Effect of Environmental Conditions and Toxic Compounds on the Locomotor Activity of *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

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ABSTRACT In this work, we evaluated the effect of environmental variables such as temperature, humidity, and light on the locomotor activity of *Pediculus humanus capitis*. In addition, we used selected conditions of temperature, humidity, and light to study the effects of cypermethrin and *N*,*N*-diethyl-3-methylbenzamide (DEET) on the locomotor activity of head lice. Head lice increased their locomotor activity in an arena at 30°C compared with activity at 20°C. When we tested the influence of the humidity level, the locomotor activity of head lice showed no significant differences related to humidity level, both at 30°C and 20°C. Concerning light influence, we observed that the higher the intensity of light, the slower the movement of head lice. We also demonstrated that sublethal doses of toxics may alter locomotor activity in adults of head lice. Sublethal doses of cypermethrin induced hyperactivated responses in adult head lice. Sublethal doses of DEET evocated hypoactivated responses in head lice. The observation of stereotyped behavior in head lice elicited by toxic compounds proved that measuring locomotor activity in an experimental set-up where environmental conditions are controlled would be appropriate to evaluate compounds of biological importance, such as molecules involved in the host–parasite interaction and intraspecific relationships.

KEY WORDS *Pediculus humanus capitis*, locomotor activity, light, temperature, humidity

The blood-sucking insect *Pediculus humanus capitis* (De Geer 1767) is a cosmopolitan parasite that affects the head of their human hosts. Head lice represent an infectious disease affecting mostly school children, where their presence cause itching, different skin reactions, and secondary infections (Heukelbach 2010).

Pediculosis is widespread throughout the world and does not discriminate on socioeconomic status grounds (Falagas et al. 2008). The main mechanism by which louse transmission is assumed to occur is the direct host-to-host contact; this includes head-to-head and body-to-head contact (Speare et al. 2002). Canyon et al. (2002) found that lice would transfer from one hair to another when hairs were moved slowly in specified alignment. Consequently, the risk of transmission of infestation by displaced lice or louse eggs is epidemiologically insignificant compared with the risks of lice transferring from one person to another during physical contact (Burgess 2004). In this context, the knowledge of the behavior of lice is a major key to understand the transmission dynamics.

Research on the locomotor activity of Phthiraptera belongs mainly to the first half of the 20th century. Hase (1915) stated that large specimens of *Pediculus humanus humanus* (body lice; Linnaeus 1758) walked faster than small ones. Nuttall (1917) collected data from different authors that made detailed observations about the locomotor activity of body lice on different surfaces and at different angles. Nuttall (1917) also developed a simple apparatus to assess the speed of head and body lice to climb along human hair. Later, Wigglesworth (1941) showed that body lice elicited a preference for 29–30°C against lower or higher temperatures. Also, the rate of movement was always greater on the warmer side. In terms of relative humidity (RH), body louse preferred to remain on the dry side (<10% RH). It seemed that these insects chose a low- or medium-humidity environment and avoided any change once they became adapted to a given humidity. Concerning light inference, body lice would be photonegative, moving away from a source of light by a negative phototactic behavior (Nuttall 1917, Wigglesworth 1941, Buxton 1946). Wigglesworth (1941) also demonstrated that a sudden increase in illumination caused body lice to become arrested, they stopped in a state of akinesis and once they were disturbed by touching, they usually moved at a slower pace and often hesitated or stopped afterwards.

Several chemical compounds are modifiers of locomotor activity and they can influence insect behavior

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through their detection by a functional sensory system or by disrupting the normal function of the sensory or central nervous system and hormonal system (Georghiou 1972, Haynes 1988). An increase in locomotor activity (i.e., hyperactivity) or the lack of it (i.e., prostration, paralysis, hypoactivation) in insects are considered symptoms of poisoning with neurotoxic substances such as pyrethroids (Gammon 1978, Miller and Adams 1982, Benoit et al. 1985, Alzogaray et al.1997, Sfara et al. 2013). Alzogaray et al. (1997, 2001) evaluated the locomotor activity of nymphs of *Triatoma infestans* (Hemiptera: Reduviidae) exposed to several pyrethroids, finding hyperactivity as one of the symptoms of poisoning. More specifically, cypermethrin was proven to have hyperactivant effect in kissing bugs (Alzogaray 2001) and wolf spiders (Baatrup and Bayley 1992). Sfara et al. (2013) found a decrease of locomotor activity in *Blatella germanica* (Dictyoptera: Blattidae) exposed to *N,N*-diethyl-3-methylbenzamide (DEET), the active ingredient of most insect repellents used worldwide (Debboun et al. 2007), and described it as a consequence of toxic effects.

With relation to *P. h. capitis*, heavy infestations might lead to increased locomotor activity in the head-to-head transmission. Consequently, the study of the factors that affect locomotor activity of head lice is pertinent to a more efficient control of pediculosis.

In this work, we evaluated the effect of environmental variables such as temperature, humidity, and light on the locomotor activity of *P. h. capitis*, and we proposed the optimal conditions for comparative behavioral assays. Moreover, we used the selected conditions to study the effects of cypermethrin and DEET in the locomotor activity of head lice.

Materials and Methods

Insects. Head lice were collected from the heads of infested children from seven elementary schools in Buenos Aires, Argentina, using a fine-toothed antilouse comb according to a protocol approved by an ad hoc committee of the Research Center of Pests and Insecticides. Back at the laboratory, the adults were selected and used in the experiments one hour later. During this hour, the selected insects were kept in plastic trays in an environmental chamber (Ambi-Hi-low Lab-Line, IA) set at 18 ± 0.5°C, 70–80% RH. Low temperature and high humidity was demonstrated to minimize the average mortality of head lice according to Picollo et al. (1998). The experiments were performed during the same period of the day (13:00–16:00 hours).

Quantification of Locomotor Activity. The arena was a modification of Sfara et al. (2013). Briefly, we developed an experimental area consisting of a circular Whatman no. 1 filter paper divided in 0.25-cm^2 squares (GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, UK; diameter: 55 mm) inside a glass petri dish (55 mm diameter). A glass ring (high: 4 cm; diameter: 5 cm) was used to prevent insects from leaving the arena. An adult of *P. h. capitis* was gently placed on the arena. Locomotor activity was quantified by counting the number of crossings to each square of the arena that insects made during 3 minutes. The arena was placed on top of a heating plate (Fábrica de aparatos científicos, Buenos Aires, Argentina). Four arenas were simultaneously recorded. This device was set in an environmental chamber (RH levels between 20 and 100%) illuminated with an LED strip of cold white light with a dimmer (RF Wireless LED Dimmer, China). The locomotor activity of the insects was recorded with a weatherproof infrared camera (KIR-J639CE20, Sony, China) connected to a monitor (LG, China) and a digital video recorder (DVR5104HE, Dahua Technology Co. ltd, Hangzhou, China). The camera was suspended 50 cm above the arena surfaces (Fig. 1). We tested 15 adult head lice individually per treatment.

Influence of Temperature on Locomotor Activity. The arenas were placed over the heating plate at two different temperatures: 30 ± 0.5°C or 20 ± 0.5°C, 50% RH, and diffuse light (200 ± 0.1 lux). The temperature of the experimental plate was measured with a digital thermometer (TFA Dostman) after a stabilization period of 5 min.

Influence of Humidity on Locomotor Activity. Three different RH levels were tested: high RH (100% RH), medium RH (50% RH), and low RH (20% RH). Values of RH were recorded by a hygrometer (TFA Dostman, China). During the assays, the arenas were kept at 30 ± 0.5°C or 20 ± 0.5°C and diffuse light (200 ± 0.1 lux).

Influence of Light on Locomotor Activity. We tested the locomotor activity at three different intensities of light: low-intensity light (21 ± 0.1 lux), medium-intensity light (553 ± 0.1 lux), and high-intensity light (1726 ± 0.1 lux). The illumination level was measured with a digital lux meter (Lux & fc, ST-1301, China). During assays, the arenas were kept at 30 ± 0.5°C and 50% RH.

Effect of Toxic Compounds in the Locomotor Activity. To determine whether the selected experimental conditions elicited the optimal locomotor activity responses, we tested the effect of two toxic compounds that modify locomotor activity in other insects. Cypermethrin (99.1%) was obtained from Chemotecnica SA, Buenos Aires, Argentina, and DEET (97%) was from Aldrich (Milwaukee, WI). Chemicals were dissolved in acetone to produce six concentrations of cypermethrin that ranged from 0.002 to 10 mg/ml, and five concentrations of DEET that ranged from 1 to 60 mg/ml. Batches of randomly selected lice were individually treated with 0.1 µl on the dorsal abdomen, by using a 5 -µl Hamilton syringe. The insects were treated under a stereoscopic microscope to ensure that each drop was placed onto the abdomen of each specimen. Each dilution, including acetone for controls, was replicated at least three times, with 10 insects per replicate. After treatment, insects were placed on a 7-cm-diameter Whatman no. 1 filter disk moistened with 0.5 ml of water and incubated in a plastic petri dish (Vassena et al. 2003). Head lice were kept at 18 ± 0.5°C and 70–80% RH. Mortality was assessed at 18 h. Treated lice were transferred to the center of a new 7-cm filter paper disk. The criterion for mortality

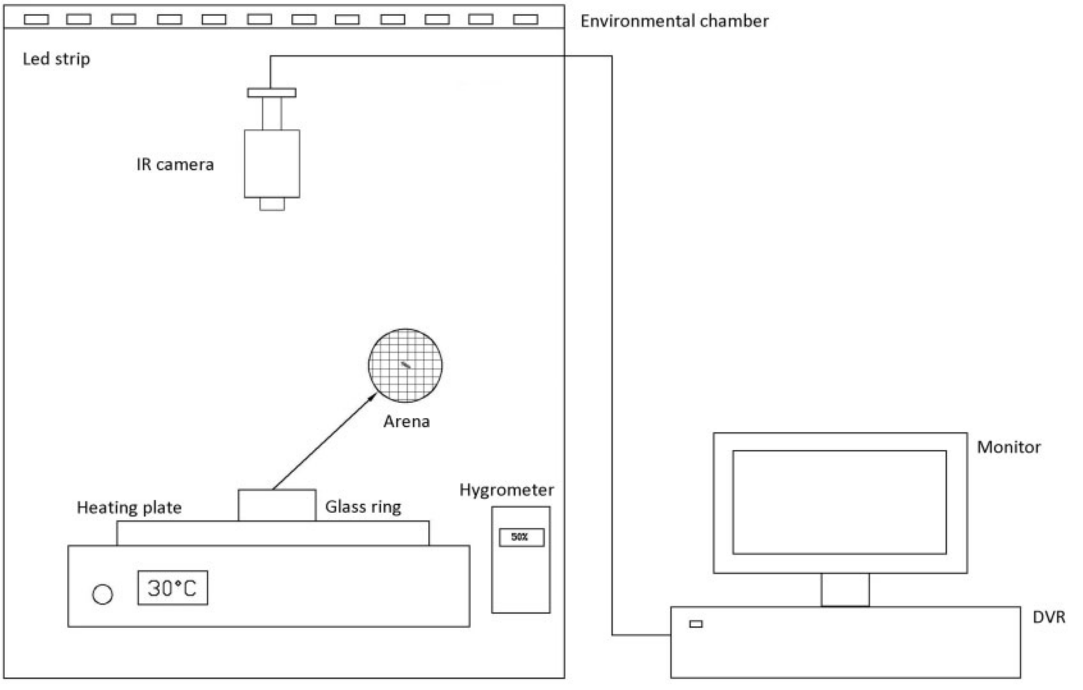

Fig. 1. Experimental device used to determine the locomotor activity of lice.

was inability to walk from the center to the border of the filter paper disk within a period of 15 s (Picollo et al. 1998). Dose–response data were subjected to probit analysis to estimate the sublethal doses (micrograms per insect) required to kill between 10 and 25% of the treated specimens (LD_{10}–LD_{25}). Once the sublethal doses were calculated for both toxics, final doses for cypermethrin and DEET were 0.001 µg and 3 µg per insect, respectively. Control lice were treated with acetone. After the topical application, an insect was taken to the experimental chamber and its locomotor activity was recorded. Locomotor activity was quantified at 0, 10, 20, and 30 minutes after topical application for each head louse. We tested 15 adult head lice individually for cypermethrin and for DEET. Evaluations were conducted at 30 ± 0.5°C, 21 ± 0.1 lux, and 50% RH.

Statistical Analysis. Locomotor activity was quantified by counting the number of crossings to each square of the arena during 3 minutes. In the experiments of temperature, humidity, light, and toxic compounds, the mean and the standard error (SE) was calculated for each treatment.

The effect of temperature, humidity, and light on the measured variable was tested using one-way analysis of variance (ANOVA). The homogeneity of variances was ascertained by Levene's test (temperature, $P = 0.6876$; humidity, $P = 0.1464$ (20°C), $P = 0.3498$ (30°C); light, $P = 0.0994$), and the normality of the data was ascertained graphically (Q-q plot). Tukey's test was used for post hoc comparison when it was suitable.

A two-way ANOVA for repeated measures was performed to assess the effect of two factors: the toxic compounds (cypermethrin and DEET) and the exposure time. The normality was ascertained (Shapiro–Wilk, cypermethrin, $P = 0.4954$; DEET $P = 0.9874$) as well as the homogeneity of covariance matrix using the Box test (cypermethrin, $P = 0.716$; DEET, $P = 0.580$). The model's assumption of sphericity (Mauchly's sphericity test) was not fulfilled for cypermethrin ($P = 0.006$) and DEET ($P = 0.002$). Nonetheless, the deviation from sphericity was solved by adjusting the degrees of freedom with the Greenhouse–Geisser correction. The Infostat v2012 statistical package was used for Shapiro–Wilk, Q-q plot, Levene's test, and ANOVA (http://www.infostat.com.ar). The assumptions of homogeneity of covariance matrix and sphericity were performed in IBM® SPSS® Statistics Version 9. The threshold for significance was at $P < 0.05$ in all the cases.

The results of the topical effect of cypermethrin and DEET were expressed as a locomotor activity index (LAI) = *[(treatment mean activity* $_{(Xmin)}$ *− control mean activity* $_{(xmin)}$*)/control mean activity* $_{(xmin)}$*]*. Positive LAI values (>0) indicate hyperactivation and negative LAI values (<0) indicate hypoactivation.

For estimation of the sublethal doses, data were analyzed using the Polo PC program (Le Ora software 2002). Mortality data were corrected using Abbott's formula (Abbott 1925). Dose–response data of *P. h. capitis* were subjected to probit analysis (Litchfield and Wilcoxon 1949) to estimate the sublethal doses.

Results

The effect of the temperature in the locomotor activity of head lice was determined. Head lice increased

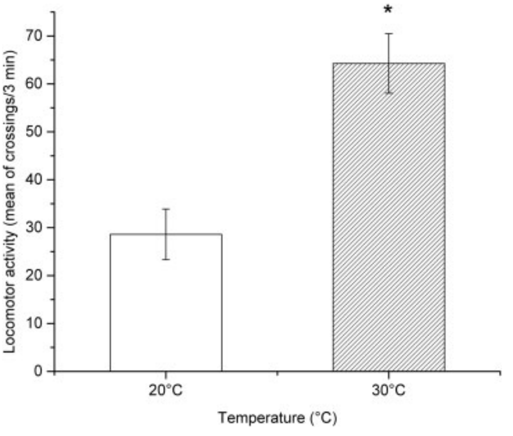

Fig. 2. Comparison of the mean locomotor activity of head lice tested in arenas at 20°C and 30°C. Asterisk denotes significant differences ($P < 0.05$, one-way ANOVA).

their locomotor activity at 30°C ($F = 19.13$; df = 1; $P = 0.0002$; Fig. 2).

When the RH level was tested, the locomotor activity of head lice showed no significant differences with the change in the humidity level, both at 30°C ($F = 1.08$; df = 2; $P = 0.3498$) and at 20°C ($F = 2.01$; df = 2; $P = 0.1464$; Fig. 3).

Results obtained from the exposure to variable degrees of light are shown in Fig. 4. Mean of locomotor activity was 17.94 ± 4.8, 39.72 ± 8.12, and 72.05 ± 5.62 for low-intensity light, medium-intensity light, and high-intensity light, respectively. There were significant differences among the three treatments ($F = 18.43$; df = 2; $P < 0.0001$). Moreover, we observed that the higher the intensity of light, the slower the movement of head lice (post hoc analysis with Tukey's test, $P < 0.05$).

The two-way ANOVA for repeated measures performed for cypermethrin showed that there was a significant effect on the locomotor activity of the insects treated ($F = 5.83$; df = 1; $P = 0.0221$; Fig. 5). Insects topically treated with cypermethrin showed hyperactivated behavior. There was also a significant effect of the factor time in the responses of head lice ($F = 10.15$; df = 3; $P > 0.0001$). However, a nonsignificant interaction between the two factors was found ($F = 1.55$; df = 2.164; $P = 0.218$).

Lice exposed to DEET elicited hypoactivated behavior (F 6.45; df = 1; $P = 0.0153$; Fig. 6). There was a significant effect of the factor time in the responses of head lice ($F = 32.32$; df = 3; $P > 0.0001$). The interaction of these factors was significant ($F = 3.39$; df = 2.392; $P = 0.03$). Thus, data were analyzed to determine the simple effects. Simple effects test reveals the degree to which one factor is differentially effective at each level of a second factor. At 10, 20, and 30 minutes, the exposed insects to DEET significantly diminished their locomotor activity compared with the control (10 min, $P = 1.72 \times 10^{-6}$; 20 min, $P = 0.0003$; 30 min, $P = 0.0001$).

The LAI for cypermethrin and DEET is presented in Fig. 7. The LAI values for cypermethrin showed

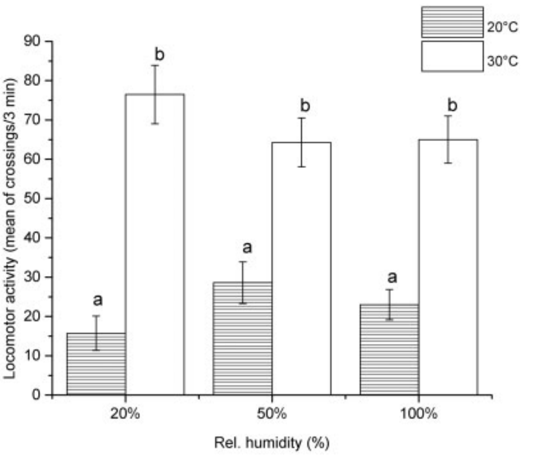

Fig. 3. Comparison of the mean locomotor activity of head lice exposed to arenas at different humidity levels and specific temperature. Different letters indicate significant differences among treatments ($P < 0.05$ one-way ANOVA).

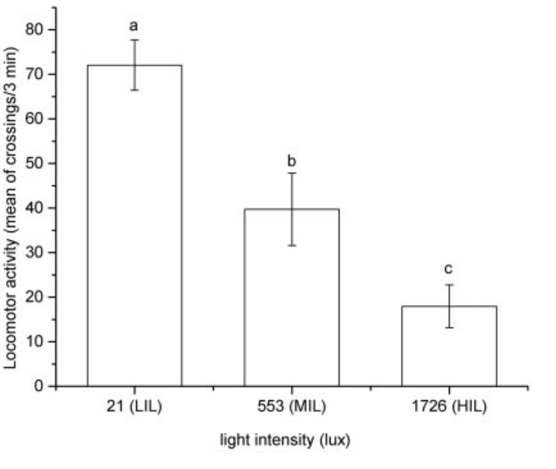

Fig. 4. Comparison of mean locomotor activity of insects exposed to different light intensities (mean ± SE). Different letters indicate significant differences among treatments ($P < 0.05$, Tukey's test). LIL, low-intensity light; MIL, medium-intensity light; and HIL, high-intensity light.

hyperactivation immediately after the topical application. There was a peak of hyperactivation at 20 minutes. The LAI values for DEET showed hypoactivation. The effect of DEET appeared to be not immediate: mean of crossings with DEET started to become significantly different after 10 minutes of the initial exposure.

Discussion

One of the main obstacles in the control of pediculosis is the transmission from host-to-host. Transmission from host-to-host tends to go unnoticed, making the prevention of reinfestation extremely difficult. Reinfestation is a highly controversial social and economic transmission issue that even effective pediculicides are unable to solve (Heukelbach 2010). Locomotor activity

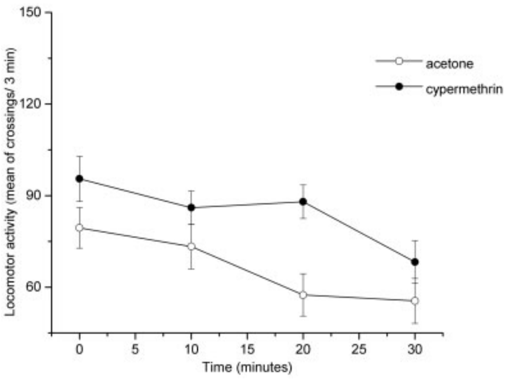

Fig. 5. Effect of topical application with cypermethrin on the locomotor activity of head lice.

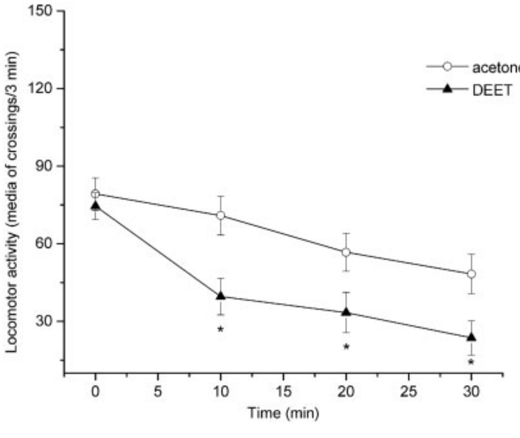

Fig. 6. Effect of topical application with DEET on the locomotor activity of head lice. Asterisks indicate the means of crossings with DEET that are significantly different at $P < 0.05$.

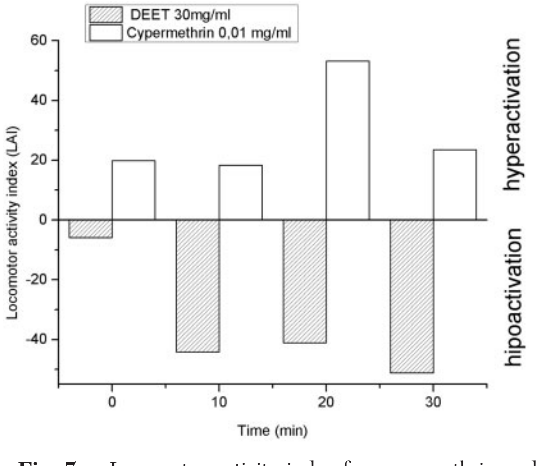

Fig. 7. Locomotor activity index for cypermethrin and DEET (LAI).

of lice is the determining behavior to understand the dynamics of this problem.

This work aimed to understand how environmental conditions could modify the locomotor activity of head lice within an experimental set-up specially designed. The novelty of our study is the development of an experimental device that allowed us to standardize the study of head louse response. Head lice responded successfully under the tested conditions of temperature (30°C) and light (low-intensity light); these conditions are similar to the natural environment of head lice in a human head.

Physical parameters like temperature and humidity have been widely studied as cues that modify life-history parameters such as mortality and oviposition (Berger et al. 2008, Gallardo et al. 2009). It has been demonstrated that temperature represents classical short-distance stimulation in blood-sucking insects who feed on vertebrate hosts (Lehane 1991, Flores and Lazzari 1996). Heat is not only an attracting agent but it can also give information about the host animal (Buxton 1946). We propose that higher activity of lice in an arena at 30°C would be associated to the recognition of heat as a short-distance host cue. In addition, enhanced behavior in warmer environments could give information about the zones that head lice choose to live. Considering that temperatures near the head scalp are considerably higher than distal hairs, increased locomotor activity not only can be associated to host finding but also to the search of an area to feed and lay eggs as well as search of potential mates.

Concerning the influence of humidity in louse locomotor activity, we found no influence in the assays conditions. The degree of involvement of humidity in behavioral studies tends to be unclear and has been reported as important as a synergistic agent with other stimuli (Lehane 1991). Barrozo et al. (2003) showed that *Triatoma infestans* nymphs elicited positive orientation and more activity owing to the synergistic effect of warm and wet sources. Nonetheless, when we combined high temperatures and high humidity, we could not find such synergistic effect.

Previous evidence on the study of the light effects on locomotor response of human lice is contradictory: the first studies demonstrated a tendency of head lice to photonegative behavior, moving in a contrary direction from a light source, describing it as a negative phototaxia (Nuttall 1917, Wigglesworth 1941, Buxton 1946); on the contrary, Mougabure-Cueto et al. (2011) found that body and head lice exhibited a photopositive response, as they preferred light to total darkness. In this study, we observed that head lice exposed to high-intensity light reduced their locomotor activity and after certain time, they definitively stopped. Thus, in accordance with the studies of Mougabure-Cueto et al. (2011), we suggest that the photopositive behavior found in head lice would be a kinesis. Kinesis is the simplest type of locomotor response of an animal to a certain stimulus, moving in a direction that is related only to the intensity of that stimulus, disregarding any spatial properties that the stimulus might possess (Matthews and Matthews 1978). This suggestion does not ignore the existence of a real taxis toward light, and further studies on this matter should be done.

We demonstrated that sublethal doses of toxic compounds may alter locomotor activity in adults of head lice. This is the first time that the modification of locomotor activity of head lice by toxic compounds is shown. Moreover, the study of the effect of chemicals on locomotor activity allowed an interesting approach that represents important information on the migration of head lice among hosts when exposed to toxic compounds. Sublethal doses of cypermethrin induced hyperactivated responses in adult head lice, as was proven with other arthropods like spiders and kissing bugs (Baatrup and Bayley 1992, Alzogaray et al. 2001). Alzogaray et al. (1997) found that 2 ng per insect of deltamethrin as a topical treatment on *Rhodnius prolixus* nymphs evocated no significant hyperactivated behavior. However, our results showed hyperactivation of head lice when exposed to similar doses of cypermethrin (1 ng per insect). Sublethal doses of DEET evocated hypoactivated responses in head lice. Similarly, Sfara et al. (2013) found that *Blatella germanica* were hypoactivated under DEET treatment.

The observation of stereotyped behavior in head lice elicited by toxic compounds proved that measuring locomotor activity in our experimental set-up controlling environmental conditions would be an appropriate parameter to evaluate compounds of biological importance such as molecules involved in the host–parasite interaction and intraspecific relationships. Studies focusing on locomotor activity should be encouraged for more successful and accurate control of the transmission and reinfestation.

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