How do ectoparasitic nycteribiids locate their bat hosts?

S. I. LOURENÇO* *and* J. M. PALMEIRIM

Centro de Biologia Ambiental, Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

(*Received 26 March 2008; revised 18 May 2008; accepted 29 May 2008; first published online 29 July 2008*)

SUMMARY

Nycteribiids (Diptera: Nycteribiidae) are specific haematophagous ectoparasites of bats, which spend nearly all their adult lives on hosts. However, females have to leave bats to deposit their larva on the walls of the roosts, where they later emerge as adult flies. Nycteribiids had thus to evolve efficient sensorial mechanisms to locate hosts from a distance. We studied the sensory cues involved in this process, experimentally testing the role of specific host odours, and general cues such as carbon dioxide, body heat, and vibrations. As models we used two nycteribiids (*Penicillidia conspicua* and *Penicillidia dufourii*) and their primary bat hosts (*Miniopterus schreibersii* and *Myotis myotis*, respectively). Carbon dioxide was the most effective cue activating and orientating the responses of nycteribiids, followed by body heat and body odours. They also responded to vibration, but did not orientate to its source. In addition, sensory cues combined (carbon dioxide and body heat) were more effective in orientating nycteribiids than either cue delivered alone. Results suggest that nycteribids have some capacity to distinguish specific hosts from a distance, probably through their specific body odours. However, the strong reliance of nycteribiids on cues combined indicates that they follow these to orientate to nearby multispecies bat clusters, where the chances of finding their primary hosts are high. The combination of sensory cues seems therefore an effective strategy used by nycteribiids to locate bat hosts at a distance.

Key words: ectoparasitism, bat flies, Nycteribiidae, host location, sensory cues, Chiroptera.

INTRODUCTION

Nycteribiid flies (Diptera: Nycteribiidae) are a distinctive family of widespread haematophagous ectoparasites exclusively associated with bats (Marshall, 1970, 1981; Dick and Patterson, 2006). They exhibit a high degree of host specificity, with most species parasitizing a single bat species (monoxenous), or a group of phylogenetically close bat species, usually from the same genus (stenoxenous) (Marshall, 1981; ter Hofstede, 2004; Dick and Patterson, 2006).

Through evolution, these animals acquired a high degree of morphological specialization to their parasitic life style: they are wingless, have reduced compound eyes and dorso-ventrally flattened bodies with combs, which help them to anchor to the hair of bats (Marshall, 1981; Lehane, 2005). Moreover, as with most obligate parasites, the life cycles of nycteribiids became intimately associated with those of their hosts (Lehane, 2005; Dick and Patterson, 2006). Adult individuals spend nearly all their lives on the fur of their bat hosts, where they feed on blood, encounter mates and reproduce (Marshall, 1970, 1981; Lehane, 2005). However, nycteribiids have a viviparous reproduction, and females often have to leave their hosts to deposit a full-grown larva on the walls of bat roosts, one at a time (e.g. Ryberg, 1947; Ching and Marshall, 1968; Marshall, 1970). This larva immediately pupates, metamorphoses attached to the walls, and emerges about 4 weeks later as an adult fly (e.g. Ryberg, 1947; Ching and Marshall, 1968; Marshall, 1970). This phase of reproduction away from the host required the evolution of effective sensorial mechanisms by nycteribiids to locate and recognize suitable hosts from a distance. However, this may be a complex task because bats are highly mobile and nycteribiids perish quickly when away from hosts (*unpublished personal observations*). Furthermore, because many bat species regularly roost in multi-species aggregations (Palmeirim, 1990), nycteribiids have to be able to distinguish them. Survival of nycteribiids thus largely depends on their ability to efficiently locate suitable hosts in a vast environment and in a limited amount of time.

A substantial amount of information is available on the host location behaviour of some groups of ectoparasites. These are known to exploit a wide variety of sensory cues to locate and recognize their hosts at some distance, ranging from general cues, delivered by all potential hosts, like light (e.g. Humphries, 1968; Poulin *et al*. 1990; Mikheev *et al*. 1998; Bandilla *et al*. 2007), vibration (e.g. Lawrence, 1981; Poulin *et al*. 1990), heat (e.g. Wigglesworth, 1941; Meyrowitsch *et al*. 1991, Kilpinen and Mullens, 2004), and carbon dioxide (e.g. Gillies, 1980; Takken and Knols, 1999; Guerenstein and Hildebrand, 2008), to specific cues like particular chemical

* Corresponding author: Tel: 00351 217500000. E-mail: silourenco@fc.ul.pt

Parasitology (2008), **135**, 1205–1213.
doi:10.1017/S003118200800468X Printed in the United Kingdom

compounds present in host body odours (e.g. Vaughan and Mead-Briggs, 1970; Osterkamp *et al*. 1999; Costantini *et al*. 2001; Krasnov *et al*. 2002; Smallegange *et al*. 2005). However, knowledge on the mechanisms involved in host location by nycteribiids is scarce, and mostly limited to small descriptions in broader autoecology studies of these parasitic species (e.g. Ryberg, 1947; Ching and Marshall, 1968; Marshall, 1970). According to these authors, mechanical vibrations, carbon dioxide and heat are likely to play a role in the emergence behaviour of nycteribiids. However, these same authors do not mention what cues are involved in host location after emergence, apart from Marshall (1970) who briefly stated that host location is done by random movements.

To our knowledge, this is the first experimental study that attempts to determine how nycteribiids locate their hosts at some distance. For this, we tested the role of sensory cues known to be involved in host searching behaviour by other haematophagous ectoparasites, namely body heat, vibration and olfactory cues, including carbon dioxide and host specific odours. In addition, we tested whether these parasites are able to discriminate between their primary bat host species and an alternative bat host from some distance. As models we used 2 nycteribiid species from the same genus, *Penicillidia conspicua* and *Penicillidia dufourii*, and their primary bat host species, *Miniopterus schreibersii* and *Myotis myotis*, respectively.

MATERIALS AND METHODS

Study species

The studied hosts are two temperate zone bats, the Schreibers' bat (*M. schreibersii*) (Kuhl, 1817) (Chiroptera: Miniopteridae) and the greater mouse-eared bat (*M. myotis*) (Borkhausen, 1797) (Chiroptera: Vespertilionidae). Both bats usually carry heavy loads of ectoparasites (Lourenço and Palmeirim, 2007). In the Mediterranean region, they roost almost exclusively in caves and mines, but further north *M. myotis* roosts mostly in buildings (Palmeirim, 1990; Rodrigues *et al*. 2003). In southern Europe, the two bats are highly gregarious, forming large nursing colonies, where individuals of both species often mix (Palmeirim, 1990; Rodrigues *et al*. 2003).

The nycteribiids *P. conspicua* Speiser, 1901 and *P. dufourii* (Westwood, 1935) (Diptera, Nycteribiidae) are morphologically similar species, characterized by the presence of a pair of ocelli and by atypical large bodies and long legs which allow them to live largely on the surface of the fur of the bat (Marshall, 1981). Both species are considered to be host specific; *P. conspicua* has a clear preference for *M. schreibersii*, although it can sporadically be found on *M. myotis* (Estrada-Peña *et al*. 1991; Imaz *et al*. 1999), whereas *P. dufourii* is mostly associated with *M. myotis* and *M. blythii*, although it can sporadically be found on other *Myotis* sp. and on *M. schreibersii* (Estrada-Peña *et al*. 1991; *unpublished personal observations*). These two parasites species can often be found cohabiting in the same bat colonies, because their hosts often cluster together (Palmeirim, 1990; Rodrigues *et al*. 2003).

Data collection

Bats and their nycteribiids were collected during the spring and summer of 2006 in 4 roosts of the region of Moura in southern Portugal (38° 08′N, 7° 26′W). We captured an average of 15 bats of each species per visit to a roost. These were caught with the help of a harp trap placed at the entrance of roosts (Lourenço and Palmeirim, 2007), and under a permit (57/2006/CAPT) issued by Instituto para a Conservação da Natureza e da Biodiversidade. Captures took place early in the morning (~07.00) and bats were released 12 h later (~19.00), so that they did not miss any foraging night. Each bat was placed in a separate cotton bag, to avoid the mixing of their parasites, and brought to captivity where experiments took place. These hosts had to be held in captivity to prevent starvation of the flies. While in captivity, bats were kept in total darkness under ambient conditions, similar to those of their roosts (~17 °C and ~75% relative humidity). Collection of nycteribiids for the experiments was made by directing them into a plastic tube, avoiding the use of forceps, since these can harm their hind legs and hence affect their behaviour. After collection, nycteribiids were sexed and identified. All nycteribiids used in the experiments were adult females and no individual was used more than once.

Experimental setup and testing procedures

We designed distinct experiments to test the responses of nycteribiids to potential sensory cues (specific host body odours, heat, vibration, and carbon dioxide), and to test whether they can discriminate between their primary host and an alternative bat host at a distance. All experiments took place between 09.00 and 17.00, since this is the time when nycteribiids deposit their larva on the cave walls and need to locate suitable roosting bats (Marshall, 1970). Experiments were conducted in total darkness and under controlled ambient conditions (~17° C and ~75% relative humidity). The activity of nycteribiids during tests was recorded continuously with a digital camcorder (Panasonic NV-15) using reflected infra-red illumination (not thermal infra-red), which allowed filming without disturbing their behaviour.

Response of nycteribiids to host body odours

To test the responses of *P. dufourii* and *P. conspicua* to body odours of their primary hosts we used a glass

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

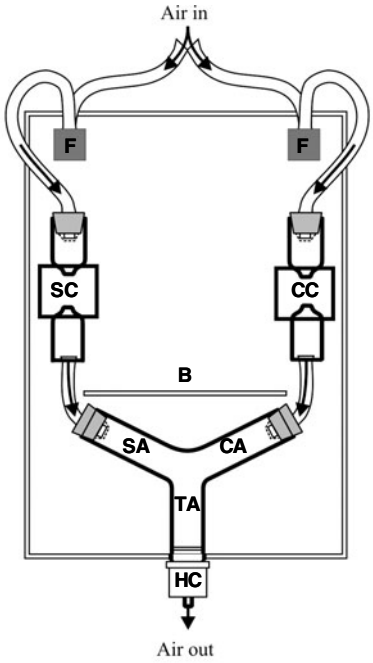

Fig. 1. Y-tube olfactometer (adapted from Jackson *et al.* 2000) (not drawn to scale). Arrows indicate the path of air flow. F – flowmeters, SC – stimulus chamber, CC – control chamber, B – opaque barrier to prevent nycteribiids from seeing odour cues, SA – stimulus arm, CA – control arm, TA – test arm, HC – holding chamber.

Y-tube olfactometer (stem: length 10 cm and internal diameter 2 cm, each arm: length 12 cm and internal diameter 2 cm) (Fig. 1). Air flowed from an aquarium pump into 2 separate flowmeters (Matheson FM-1000 flowmeter) adjusted to 1000 ml/min flow. From there, airflow moved into a stimulus chamber (containing an odour cue) and a control chamber (without an odour cue), and subsequently into 2 choice arms (Fig. 1). The air then converged into the test arm, at the end of which there was a holding chamber where nycteribiids were placed prior to each test. The olfactometer was surrounded by a white frame to minimize visual distractions from the room (Fig. 1). Ten min before each test began, the air pump was turned on, and we placed an odour cue – a piece of cotton rubbed on the urine and fur of the host – in the stimulus chamber, and a piece of clean cotton in the control chamber. A single nycteribiid was then placed in the holding chamber and 5 min later the net separating the holding chamber from the test arm was removed. Each test began when the net was removed and lasted for 10 min. For each test, we randomly switched the position of the stimulus and control chambers in order to avoid directional biases. After each test, the olfactometer was dismantled and cleaned with 80% alcohol and distilled water (Jackson *et al.* 2002), to prevent any potential influences from traces of previously tested parasites. To exclude possible biases in the movements of nycteribiids caused by the airflow within the olfactometer, we ran 'blank' tests, during which both testing chambers were empty (i.e. no odour source was placed in the stimulus or in the control chamber) and the air was turned on. Nycteribiids rarely waved their legs and did not move around in the olfactometer, which demonstrates that their responses are not influenced by the airflow.

Response of nycteribiids to heat

The responses of nycteribiids to heat were tested in an experimental arena (Fig. 2A). This consisted of a circular glass (30 cm diameter) covered with white paper. The arena was placed inside a black plastic box (50 × 100 cm and 40 cm high) filled with distilled water to prevent nycteribiids from escaping. The plastic box was covered with a black lid fitted with a transparent window through which the arena could be viewed and filmed. The arena was divided in 4 equal quadrants; 1 was used as a stimulus quadrant and the remaining 3 as controls (Fig. 2A). The heat cue was simulated using a glass container (6 cm diameter, 7 cm high) filled with water at 35 °C (±1) to replicate bat body temperature, and sealed with Parafilm®. Prior to each test, we placed this container, hereafter named stimulus container, in the middle of the stimulus quadrant, and 3 identical control containers with water at room temperature (~17 °C) in the 3 control quadrants (Fig. 2A). After all containers were positioned in the arena, a nycteribiid was placed at the top of a wire (0·3 cm diameter, 5 cm long) in its centre (Fig. 2A), so that it could choose any direction on its way down. Tests started immediately after that and lasted for 10 min. The positions of the stimulus and control quadrants were switched between tests to exclude directional bias. After each test, the white paper surface was replaced to prevent any chemical traces of previously tested parasites.

Response of nycteribiids to carbon dioxide

The response of nycteribiids to carbon dioxide was tested in the described arena. We inserted 4 identical plastic tubes (5 mm diameter) through holes in the outer box, which delivered air to the centre of each quadrant of the arena (Fig. 2B). During tests, the tubes in the control quadrants delivered charcoal–filtered air at 1000 ml/min (flowmeters Matheson FM-1000 flowmeter). The tube of the stimulus quadrant delivered air at the same rate, but with a concentration of carbon dioxide above the normal atmospheric concentration of 350 ppm (Gillies, 1980). We tried increasing concentrations until reaching a level at which nycteribiids responded (~2000 ppm above atmospheric concentration). Carbon dioxide was delivered from a pressurized cylinder with outflow pressure regulated by a

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

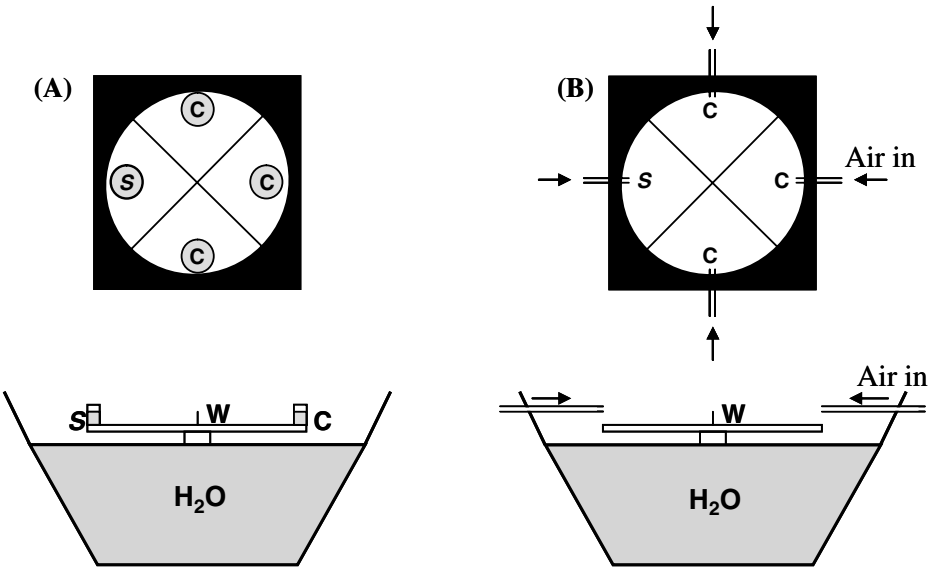

Fig. 2. Top and lateral views of the arena used to test the responses of nycteribiids to (A) heat and (B) carbon dioxide. (A) *S* – stimulus container (at 35 °C), C – control containers (at room temperature), W – wire. (B)*S* – stimulus tube (with flow of carbon dioxide), C – control tubes (with flow of air), W – wire.

manometer (RBD-30 Carburos Metalicos), and mixed with charcoal-filtered air in an airtight box. All other test procedures were analogous to those described for the heat tests.

Response of nycteribiids to vibrations

The response of nycteribiids to substrate vibrations was tested in the described arena. The vibration stimulus was a gentle continuous scratching of the edge of one of its quadrants with a piece of wire (0·3 cm diameter, 50 cm long). Each test consisted of a control period of 5 min without any vibration stimulus, and a 5 min period during which the edge of the arena was scratched as uniformly as possible. The position of the scratched quadrant (i.e. stimulus quadrant) varied between tests. All other test procedures were analogous to those described for the heat tests.

Response of nycteribiids to a combination of carbon dioxide and heat

Bat hosts always provide more than one potential sensory cue simultaneously, such as body heat and exhaled carbon dioxide. To determine how nycteribiids responded to this particular combination of cues, we ran experiments in an arena in which we provided them simultaneously. To do this we combined the procedures described above for the heat and carbon dioxide tests. The stimulus quadrant had a container at 35 °C and a flow of air with a concentration of carbon dioxide above normal atmospheric levels, whereas the control quadrants had containers at ambient temperature and flows of charcoal–filtered air.

Ability of nycteribiids to discriminate their primary host

We used the olfactometer (Fig. 1) to test whether nycteribiids were able to discriminate their primary host from an alternative bat host at a distance. The methodology was similar to that employed to test the responses to host body odours, but this time we placed 2 live bats in the chambers of the olfactometer (i.e., the primary bat host species of the tested nycteribiid in one chamber, and its alternative host in the other).

Data analysis

All the experiments were video-recorded continuously during the 10 min of its duration, and the recordings were used to quantify the behavioural responses of nycteribiids to the different cues. We used the following parameters to quantify the responses: (1) latency of movement (time from beginning of the test to the first movement); (2) latency of choice (time from first movement to the choice of a host); and (3) choice (considered as the arm or quadrant where the nycteribiid spent most time, and at least 1 continuous min). The sample size (*n*) values given in Table 1 only include the individuals that responded to the cues.

We calculated confidence intervals of 95% for percentages using the Wilson score method (Newcomb, 1998). Chi-square goodness of fit tests (Sokal and Rohlf, 1995) were used to analyse the choices of nycteribiids. Between-species comparisons were analysed with non-parametric U-Mann Whitney tests (Sokal and Rohlf, 1995). All statistical analyses were performed using Excel (2002) and

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

Table 1. Time lag (mean ± S.D.) (in sec) from the beginning of the test to the first movement of parasite (latency of movement), and from time of first movement to choice of a host (latency of choice)

(The sample sizes (*n*) only include the individuals that responded to the cues.)

	P. conspicua			*P. dufourii*		
Cues	Movement	Choice	*n*	Movement	Choice	*n*
Primary host	10 ± 8	182 ± 121	42	8 ± 5	14 ± 7	39
Host odour	102 ± 41	173 ± 108	27	88 ± 40	143 ± 114	32
Heat	58 ± 23	92 ± 33	29	70 ± 42	109 ± 52	24
CO_2	17 ± 9	10 ± 7	41	24 ± 10	21 ± 14	39
CO_2 + Heat	11 ± 7	6 ± 5	40	13 ± 10	12 ± 10	39

SPSS (version 12). *P*-values $\leqslant 0{\cdot}05$ were considered to be statistically significant.

RESULTS

Response of nycteribiids to host body odours

About half of the tested individuals (47% of *P. conspicua* and 53% *P. dufourii*) responded to host odours by moving around in the olfactometer. In both species, the responsive individuals showed a slight tendency to prefer the arm with the odour cue, although this was not statistically significant (*P. conspicua*, $\chi^2 = 1{\cdot}2$, D.F. = 1, $P = 0{\cdot}27$; *P. dufourii*, $\chi^2 = 2{\cdot}9$, D.F. = 1, $P = 0{\cdot}09$; Fig. 3). Both nycteribiid species took a similar amount of time to initiate their movements towards the odour cues (U = 249, $P = 0{\cdot}57$), and to choose between one of the arms (U = 212, $P = 0{\cdot}17$) (Table 1).

Response of nycteribiids to heat

Heat was more effective than odours in stimulating the movements of *P conspicua* (64%) and *P. dufourii* (54%). These nycteribiids were attracted to the stimulus quadrant about twice as often as to any of the control quadrants (*P. conspicua*, $\chi^2 = 23{\cdot}7$, D.F. = 3, $P = 0{\cdot}009$; *P. dufourii*, $\chi^2 = 9{\cdot}4$, D.F. = 3, $P = 0{\cdot}02$) (Fig. 4A). Both nycteribiid species spent a similar amount of time to start moving towards heat (Table 1), and these responses were quicker than the ones observed to host odours. Likewise, they took similar time to make their choice for a host (Table 1).

Response of nycteribiids to vibration

The large majority of *P. conspicua* (80%) and *P. dufourii* (85%) exhibited a behavioural response to vibration, waving their front legs up in the air, but remained at the top of the wire. This behaviour was not detected in the absence of the vibration stimulus. Only 22% of *P. conspicua* and 14% of *P. dufouri* exposed to vibrations descended from the wire but remained near its base and did not walk towards any of the quadrants.

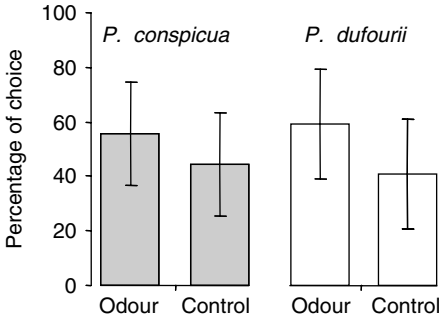

Fig. 3. Percentage (± 95% confidence intervals) of *Penicillidia conspicua* and *P. dufourii* that chose the odour arm versus the control arm in olfactometer tests.

Response of nycteribiids to carbon dioxide

Carbon dioxide was effective in activating movements from most tested individuals (*P. conspicua*, 91%, *P. dufourii*, 86·6%). Moreover, the large majority of these were attracted to the quadrant where the increased concentration of carbon dioxide was being released (*P. conspicua*, $\chi^2 = 86{\cdot}0$, D.F. = 3, $P = 0{\cdot}001$; *P. dufourii*, $\chi^2 = 130{\cdot}1$, D.F. = 3, $P = 0{\cdot}001$) (Fig. 4B). Additionally, both species were equally fast in activating their movements in response to carbon dioxide (U = 122, $P = 0{\cdot}44$) and when choosing the carbon dioxide quadrant (U = 408, $P = 0{\cdot}45$) (Table 1).

Response of nycteribiids to carbon dioxide and heat combined

The combination of carbon dioxide and heat resulted in a very high proportion of active responses (*P. conspicua*, 93%. *P. dufourii*, 88%). In addition, both species chose the quadrant which delivered heat and carbon dioxide combined far more often than the remaining quadrants (*P. conspicua*, $\chi^2 = 123{\cdot}9$, D.F. = 3, $P = 0{\cdot}0001$; *P. dufourii*, $\chi^2 = 162{\cdot}2$, D.F. = 3, $P = 0{\cdot}002$) (Fig. 4C). Also, the number of correct choices made by *P. dufourii* and *P. conspicua* when carbon dioxide and heat were delivered combined, increased by about 10%, compared to carbon dioxide delivered alone (Fig. 4B, C) and about 100%

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

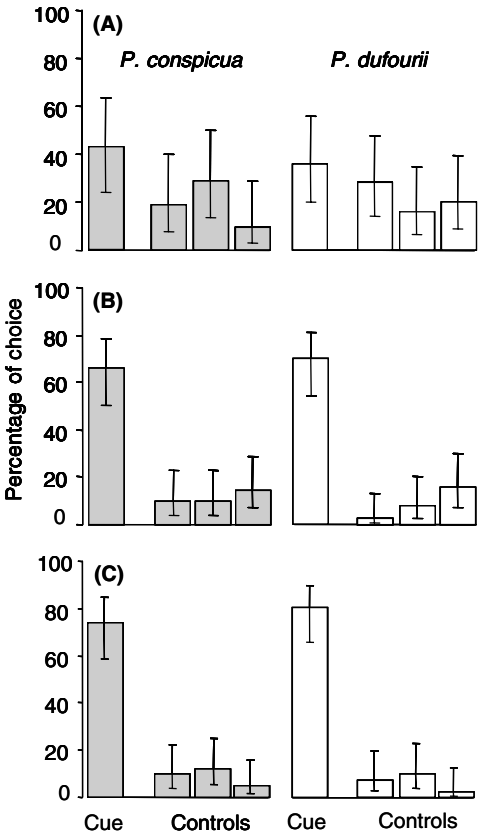

Fig. 4. Percentage (±95% confidence intervals) of *Penicillidia conspicua* and *P. dufourii* that chose the stimulus quadrant versus the control quadrants in arena tests. Stimulus quadrant delivered (A) heat, (B) air enriched with carbon dioxide and (C) heat and air enriched with carbon dioxide.

compared to heat delivered alone (Fig. 4A–C). *P. conspicua* and *P. dufourii* showed a similar latency to move (U = 334, $P = 0{\cdot}67$), and to choose a quadrant (U = 438, $P = 0{\cdot}54$). Both species took less time to respond and to make a choice when cues were delivered together than when delivered alone (Table 1).

Ability of nycteribiids to discriminate their primary host

All tested individuals of both nycteribiid species were able to choose a host within the time of experiment. However, the rate of correct choices differed between the two species (Fig. 5). Indeed, *P. dufourii* consistently chose the arm of the olfactometer with cues of its primary host, *M. myotis* ($\chi^2 = 15{\cdot}22$, D.F. = 1, $P < 0{\cdot}001$). In contrast, *P. conspicua* chose more frequently the arm of its alternative host, although this was not significant ($\chi^2 = 0{\cdot}82$, D.F. = 1, $P = 0{\cdot}36$) (Fig. 5). *P. conspicua* and *P. dufourii* were equally fast in moving in the presence of bats (U = 674, $P = 0{\cdot}92$) (Table 1). However, they differed in their latency of choice (U = 98, $P < 0{\cdot}001$), with *P. dufourii* rapidly choosing a bat and *P. conspicua* exhibiting a long

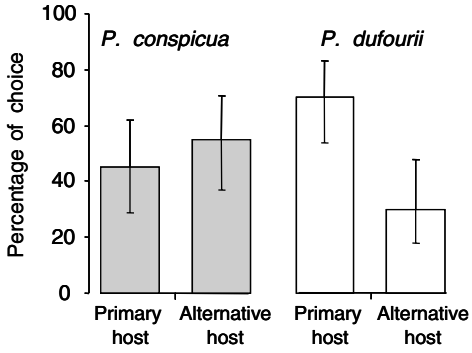

Fig. 5. Percentage (±95% confidence intervals) of *Penicillidia conspicua* and *P. dufourii* that chose their primary host species versus the alternative species in olfactometer tests.

exploratory behaviour at the junction of the arms before making a choice (Table 1).

DISCUSSION

Which sensory cues are involved in host location by nycteribiids at a distance?

Our results suggest that carbon dioxide is the most efficient cue used by nycteribiids to locate their hosts from a distance. This cue on its own promptly activated the movements from both *P. conspicua* and *P. dufourii*, and clearly attracted them. The decisive role of carbon dioxide in the activation and orientation behaviour of haematophagous ectoparasites is widely recognized (Marshall, 1981; Lehane, 2005; Guerenstein and Hildebrand, 2008). This is considered a long-range cue, delivered in high amounts by the breathing of vertebrates, and also through their skin. For example, human breath contains levels of carbon dioxide of about 45 000 ppm, against the atmospheric concentration of about 350 ppm (Barrozo and Lazzari, 2004). Our tests revealed that nycteribiids of both species are very sensitive to small increases of carbon dioxide, responding to concentrations as low as 2000 ppm above those of normal atmospheric levels. Lower concentrations did not evoke behavioural responses. This is the first demonstration of the importance of carbon dioxide as a long-range cue for adult nycteribiids.

Although it seems that nycteribiids are able to find hosts using just carbon dioxide to guide them, our results suggest that other cues, such as body heat and host odours may also be involved in the process. In fact, simulated body heat alone activated the movements in both *P. conspicua* and *P. dufourii*. In addition, they appeared to be attracted to the heated container. The use of host body heat as a cue by nycteribiids is not surprising, because even though heat is generally considered a close-range stimulus (Lehane, 2005), some other small ectoparasites use it from as far as 2 m (e.g. Wigglesworth, 1941). Furthermore, the thermally stable and homogeneous

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

conditions found in underground roosts are presumably suitable for the use of this sensory cue. Specific host odours were also able to activate the movement of nycteribiids, but were less successful than heat or carbon dioxide at directing them. Such a weak directional response to the odours of their primary hosts is not in line with findings for other host-specific haematophagous parasites (e.g. Vaughan and Mead-Briggs, 1970; Osterkamp *et al*. 1999; Costantini *et al*. 2001; Krasnov *et al*. 2002; Smallegange *et al*. 2005), including species of the closely related family Streblidae (Overal, 1980). Why don't nycteribiids rely more on specific odour cues, which would orientate them directly to their specific hosts? These two host species, like many other cave bats, often form dense mixed clusters (Palmeirim, 1990), which release large amounts of different sensory cues. We presume that the specific body odours of the various bat species present in these clusters blend, and consequently these might not be such efficient host discriminating cues for nycteribiids. Vibration was the only tested cue that elicited a behavioural response of nycteribids but did not orientate their responses. Marshall (1970) and Ryberg (1947) had already noted that touching the pupa caused the adult nycteribiids to emerge, and suggested that at least the pupae are sensitive to mechanical vibration.

Our results also revealed that nycteribiids rely on a combination of sensory cues to enhance their ability to locate bats. In fact, they responded stronger and more efficiently to the combination of carbon dioxide and heat than to either of the stimuli alone. This capacity of nycteribiids to take advantage of cues combined for locating hosts has also been described for many other haematophagous parasites (e.g. Gillies, 1980; Osterkamp *et al*. 1999; Takken and Knols, 1999; Barrozo and Lazzari, 2004; Smallegange *et al*. 2005). Lehane (2005) suggested that this strategy increases the certainty of the presence and nature of a host, since one cue alone has a higher chance of not being host originated, and therefore maximizes the chances of host encounter while minimizing energy consumption.

Are nycteribiids able to discriminate their primary hosts from other bats at a distance?

The nycteribids species responded differently when exposed to their primary hosts and an alternative bat in the olfactometer. *P. dufourii* tended to quickly select the side of its primary host (*M. myotis*), while *P. conspicua* spent far more time in exploratory behaviour at the junction of the arms of the olfactometer, and in the end was unable to choose its primary host (*M. schreibersii*). This apparent difference in host location behaviour between the two nycteribiids is surprising, because *P. conspicua* and *P. dufourii* are closely related species and exhibited similar responses to all sensory cues. How can this difference be explained?

Body heat and carbon dioxide are general cues, released by all vertebrates (Lehane, 2005), and therefore have a low potential to allow discrimination between host species. However, hosts with greater body masses or metabolic rates are likely to emit these general cues in larger amounts. Thus, as *M. myotis* has approximately twice the body mass of *M. schreibersii* (Palmeirim *et al*. 1999), it presumably delivers stronger general cues than the latter. We presume that in the experiments, when *P. dufourii* reached the junction of the arms of the olfactometer, it received from the side of its primary bat host (*M. myotis*) both specific odour cues and a great amount of general cues. All cues combined might have been responsible for the strong attraction of this nycteribiid towards the *M. myotis* side, explaining the high percentage of correct choices and the short time needed to make them. In contrast, when *P. conspicua* reached the junction of the stimulus and control arms, it probably received contradictory cues: specific odour cues from the side of its primary host (*M. schreibersii*), but stronger general cues from the side of its alternative host *M. myotis*. This conflict may explain why *P. conspicua* did not significantly choose any of the arms and took so much time at their junction. Hence, the most parsimonious interpretation for these results is that nycteribiids have some capacity to discriminate their primary bat hosts from other bats at some distance, probably by their odours. However, specific cues seem to be unable to counter the attraction of general cues combined, to which we found nycteribids to be very sensitive. Nevertheless, these results can not be considered entirely conclusive and the issue deserves further research.

Altogether, our results suggest that in order to find a host, nycteribids initially rely on the combination of several cues, such as carbon dioxide and body heat, rather than only on specific host odour cues. These general cues may orientate them to individual bats or to large multispecies clusters, where the chance of finding their primary hosts is high. Even if nycteribiids do not directly find their primary bat hosts, this may not be a major problem, as they are most likely able to survive on alternate hosts, presumably until they have an opportunity to change to their preferred bat species. We assume that for *P. conspicua* and *P. dufourii* these opportunities might be common, as their hosts often form mixed clusters in southern Europe. In this work, we have only considered the cues important in host location. Once in physical contact with potential hosts, nycteribiids might use different cues. In fact, these may discriminate their primary hosts mainly through specific bat skin emanations, which they likely recognize by tarsal contact (Marshall, 1981).

https://doi.org/10.1017/S003118200800468X Published online by Cambridge University Press

We thank friends and colleagues who helped with fieldwork, namely S. Vinuesa, J. T. Marques, L. Rodrigues, M. Augusto, M. Lecoq, R. Correia, R. Ferreira, R. Moreira, F. Amorim and B. Pinto. We are very grateful to M. Lecoq for logistical support, H. U. Schnitzler and I. Kaipf for training on keeping bats in captivity, and A. Gracio (IHMT) for introduction to mite identification. A. Cerveira and two anonymous reviewers provided helpful comments on an earlier version of the manuscript. This work was part of a Ph.D. thesis that is supported by the Fundacão para a Ciência e Tecnologia (FCT), co-financed by the European Regional Development Fund.

REFERENCES

Bandilla, M., Hakalahti-Sirén, T. and Valtonen, E. T. (2007). Experimental evidence for a hierarchy of mate- and host-induced cues in a fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura). *International Journal for Parasitology* **37**, 1343–1349. doi:10.1016/j.ijpara.2007.04.004.

Barrozo, R. B. and Lazzari, C. R. (2004). Orientation behaviour of the blood-sucking bug *Triatoma infestans* to short-chain fatty acids: synergistic effect of L-lactic acid and carbon dioxide. *Chemical Senses* **29**, 833–841. doi:10.1093/chemse/bjh249.

Ching, L. M. and Marshall, A. G. (1968). The breeding biology of the bat-fly *Eucampispoda sundaicum* Theodor, 1955 (Diptera: Nycteribiidae). *Malayan Nature Journal* **21**, 171–180.

Costantini, C., Birkett, M. A., Gibson, G., Ziesmann, J., Sagnon, N. F., Mohammed, H. A., Coluzzi, M. and Pickett, J. A. (2001). Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. *Medical and Veterinary Entomology* **15**, 259–266. doi:10.1046/j.0269-283x.2001.00297.x.

Dick, C. W. and Patterson, B. D. (2006). Bat flies: obligate ectoparasites of bats. In *Micromammals and Macroparasites: From Evolutionary Ecology to Management* (ed. Morand, S., Krasnov, B. and Poulin, R.), pp. 179–194. Springer-Verlag Publishing, Tokyo.

Estrada-Peña, A., Balcells, E. and Serra-Cobo, J. (1991). Los artropodos ectoparasitos de murciélagos en España: In *Los Murciélagos de España y Portugal* (ed. Benzal, J. and De Paz, O.), pp. 253–279. Colección Técnica. ICONA.

Gillies, M. T. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research* **70**, 525–532.

Guerenstein, P. G. and Hildebrand, J. G. (2008). Roles and effects of environmental carbon dioxide in insect life. *Annual Review of Entomology* **53**, 161–178. doi:10.1146/annurev.ento.53.103106.093402.

Humphries, D. A. (1968). The host-finding behaviour of the hen flea, *Ceratophyllus gallinae* (Schrank) (Siphonaptera). *Parasitology* **58**, 403–414.

Imaz, E., Aihartza, J. R. and Totorika, M. J. (1999). Ectoparasites on bats (Gamasida, Ixodida, Diptera) in Biscay (N. Iberian peninsula). *Miscelanea Zoológica* **22**, 21–30.

Jackson, R. R., Clark, R. J. and Harland, D. P. (2002). Behavioural and cognitive influences of kairomones on an araneophagic jumping spider. *Behaviour* **139**, 749–775.

Kilpinen, O. and Mullens, B. A. (2004). Effect of food deprivation on response of the mite, *Dermanyssus gallinae*, to heat. *Medical and Veterinary Entomology* **18**, 368–371.

Krasnov, B. R., Khokhlova, I. S., Oguzoglu, I. and Burdelova, N. I. (2002). Host discrimination by two desert fleas using an odour cue. *Animal Behaviour* **64**, 33–40. doi:10.1006/anbe.2002.3030.

Lawrence, P. O. (1981). Host vibration – a cue to host location by the parasite, *Biosteres longicaudatus*. *Oecologia* **48**, 1432–1939.

Lehane, M. J. (2005). *The Biology of Blood-Sucking Insects*. 2nd Edn. Cambridge University Press, Cambridge, UK.

Lourenço, S. I. and Palmeirim, J. M. (2007). Can mite parasitism affect the condition of bat hosts? Implications for the social structure of colonial bats. *Journal of Zoology* **273**, 161–168. doi:10.1111/j.1469-7998.2007.00322.x.

Marshall, A. G. (1970). The life cycle of *Basilia hispida* Theodor 1957 (Diptera:Nycteribiidae) in Malaysia. *Parasitology* **61**, 1–18.

Marshall, A. G. (1981). *The Ecology of Ectoparasitic Insects*. Academic Press, London, UK.

Meyrowitsch, D., Christensen, N. O. and Hindsbo, O. (1991). Effects of temperature and host density on the snail-finding capacity of cercariae of *Echinostoma caproni* (Digenea: Echinostomatidae). *Parasitology* **102**, 391–395.

Mikheev, V. N., Valtonen, E. T. and Rintamaki-kinnunen, P. (1998). Host searching in *Argulus foliaceus* L. (Crustacea: Branchiura): the role of vision and selectivity. *Parasitology* **116**, 425–430.

Newcombe, R. G. (1998). Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine* **17**, 857–872.

Osterkamp, J., Wahl, U., Schmalfuss, G. and Haas, W. (1999). Host-odour recognition in two tick species is coded in a blend of vertebrate volatiles. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **18**, 59–67.

Overal, W. L. (1980). Host-relations of the bat fly *Megistopoda aranea* (Diptera: Streblidae) in Panamá. *The University of Kansas Science Bulletin* **52**, 1–20.

Palmeirim, J. M. (1990). Bats of Portugal: zoogeography and systematics. *The University of Kansas, Museum of Natural History* **82**, 1–53.

Palmeirim, J. M., Rodrigues, L., Rainho, A. and Ramos, M. J. (1999). Chiroptera. In *Mamíferos Terrestres de Portugal Continental, Açores e Madeira* (ed. Mathias, M. L., Santos-Reis, M., Palmeirim, J. M. and Ramalhinho, M. G.), pp. 41–95. Instituto da Conservação da Natureza e Centro de Biologia Ambiental, Lisboa.

Poulin, R., Curtis, M. A. and Rau, M. E. (1990). Responses of the fish ectoparasite *Salmincola edwardsii* (Copepoda) to stimulation, and their implication for host-finding. *Parasitology* **100**, 417–421.

Rodrigues, L., Zahn, A., Rainho, A. and Palmeirim, J. M. (2003). Contrasting the roosting behaviour and

phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. *Mammalia* **67**, 321–335.

Ryberg, O. (1947). *Studies on Bats and Bat Parasites*. Bokförlaget Svensk Natur, Stockholm.

Smallegange, R. C., Qiu, Y. T., van Loon, J. J. and Takken, W. (2005). Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Chemichal Senses* **30**, 145–152. doi:10.1093/chemse/bji010.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd Edn, W. H. Freeman and Company, New York, USA.

Takken, W. and Knols, B. G. J. (1999). Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology* **44**, 131–157.

ter Hofstede, H. M., Fenton, M. B. and Whitaker, J. O., Jr. (2004). Host and host-site specificity of bat flies (Diptera: Streblidae and Nycteribiidae) on Neotropical bats (Chiroptera). *Canadian Journal of Zoology* **82**, 616–626. doi:10.1139/z04-030.

Vaughan, J. A. and Mead-Briggs, A. R. (1970). Host-finding behaviour of the rabbit flea, *Spilopsyllus cuniculi* with special reference to the significance of urine as an attractant. *Parasitology* **61**, 397–409.

Wigglesworth, V. B. (1941). The sensory physiology of the human louse *Pediculus humanus corporis* de Geer (Anoplura). *Parasitology* **33**, 67–109.