feces and likewise 30 control replicates were performed.

Chemical Analysis of Feces Extracts

The extracts were centrifuged (6,000 rpm, IEC HN Centrifuge, Des Moines, IA) and the supernatants were filtered (Ministart filter unit, 0.20 µm, Sartorius, Goettingen, Niedersachsen, Germany). Chemical

analysis was performed in an HPLC-UV-MS system (Alliance e2695 system from Waters Corp., Milford, MA) using an analytical reverse-phase column (Varian RP C-18, 250 mm × 4.6 mm i.d., 5-µm particle size, St. Louis, MO). The solvent used was 10 mM of CH_3COONH_4 pH: 4.7 (buffer) and methanol, at a flow of 0.3 ml/min. The liquid chromatograph gradient conditions were as follows: mobile phase buffer (Phase A) and methanol (Phase B), with the solvent gradient changed according to these conditions: from 0 to 5 min, it was 90% (A):10% (B). Then, it was modified between 5 and 30 min to 10% (A): 90% (B), and kept for 10 min, with each run ending in a total time of 40 min. The column temperature was set at 30°C for all the runs and the injection volume was 10 µl. For the UV analysis, the chromatogram was acquired using a photodiode array detector (wavelength 190–800 nm) at 30°C, using 1.2-nm slit width and 1-nm bandwidth and the mass spectrometer was operated in the positive mode with an acquisition range of *m/z* 50–1,200 TIC (total ion chromatography). The signals were also acquired in SIR code (simple ion recording). The UV and mass spectra obtained for each peak were compared with bibliographic data. All data processing was conducted using MassLynx v4.1 software (Waters Corp.). The identified compounds were confirmed by injection of analytical standards (Sigma Aldrich).

Chemical Analysis of Volatiles

The chemical composition of volatiles derived from starved lice and from fed lice (containing lice and feces volatiles) was comparatively analyzed to identify the feces' volatile compounds.

Analyses were done in a Shimadzu QP2010-UltraGas chromatograph-Mass spectrometer (GC-MS) with a polar DB-WAX capillary column (30 m, 0.32 mm i.d, d.f 0.25 µm, Agilent Technologies, Santa Clara, CA) with helium as carrier gas (total flow = 4.82 ml/min). The fiber was injected in the GC split-less injector mode at 240°C. The GC oven temperature was 50°C (4-min hold) followed by a ramp of 8°C/min to 230°C, and held isothermal for 5 min. The detector was operated at 70 eV, scanning from 40 to 350 *m/z*, with a 245°C interface temperature.

Identification of individual volatiles was carried out using standard reference samples (Sigma–Aldrich), based on the comparison with the retention index from the literature and/or by the comparison of the mass spectrum (MS) against the Wiley Mass Spectra Library (Mc Lafferty 2005). The MS of the compounds in the chromatogram corresponding to siloxanes, room air, liquid soap, solvents (e.g., traces of chloroform, column, and septa), as well as solvents commonly employed in cosmetic products and room air products (e.g., 2-butoxy ethanol), were omitted in the analysis. The areas of the remaining compounds were normalized accordingly.

Statistical Analysis

Binary data obtained with the olfactometer (1: proximity to the treated paper or 0: proximity to the untreated paper) were analyzed using a generalized linear model with a binomial error structure with *n*: 1 (Bernoulli distribution) and logit link function (Zuur et al. 2009). For the circular arena data, one-way analysis of variance was applied to test for significant differences in the average time of permanence in the solvent paper area or the feces extract area. Key assumptions of independent observations and randomness were assured by experimental design (i.e., each louse was assigned only to one trial, and the treated paper was assigned randomly to the right or left side of the olfactometer and the circular arena.)

In the olfactometer test, the absence of outliers was assessed visually by plotting the Pearson residuals against predicted values, and the absence of influential data points was checked graphically using Cook's distance values, which were always ≤1. In the circular arena, normality and heteroscedasticity were graphically ascertained through a Q-Q plot and a plot of residuals versus predicted values. All above analyses were conducted using lme4 (Bates et al. 2013) and MASS packages (Venables and Ripley 2002) from the R software (R Core Team 2013).

Results

Behavioral Response to Fecal Extracts

Bioassays in the divided experimental arena showed that head lice were attracted to methanol extracts of their feces ($P \leq 0.05$). In contrast, they were not attracted to dichloromethane or hexane extracts of the feces ($P \geq 0.05$; Fig. 1). These results indicated that only feces' methanol extract contained compounds capable of producing head louse attraction.

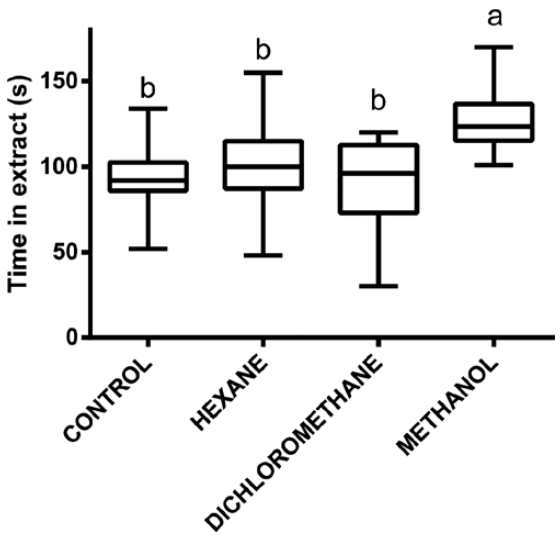

Fig. 1. Boxplots showing the response of *Pediculus humanus capitis* to head louse feces extracts in hexane, dichloromethane, and methanol. The methanol response was significantly different from the control ($n = 20$)

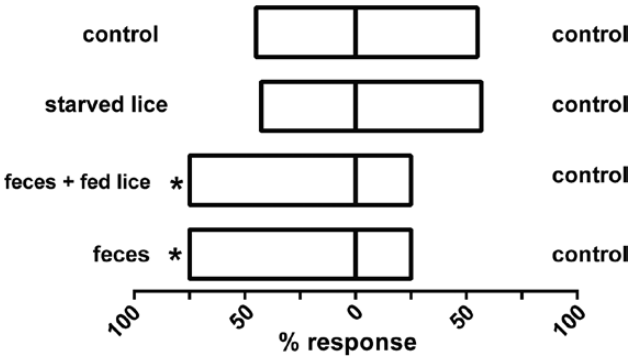

Fig. 2. Choice response of head lice in a dual-choice olfactometer to airborne volatiles from starved lice, feces + fed lice, and feces. Each horizontal bar marks the preference for an arm in the olfactometer. Asterisks denote a significant difference between the treatment and control ($P < 0.05$).

Behavioral Response to Fecal Volatiles

Olfactometric bioassays showed that head lice were preferentially oriented towards the arm with volatiles emanated by louse feces or lice plus feces, when compared with the control arm (lacking a stimulus; $P < 0.05$). Conversely, the head lice did not respond to the volatiles emanated by the starved head lice, suggesting that louse-attracting volatile compounds were emanated from feces (Fig. 2).

Chemical Analysis of Extracts

The HPLC analysis of the methanol extract of feces—the only extract that produced head louse response—showed three peaks (Fig. 3). The comparison of peak retention times with standards, UV spectrum, and fragmentation pattern of MS jointly indicated that the largest peak was hypoxanthine (12.17 min) and the second-largest peak was uric acid (9.86 min).

The identities of uric acid and hypoxanthine were confirmed by the comparison of the HPLC-MS retention times with corresponding

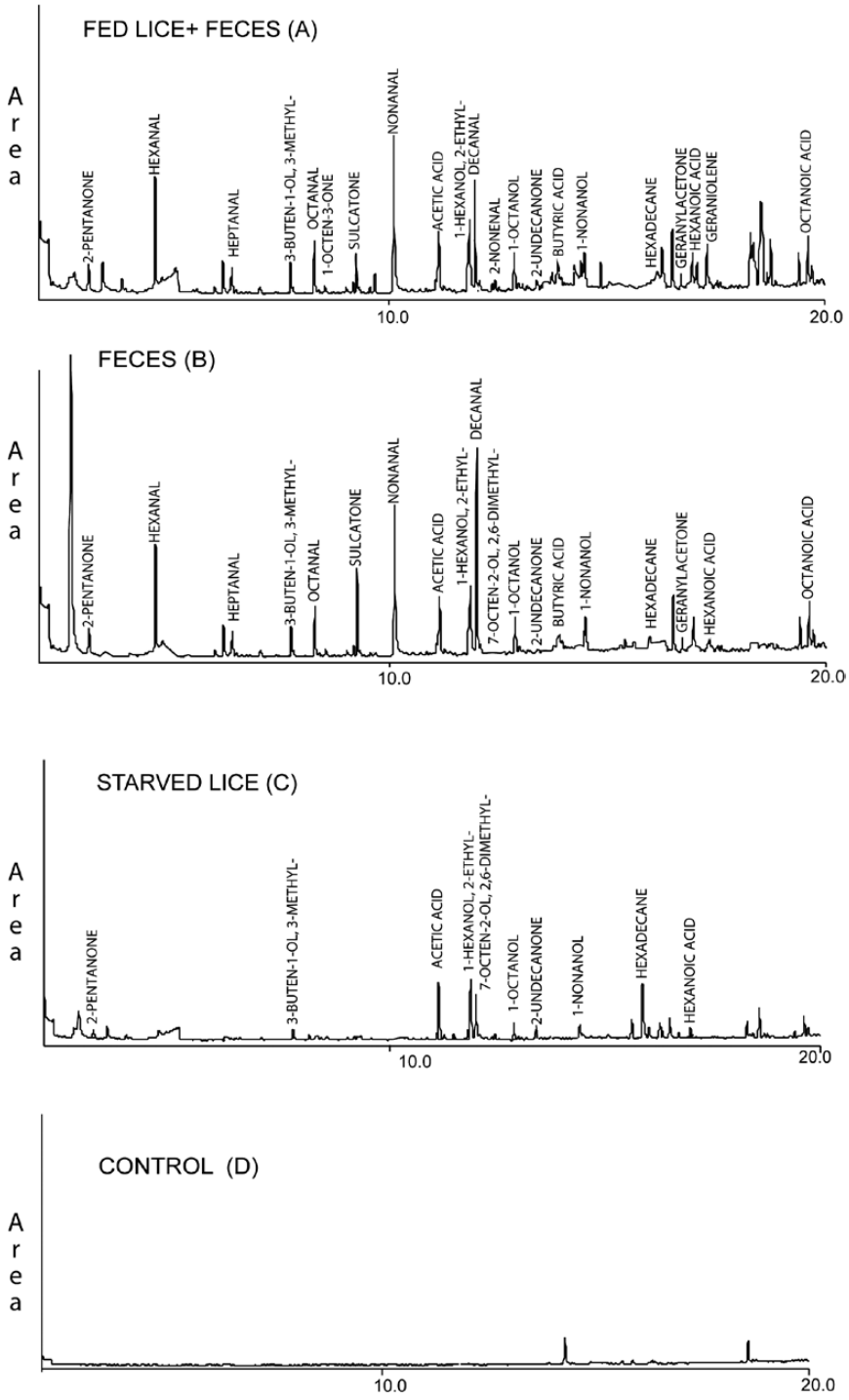

Fig. 3. Gas chromatogram of the volatiles collected from samples of feces plus lice, only feces, and starved lice, injected in a polar DB-WAX capillary column.

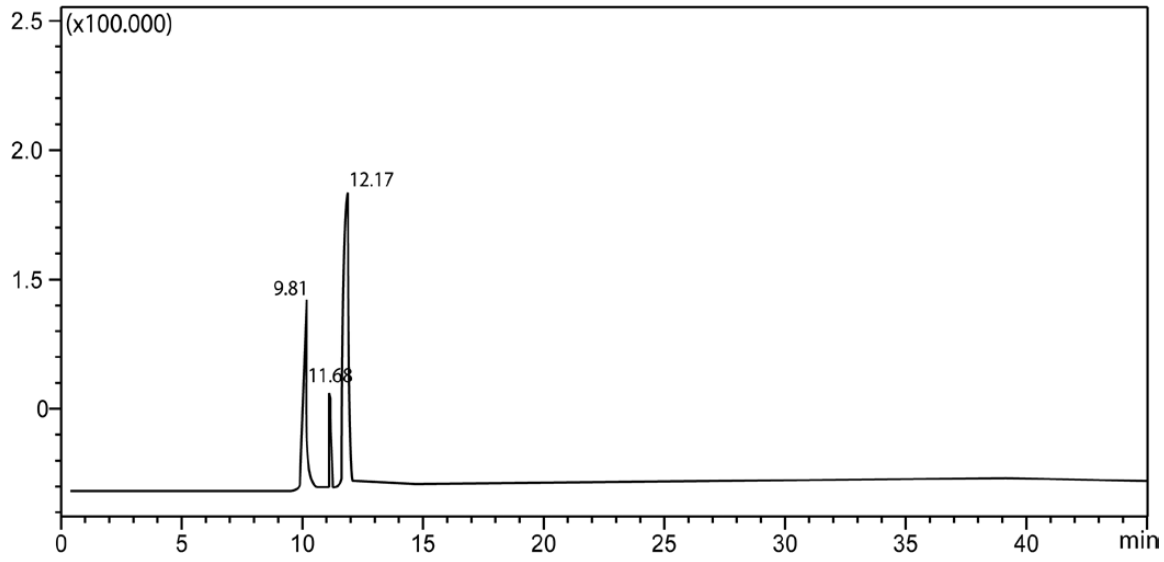

Fig. 4. HPLC- MS chromatogram of feces of head lice in methanol. The analysis revealed three peaks: hypoxanthine (12.17 min) and acid uric (9.81 min) that were confirmed by standard patron, UV, and MS spectrum. The peak at 11.68 min could not be resolved.

Table 1. Area of the volatile compounds identified in the samples of lice plus feces, only feces, and starved lice

Compounds	CAS number	Relative area in headspace[a]		
		Feces + fed lice	Feces	Starved lice
2-Pentanone[b,c]	107-87-9	3.8	3.8	6.9
Hexanal[b,c]	66-25-1	12.0	12.0	–
Heptanal[b,c]	111-71-7	2.9	2.9	–
3-Methyl-3-buten-1-ol[c]	763-32-6	3.1	3.1	5.1
Octanal[b,c]	204-683-8	6.4	6.4	–
1-Octen-3-one[b,c]	4312-99-6	<1	–	–
Sulcatone[b,c]	110-93-0	1.2	6.2	–
Nonanal[b,c]	124-19-6	20.8	13.8	–
Acetic acid[b,c]	64-19-7	10.2	10.2	12.2
2-Ethyl-1-hexanol[c]	104-76-7	4.1	4.1	15
Decanal[b,c]	112-31-2	10.9	17.6	–
2,6-Dimethyl-7-octen-2-ol[c]	18479-58-8	<1	<1	6.3
2-Nonenal[b,c]	60784-31-8	1.6	–	9.4
1-Octanol[b,c]	111-87-5	4.3	4.3	–
2-Undecanone[c]	112-12-9	<1	<1	5.0
Butyric acid[b,c]	107-92-6	3.4	3.4	–
1-Nonanol[b,c]	143-08-8	3.9	3.4	8.9
Hexadecane[b,c]	208-878-9	–	<1	16.0
Hexanoic acid[b,c]	142-62-1	3.6	3.6	5.6
Geranylacetone[b,c]	3796-70-1	2.3	2.6	9.6
Geraniolene[b,c]	6709-39-3	2.3	–	–
Octanoic acid[b,c]	124-07-2	2.5	2.5	–

[a]Peak was calculated based ion GC-MS analysis.
[b]Standard analysis.
[c]Compared literature RI (Kovats) with relative RI (Kovats).
– Compound not detected.

authentic standards. The identity of the third peak in the chromatogram could not be confirmed, but according to its retention time and mass spectrum, we hypothesized it could be guanine or iso-guanine.

Chemical Analysis of Fecal Volatiles

Figure 4 shows the gas traces of volatiles emanated from the samples of feces plus lice, feces, or starved lice, with their respective content listed in Table 1. The volatiles emanated from samples of louse feces alone contained 19 identifiable substances: 2-pentanone, hexanal, heptanal, 3-methyl- 3-buten-1-ol, octanal, sulcatone, nonanal, acetic acid, 2-ethyl-1-hexanol, decanal, 1-octanol, butyric acid, 1-nonanol, geranylacetone, hexanoic acid, octanoic acid, as well as traces of 2-undecanone, 2-6-dimethyl-7-octen-2-ol and hexadecane.

When the volatiles emanated from feces plus lice were analyzed, three more compounds were identified: 1-octen-3-one, 2-nonenal

and geraniolene. In addition, differences in the proportions of nonanal, decanal, and sulcatone between the volatiles of both groups of samples (i.e., feces and feces plus lice) were detected; however, their major compounds were the same (i.e., decanal, nonanal, hexanal, acetic acid), together representing ~60% of all identified compounds. For starved lice, from this group of samples only 11 compounds were identified, the majority being 2-ethyl-1-hexanol, hexadecane, and acetic acid. Unlike fecal samples, the volatiles emitted by starved head lice did not contain aldehyde compounds.

Discussion

Body lice (*P. humanus humanus*) were aggregated by the aqueous extracts of their feces, which contain nitrogenous compounds, such as amino acids and purines (Mumcuoglu et al. 1986). This aggregation behavior has been well studied in different species of ticks, for which it was demonstrated that two components of the excreta (xanthine and hypoxanthine) were components of the arrestment pheromone (Dusbábek et al. 1991a). The sheep tick *Ixodes scapularis* (Ixodida: Ixodidae) strongly responded to fecal exudates containing guanine, xanthine, and hematin (Sonenshine et al. 2003). Similar results were found for *Ixodes ricinus* (Linnaeus) (Ixodida: Ixodidae) by Grenacher et al. (2001), who provided evidence for an arresting stimulus detected by the antennae of conspecific feces, being the major components purines and their derivatives.

In this study, we found that head lice were attracted by methanolic extract of the feces but not by the hexane or dichloromethane extracts. This different extraction capacity was related to the polarity of the solvent: methanol is a polar solvent able to extract polar compounds, hexane is a nonpolar solvent, and dichloromethane is a semipolar solvent that can extract hydrocarbons and other nonpolar compounds. Our results suggested the polar nature of bioactive chemicals present in head louse feces. Furthermore, their chemical identification demonstrated the presence of hypoxanthine, uric acid, and another purine identified either as guanine or iso-guanine. As was previously commented, the presence of purines was also reported in feces extracts from body lice (Mumcuoglu et al. 1986) and ticks (Grenacher et al. 2001). Hemoglobin, xanthine, hypoxanthine, uric acid, and ammonium salts were found in body lice feces, and guanine, xanthine, uric acid, and 8-aza-guanine were found in ticks. In our study, the olfactometer tests showed that volatiles from feces significantly attracted adult lice compared with volatiles from starved lice, suggesting the existence of active volatiles emitted by louse feces. Therefore, both the feces extracts and the volatile compounds emitted by the feces are attractants.

In the chemical analyses of fecal volatiles, we found 19 genuine identified peaks, being the major decanal, nonanal, hexanal, and acetic acid. These compounds also occur in the scalp's chemical composition, and they attracted head lice (Galassi et al. 2018) and other hematophagous insects, such as bed bugs and triatomines, when they were tested individually at different concentrations (Pires et al. 2002; Vitta et al. 2002, 2007). For example, in *Triatoma dimidiata* (Hemiptera: Reduvidae), the chemical composition of its feces showed nonanal as the main component (Galvez-Marroquin et al. 2018). In bed bug *Cimex lecturiarus* (Hemiptera: Cimicidae), initial research on the aggregation pheromone in the feces and exuvia has identified aldehydes and ketones such as nonanal, decanal, (*E*)-2-hexenal, (*E*)-2-octenal, (2*E*, 4*E*)-octadienal, benzaldehyde, (+) sulcatone, and benzyl alcohol. These compounds were essentials for attracting adult males, virgin females, and juvenile bed bugs (Siljander et al. 2008). Other compounds we found in head louse feces were detected in feces of other species. For example, 2-ethyl-1-hexanol, hexanoic acid, and acetic acid caused aggregation behavior in *T. infestans* and *Triatoma brasilensis* (Neiva) (Hemiptera: Reduvidae) (Mota et al. 2014).

Unlike other hematophagous insects that have an active search for the host, human lice are permanent parasites that spend their entire life cycle in the human head; however, pediculosis is an evidence that there is in fact a dispersion of lice among different hosts, and in consequence different populations can be found in one individual head. In this context, it is possible that compounds present in feces are used by lice to discriminate different cohorts that come from different hosts.

Several aspects of louse biology underscore the potential relevance of feces in their intraspecies communication. First, lice complete their entire life cycle on the human head, where they feed, reproduce, and oviposit. Since they spend most of their time feeding on blood, they generate a large amount of feces (Takano Lee et al 2005), so it is likely that feces would play an ecological role in their intraspecific communication. Second, the absence of cuticle external glands in the thorax and abdomen of head louse adults—according to scanning electron microscopy (SEM) images (Hatsushika et al. 1983 and Ortega Insaurralde et al. 2019) and unlike other blood-sucking insects such as bed bugs and triatomines (Usinger 1966)—increases the potential relevance of compounds present in feces as chemical mediators. Third, fecal composition may convey information about the host's physiological condition. For ticks, the volatiles generated after a feeding event evoked an aggregation response toward other ticks from a medium distance, this being interpreted as a food signal (Donze et al. 2004). Although Ortega Insaurralde et al. (2017) reported that head lice did not show differential preferences toward the scalp from different hosts, different levels of infestation do exist among the students of the same class (Toloza et al. 2009). Fecal products could, thus, account for these differences if they provided accurate intraspecific information about the physiological condition of the human host. Fourth, head louse populations have a female-to-male ratio of 3:1, as confirmed at different times and sites worldwide (Perotti et al. 2004). In this context, the chemical signals could contribute to the localization of the mate on the host. The fact that females have to be inseminated many times to maintain fertility (Takano Lee et al. 2005) suggests the need of a reliable chemical signal associated with mate searching or with aggregation behavior. The latter is, in fact, mediated by chemical clues as has been reported for other hematophagous insects, such as ticks (Bunnell et al. 2011), body lice (Mumcuoglu et al. 1986), bed bugs (Siljander et al. 2008), and kissing bugs (Galvez et al. 2018).

The secretory tissues of fecal compounds in lice are unknown. The found compounds could be metabolic byproducts of blood digestion or may be produced by microorganisms. Intestinal symbionts are essential microorganisms in several blood-sucking insects (Eichler and Schaub 2002), and their presence in feces could provide a direct potential source of aggregation compounds. Fecal microbial communities are known to contribute to animal communication (Ezenwa et al. 2012), as in the German cockroach, *Blattella germanica* (Linnaeus) (Blattodea: Blattellidae), whose intestinal bacterial communities mediate the production of the aggregation pheromone (Wada-Katsumata et al. 2015).

The present experimental work has provided the first chemical evidence of intraspecies communication among head louse individuals. The results support the existence of active substances present in the feces of *P. humanus capitis* potentially involved in its aggregation behavior, and our future studies will aim to discriminate the concentrations and proportions of chemicals associated with either an attraction

or repellence behavior. Though the attraction behavior of chemicals is naturally of academic interest, repellent activity is always sought after in the search for new pediculicidal actives for head lice control.

Acknowledgments

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References Cited

Bates, D., M. Maechler, and B. Bolker. 2013. lme4: linear-mixed effects models using S4 classes. http://CRAN.R-project.org/ package=lme4 (R package version 0.9999992).

Bunnell, T., K. Hanisch, J. D. Hardege, and T. Breithaupt. 2011. The fecal odor of sick hedgehogs (*Erinaceus europaeus*) mediates olfactory attraction of the tick *Ixodes hexagonus*. J. Chem. Ecol. 37: 340–347.

Donzé, G., C. McMahon, and P.M. Guerin. 2004. Rumen metabolites serve ticks to exploit large mammals. J. Exp. Biol. 207: 4283–4289.

Dusbábek, F., A. Jegorov, and P. Simek. 1991a. Identification of xanthine and hypoxanthine as components of assembly pheromone in excreta of Argasid ticks. Exp. Appl. Acarol. 11: 307–316.

Eichler, S., and G. A. Schaub. 2002. Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. Exp. Parasitol. 100: 17–27.

Ezenwa, V. O., N. M. Gerardo, D. W. Inouye, M. Medina, and J. B. Xavier. 2012. Microbiology. Animal behavior and the microbiome. Science. 338: 198–199.

Galassi, F. G., G. Fronza, A. C. Toloza, M. I. Picollo, and P. González-Audino. 2018. Response of *Pediculus humanus capitis* (Phthiraptera: Pediculidae) to volatiles of whole and individual components of the human scalp. J. Med. Entomol. 55: 527–533.

Gallardo, A., G. Mougabure Cueto, and M. I. Picollo. 2009. *Pediculus humanus capitis* (head lice) and *Pediculus humanus humanus* (body lice): response to laboratory temperature and humidity and susceptibility to monoterpenoids. Parasitol. Res. 105: 163–167.

Galvez-Marroquin, Z., L. Cruz-López, E. A. Malo, J. M. Ramsey, and J. C. Rojas. 2018. Behavioural and electrophysiological responses of *Triatoma dimidiata* nymphs to conspecific faecal volatiles. Med. Vet. Entomol. 32: 102–110.

Grenacher, S., T. Kröber, P. M. Guerin, and M. Vlimant. 2001. Behavioural and chemoreceptor cell responses of the tick, *Ixodes ricinus*, to its own faeces and faecal constituents. Exp. Appl. Acarol. 25: 641–660.

Hatsushika, R., S. Naramoto, and K. Miyoshi. 1983. Scanning electron microscope studies on head louse, *Pediculus humanus capitis* (Anoplura: Pediculidae). Kawasaki Med. J. 9: 109–119.

Jaffe, K. 1987. Evolution of territoriality and nestmate recognition systems in ants, pp. 295–311. *In* J. M. Pasteels and J. L. Deneubourg (eds.), From individual to collective behavior in social insects. Birkhaüser Verlag, Basel, Switzerland.

Kittler, R., M. Kayser, and M. Stoneking. 2003. Molecular evolution of *Pediculus humanus* and the origin of clothing. Curr. Biol. 13: 1414–1417.

Lorenzo Figueiras, A. N., and C. R. Lazzari. 1998. Aggregation in the haematophagous bug *Triatoma infestans*: a novel assembling factor. Physiol. Entomol. 23: 33–37.

Lorenzo Figueiras, A. N., A. Kenigsten, and C. R. Lazzari. 1994. Aggregation in the haematophagous bug *Triatoma infestans*: chemical signals and temporal pattern. J. Insect. Physiol. 40: 311–316.

Lorenzo, M. G., and C. R. Lazzari. 1996. The spatial pattern of defecation in *Triatoma infestans* and the role of faeces as a chemical mark of the refuge. J. Insect. Physiol. 42: 903–907.

McLafferty, F. W. 2005. Wiley registry of mass spectral data. J. Chem. Ecol. 26: 1367–1382

Mota, T., A. C. Vitta, A. N. Lorenzo-Figueiras, C. P. Barezani, C. L. Zani, C. R. Lazzari, L. Diotaiuti, L. Jeffares, B. Bohman, and M. G. Lorenzo. 2014. A multi-species bait for Chagas disease vectors. PLoS Negl. Trop. Dis. 8: e2677.

Mumcuoglu, K. Y., R. Galun, and R. Ikan. 1986. The aggregation response of human body louse (*Pediculus humanus*) (Insecta, Anoplura) to its excretory products. Insect Sci. Appl. 7: 629–632.

Ortega-Insaurralde, I., A. C. Toloza, P. Gonzalez-Audino, G. A. Mougabure-Cueto, A. Alvarez-Costa, G. Roca-Acevedo, and M. I. Picollo. 2015. Effect of environmental conditions and toxic compounds on the locomotor activity of *Pediculus humanus capitis* (Phthiraptera: Pediculidae). J. Med. Entomol. 52: 1036–1042.

Ortega-Insaurralde, I., A. Ceferino Toloza, P. Gonzalez-Audino, and M. Inés Picollo. 2017. Arrestant effect of human scalp components on head louse (Phthiraptera: Pediculidae) behavior. J. Med. Entomol. 54: 258–263.

Ortega Insaurralde, I., S. Minoli, A. C. Toloza, M. I. Picollo, and R. B. Barrozo. 2019. The sensory machinery of the head louse *Pediculus humanus capitis*: from the Antennae to the Brain. Front. Physiol. 10: 434.

Perotti, M. A., S. S. Catalá, A. d. e. l. V. Ormeño, M. Zelazowska, S. M. Bilíński, and H. R. Braig. 2004. The sex ratio distortion in the human head louse is conserved over time. BMC Genet. 5: 10.

Picollo, M. I., C. V. Vassena, A. A. Casadio, J. Massimo, and E. N. Zerba. 1998. Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura; Pediculidae). J. Med. Entomol. 35: 814–817.

Pires, H. H., M. G. Lorenzo, L. Diotaiuti, C. R. Lazzari, and A. N. Lorenzo Figueiras. 2002. Aggregation behaviour in *Panstrongylus megistus* and *Triatoma infestans*: inter and intraspecific responses. Acta Trop. 81: 47–52.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robinson, D., N. Leo, P. Prociv, and S. C. Barker. 2003. Potential role of head lice, *Pediculus humanus capitis*, as vectors of *Rickettsia prowazekii*. Parasitol. Res. 90: 209–211.

Siljander, E., R. Gries, G. Khaskin, and G. Gries. 2008. Identification of the airborne aggregation pheromone of the common bed bug, *Cimex lectularius*. J. Chem. Ecol. 34: 708–718.

Sonenshine, D. E., T. Adams, S. A. Allan, J. McLaughlin, and F. X. Webster. 2003. Chemical composition of some components of the arrestment pheromone of the black-legged tick, *Ixodes scapularis* (Acari: Ixodidae) and their use in tick control. J. Med. Entomol. 40: 849–859.

Takano-Lee, M., J. D. Edman, B. A. Mullens, and J. M. Clark. 2005. Transmission potential of the human head louse, *Pediculus capitis* (Anoplura: Pediculidae). Int. J. Dermatol. 44: 811–816.

Toloza, A., C. Vassena, A. Gallardo, P. González-Audino, and M. I. Picollo. 2009. Epidemiology of *Pediculosis capitis* in elementary schools of Buenos Aires, Argentina. Parasitol. Res. 104: 1295–1298.

Usinger, R. L. 1966. Monograph of Cimicidae (Hemiptera-Heteroptera). Entomological Society of America, College Park, MD.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics using S, 4th ed. Springer, New York.

Vitta, A. C., A. N. Figueiras, C. R. Lazzari, L. Diotaiuti, and M. G. Lorenzo. 2002. Aggregation mediated by faeces and footprints in *Triatoma pseudomaculata* (Heteroptera: Reduviidae), a Chagas disease vector. Mem. Inst. Oswaldo Cruz. 97: 865–867.

Vitta, A. C., T. R. Mota, L. Diotaiuti, and M. G. Lorenzo. 2007. The use of aggregation signals by *Triatoma brasiliensis* (Heteroptera: Reduviidae). Acta Trop. 101: 147–152.

Wada-Katsumataa, A., L. Zurekc, G. Nalyanyaa, G. W. Roelofsd, A. Zhangf, and C. Schal. 2015. Gut bacteria mediate aggregation in the German cockroach. Proc. Natl. Acad. Sci. USA. 112: 15678–15683.

Wigglesworth, V. 1941. The sensory physiology of the human louse *Pediculus humanus corporis* De Geer (Anoplura). Parasitology 32: 67–109.

Yong, Z., P. E. Fournier, E. Rydkina, and D. Raoult. 2003. The geographical segregation of human lice preceded that of *Pediculus humanus capitis* and *Pediculus humanus humanus*. CRBiologies 326: 565– 574.

Zuur, A. F., E. N. Ieno, N. Walker, A. Anatoly, and A. A. Saveliev. 2009. Mixed effects models and extensions in ecology with R, pp. 574–580. Springer-Verlag, New York.