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ORIGINAL PAPER

The seabird tick, *Ixodes uriae*, uses uric acid in penguin guano as a kairomone and guanine in tick feces as an assembly pheromone on the Antarctic Peninsula

Joshua B. Benoit · Giancarlo Lopez-Martinez · Seth A. Philips · Michael A. Elnitsky · Jay A. Yoder · Richard E. Lee Jr · David L. Denlinger

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Abstract In the vicinity of Palmer Station, Antarctica, the seabird tick, *Ixodes uriae*, forms large aggregations under rocks at the periphery of Adelie penguin rookeries. When the adult penguins return to the rookeries at the beginning of the nesting season the ticks leave their off-host aggregation site, attach to the penguins for a period of feeding, and then subsequently return to the aggregation site. In this study, we searched for chemical cues that may be used by the ticks to locate their aggregation sites as well as cues involved in finding penguins. Tick excreta and soil extracts from beneath tick aggregations contained a pheromone that elicited assembly behavior in unfed larvae, non-fed nymphs and non-fed adults. Guanine, the major excretory product of ticks, elicited assembly behavior, thus, guanine is likely an active component involved in assembly. Non-fed stages also responded positively to penguin guano and uric acid, the primary excretory product of penguins, suggesting that uric acid and other components of penguin guano function as a kairomone used by the non-fed ticks to locate their host. After feeding, the immature ticks' response to both the assembly and kairomones is switched off for several days, and the ticks regain responsiveness only after they have molted. Fed adult females lay eggs and die without ever regaining responsiveness. Thus, *I. uriae* relies on two closely related chemicals to regulate two critical aspects of its life: assembly and host-finding. Guanine and other components of tick excreta function as an assembly pheromone in promoting the formation of off-host aggregations, while uric acid and other components of penguin guano function as a kairomone used by the tick to locate its host.

Keywords Penguin · Tick · Assembly pheromone · Host cues · Kairomone · Antarctica

J. B. Benoit (✉) · G. Lopez-Martinez · S. A. Philips · D. L. Denlinger
Department of Entomology, The Ohio State University, 318W. 12th Ave, Columbus, OH 43210, USA
e-mail: benoit.8@osu.edu

J. A. Yoder
Department of Biology, Wittenberg University, Springfield, OH 45501, USA

M. A. Elnitsky · R. E. Lee Jr
Department of Zoology, Miami University, Oxford, OH 45056, USA

Introduction

Enormous burdens of the seabird tick, *Ixodes uriae*, can have a significant negative impact on breeding performance of penguins and other birds that nest at high latitudes (Eveleigh and Threlfall 1974; Mangin et al. 2003). Birds such as the Adelie penguin, *Pygoscelies adeliae*, are sought as hosts at the beginning of the breeding season, but during the remainder of the year the ticks can be found in large aggregations under rocks near the rookeries (Fig. 1). So dense are these tick aggregations that the underlying and surrounding soil may be whitish in appearance from tick feces (Knülle and Devine 1972; Sonenshine 1991) that have accumulated over the years. These favorable off-host microhabitats satisfy the moisture and temperature requirements, particularly for winter protection, essential for development, molting, mating, oviposition and hatching (Benoit et al. 2007). Soon after drop-off from the host, fed stages of the tick exhibit an intense, excited wandering period that prompts them to leave the immediate area of the rookery and seek a suitable off-host site, one frequently already occupied by numerous other ticks of both sexes and all developmental stages.

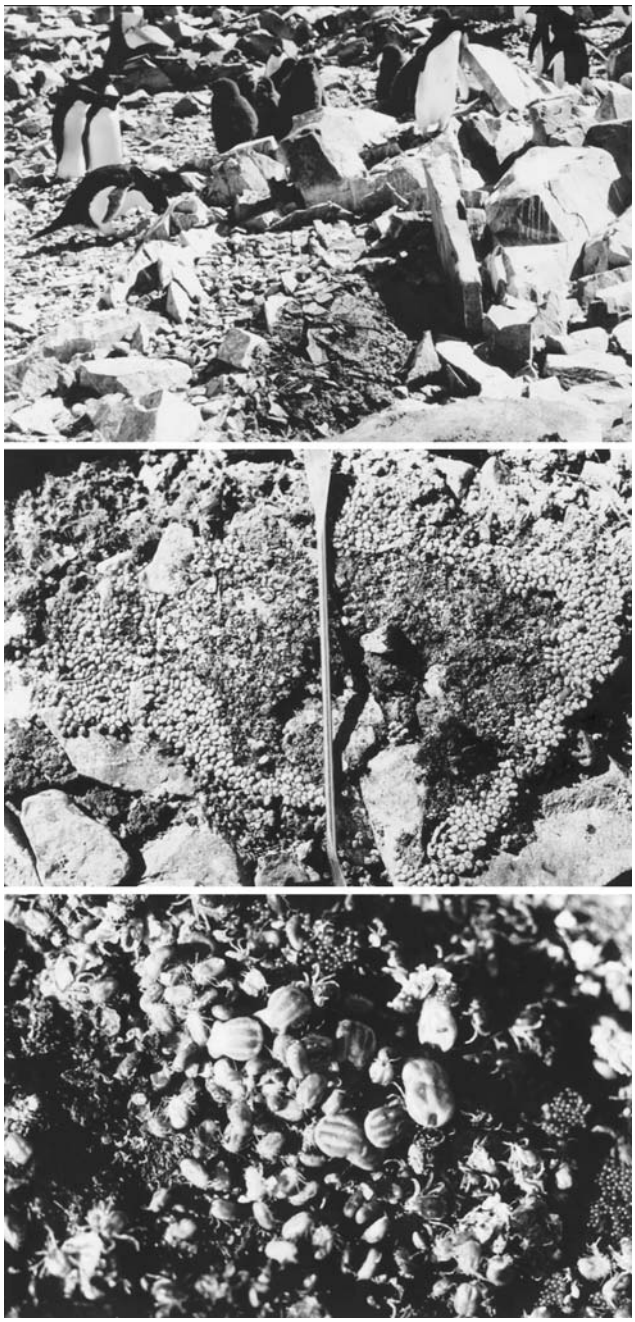

Fig. 1 Photographs of aggregations of *I. uriae* under rocks adjacent to an Adelie penguin (*Pygoscelies adeliae*) rookery near Palmer Station, Antarctica

The aggregations of *I. uriae* that we observed in the field suggest the possible use of an assembly pheromone. Though not all ticks use assembly pheromones (Sonenshine 1991), an assembly pheromone consisting of guanine and related purines is known from *I. scapularius* (Allan and Sonenshine 2002; Sonenshine 2006). The goal of this study was to determine whether *I. uriae*, living near Palmer Station, Antarctica, uses an assembly pheromone (chemicals released by ticks that attract other conspecifics) to promote the formation of its off-host aggregations, and to determine whether chemical cues emanating from the penguin rookeries are used by the ticks as kairomones (chemicals released by hosts that attract ticks) to locate their host.

Materials and methods

Tick collection and maintenance

Ixodes uriae were field collected on Humble and Comorant Islands off the Antarctic Peninsula, near Palmer Station (64°04′S, 64°03′W), January–March, 2006–2007, using aspirators and soft-tipped forceps. All ticks appeared to be healthy and exhibited regular crawling movements and heightened activity in response to CO_2 stimulation (Sonenshine 1991). Unfed larvae were used for experiments 10–14 days after hatching, and non-fed nymphs and adults were used 2–3 weeks after ecdysis. Engorged stages (larvae, nymphs and females; males do not feed) were selected while they were in the wandering stage (prior to settling for molting), indicating that they had recently completed feeding and dropped from the host. Storage conditions for ticks in the laboratory were 4 ± 1°C, 98% RH (saturated K_2SO_7 solution; Winston and Bates 1960; measured by hygrometer ±3% RH; Thomas Scientific, Philadelphia, PA), a relative humidity that is higher than the critical equilibrium humidity (CEH) of this tick species (Benoit et al. 2007), and 15:9 h photoperiod maintained in an environmental chamber.

Collection of semiochemicals

To reflect natural conditions, soil with an abundance of whitish fecal spheres (Sonenshine 1991) collected beneath aggregations of ticks was evaluated as a potential source of assembly pheromone. Control soil samples were collected from areas near penguin rookeries not inhabited by ticks. Fresh penguin guano, which has a high uric acid content (Lindeboom 1984), was obtained by collecting guano of *P. adeliae* within 5 min after deposition. Soil and fecal samples were dissolved in acetone and stored in 2 cc glass vials protected from light exposure. Commercial uric acid and guanine were diluted in acetone (HPLC-grade; Sigma Chemical Co., St. Louis, MO), and acetone served as the control. To conform to standard practice (Sonenshine 1991), natural tick assembly pheromone (tick excreta) was collected by placing a 0.5 cm filter paper disc in a mesh-covered 20 cc glass vial containing 5 or 25 non-fed ticks for 1 week. Females were tested against putative assembly pheromones collected from females, males against males, nymphs against nymphs, and larvae against larvae. Fed stages were also tested against assembly pheromones from their corresponding non-fed stages.

Attraction bioassay

The assembly pheromone assays used by Allan and Sonenshine (2002) for *I. scapularis*, modified from the two-choice, four quadrant design of Arlian and Vyszenski-Moher (1995), was used. This is a short range test conducted on a 9 cm filter paper disc enclosed within a 100 × 15 mm Petri dish. Latex gloves were worn to avoid contact with potentially attractive skin oils. The filter paper disc (Whatman No. 3; Whatman, Hillsboro, OR) was divided into four quadrants with a pencil (No. 2, Pentel, Torrance, CA), and alternating quadrants received either 25 µl acetone (control) or 25 µl of the test compound. The 25 µl spot applications did not exceed a 2 cm diameter and were placed 3 cm from the center of the filter paper disc arena and air-dried before use. Only one compound and one concentration were tested at a time. Ticks were released ten at a time (five at a time for larger engorged stages) into the center of the bioassay arena. Counts of ticks in the various quadrants (two control; two test) were made after 1 h, 24 h and 5 days. Fed ticks were subsequently tested after molting. Test temperature was 4 ± 1°C in accordance with the tick's natural environmental temperature and common standards used for polar research (Lee and Baust 1982, 1987; Worland and Block 2003). Corresponding observations were made at 25°C for comparison with other tick pheromone literature (Sonenshine 1991; Allan and Sonenshine 2002). Each concentration of a compound was tested using 150 non-fed ticks and 150 fed ticks. Acetone-only (all quadrants received 25 µl acetone) and untreated filter paper discs were used as controls to eliminate left-right hand bias.

Data were expressed as the percentage of ticks in the test quadrants and also as the relative efficacy of attraction (REA) as described by Norval et al. (1991) and Yunker et al. (1992) to allow comparison with other known tick attractants: REA = 100 × [(% unattracted to control) − (% unattracted to test)]/(% unattracted to control). The number of ticks in acetone-only control discs served as the control value in the REA calculations. Chi-square (χ^2) statistics were used to compare percentage attraction values (following arcsin transformation) using 50% attraction as the expected (E) value with $a = 0.05$, $df = 1$ (Sokal and Rohlf 1995).

Results

Response of non-fed ticks to natural pheromone and guanine

When introduced into the bioassay arena, all stages displayed typical searching behavior by waving their front pairs of legs, pausing on occasion for 1–2 min with front legs raised, then resumed crawling. Most movement in the arena stopped within 25–35 min, with ticks lowering their body, settling and forming small clusters, usually at the edge of the dish, where they remained immobile with their legs tucked under their bodies. For all stages, treatment areas were encountered passively without a redirection in crawling path or any deliberate crawling movement indicative of attraction. In all cases, upon encountering other individuals, the ticks stopped moving, became sessile and joined the cluster. Once a tick settled, there was little movement within the cluster or to adjacent clusters for as long as a day. None of the ticks were observed displaying feeding posture behavior (posterior end raised, palps splayed and making mouthpart contact with the filter paper; 40× microscopy). Tick excreta prompted significant arrestment by 67% of the larvae, 66% of the nymphs, 64% of the males and 71% of the females (Table 1). Strong arrestment responses were elicited by 0.05 M guanine for larvae (64%), 0.5 M guanine for nymphs (61%), 0.001 M guanine for males (61%), and 0.01 M guanine for females (63%) (Table 1). Highest arrestment responses noted for uric acid were 67% larvae responding to [0.05 M] uric acid, 69% nymphs to [0.005 M] uric acid, 67% males at [0.001 M] uric acid, and 69% females at [0.05 M] uric (Table 1). There was no evident behavioral dose response for guanine and uric acid concentrations. Similar results were obtained at 25°C (data not shown), however searching behavior was more excited and settling occurred more rapidly than at 4°C. Non-fed adults reacted similarly to assembly pheromone from nymphs and larvae, and we noted no other differences of responsiveness of one stage to the pheromones from other stages; we also noted no differences in the scores at 24 h and 5 days (data not shown) compared to the scores reported at 1 h in Table 1. No statistical differences were noted between the acetone and untreated controls ($P > 0.05$). We conclude that each of the non-fed stages of *I. uriae* assemble, characterized by arrestment and retention, and the assembly behavior elicited by the natural pheromone present in the feces is similar to the response elicited by guanine alone.

Responses of fed ticks to natural pheromone and guanine

After feeding, the ticks displayed a hurried crawling activity on the bottom of the Petri dish. The wandering phases of engorged stages persisted 3–5 days for fed larvae, 4–6 days for fed nymphs and 4–6 days for females. Some fed larvae and fed nymphs crawled up the sides of the Petri dish before they settled completely for apolysis (whitish appearance, for larvae and nymphs) or oviposition (females). They paused every 1–3 min and then continued crawling, typically following the edge of the Petri dish. This produced a line of ticks, most facing the edge around the

Table 1 Positive behavioral responses of non-fed *I. uriae* to the natural assembly pheromone (tick excreta), guanine, and uric acid tested at 4°C

	Larvae		Nymphs		Males		Females	
	%	REA	%	REA	%	REA	%	REA
Tick excreta								
Controls	49.3		52.6		50.7		54.7	
5 ticks	65.3*	24.5	68.7*	23.4	67.3*	24.7	66.0*	17.1
25 ticks	68.6*	28.1	66.0*	20.3	64.0*	20.8	70.6*	22.5
Guanine								
Controls	53.3		52.0		50.7		52.7	
0.001 M	61.3*	13.1	53.3	2.4	61.3*	17.3	60.7*	13.2
0.005 M	60.6*	12.0	56.7	8.3	57.3	11.5	59.3	11.1
0.01 M	58.6	9.0	57.3	9.2	58.0*	12.6	62.7*	15.9
0.05 M	64.0*	16.7	60.0*	13.3	58.7*	13.6	61.3*	14.0
0.1 M	58.0	8.1	59.3*	12.3	59.3*	14.5	58.0	9.1
0.5 M	62.0*	14.0	61.3*	15.2	60.0*	15.5	63.3*	16.7
Uric acid								
Controls	51.3		52.6		52		49.3	
0.001 M	65.3*	21.4	68.7*	23.4	60.7*	14.3	66.0*	25.3
0.005 M	61.3*	16.3	69.3*	24.1	54	3.7	64.7*	23.8
0.01 M	66.0*	22.3	60.7*	13.3	62.0*	16.1	61.3*	19.6
0.05 M	66.7*	23.1	62.7*	16.1	60.7*	14.3	68.7*	28.2
0.1 M	59.3*	13.5	62.0*	15.2	56.7	8.3	59.3*	16.9
0.5 M	64.0*	19.8	67.3*	21.8	58	10.3	65.3*	24.5

Control, acetone-only test; *M*, molar; *REA*, relative efficacy of attraction, see text for details; *, denotes a significant percentage difference compared to 50% attraction ($P < 0.05$). Maximum standard error was ±2.6%. Each proportion is based on assays of 150 ticks, 5 ticks per assay

perimeter of the dish. At 25°C, the wandering period was shortened by 1–2 days and movement was more rapid. All fed stages encountered the spot applications in the bioassay arena randomly and were unaffected and failed to stop when they made contact with the treated surfaces. No regular clustering effect was evident in response to the tested concentrations of natural assembly pheromone, guanine, or uric acid (Table 2). Fed ticks were fairly evenly distributed in different quadrants, and no significant differences were seen. Fed females were also unresponsive to excreta collected from nymphs and larvae. At 25°C, the intensity of crawling activity increased, but the ticks settled at the edge of the dish more readily than they did at 4°C. Similar results were noted after 1 day (data not shown), and as before no differences were noted between the acetone and untreated controls. Thus, the arrestment and clustering response to the natural assembly pheromones, guanine and uric acid, ceased immediately after feeding, and the ticks remained unresponsive until after molting. Although immature ticks again gained responsiveness after molting, adult females laid eggs and died without ever regaining responsiveness.

Response to soil from underneath the tick clusters

Non-fed nymphs reacted positively, displaying assembly behavior, when exposed to soil extracts containing tick excreta, whereas they were unresponsive to extracts of soil made from soil not exposed to ticks (Fig. 2). Concentrations of 0.1 g soil/ml yielded the highest response (68%). No response was evident to acetone or in the untreated controls, and similar results were noted for non-fed larvae, males, and non-fed females. The responses to soil extracts (Fig. 2) were thus similar to the responses observed using assembly pheromone collected from non-fed nymphs noted in Table 1 (ANOVA; $P > 0.05$).

In response to soil extracts, fed nymphs settled in a fairly even distribution around the edge of the Petri dish (Fig. 2) as noted in Table 2. Results at 25°C were similar to the results observed at 4°C: positive responses by non-fed nymphs and lack of response by fed nymphs. At 25°C the settling response was more rapid, and we noted no differences in the results observed at 1 and 24 h (data not presented). Thus, non-fed ticks consistently responded to the soil extracts, while fed ticks did not. We conclude that sites where ticks aggregate in nature contain a rich source of assembly pheromone.

Response to penguin guano and uric acid

Extracts of penguin guano ranging from 0.001 to 0.1 g/ml elicited a pronounced arrestment response in non-fed nymphs (Fig. 3) as well as non-fed larvae, males and non-fed females. We emphasize that penguin guano was applied as an acetone extract and was not used neat, thus the behavior modifying properties of the guano is not due to the

Table 2 Engorged *Ixodes uriae* showing a lack of response to the natural assembly pheromone (tick excreta), guanine and uric acid tested at 4°C

	Larvae		Nymphs		Females	
	%	REA	%	REA	%	REA
Tick excreta						
Controls	53.3		52		52.7	
5 ticks	52.0	−2.5	53.3	2.4	49.3	−6.9
25 ticks	54.6	2.4	56.7	8.3	59.3	−11.1
Guanine						
Controls	50.7		53.3		50	
0.001 M	49.3	−2.8	46.7	−14.1	49.3	−1.4
0.005 M	51.3	1.2	48	−11.0	48.0	−4.2
0.01 M	52.7	3.8	58	8.1	52.7	5.1
0.05 M	50.0	−1.4	50.7	−5.1	50.0	0
0.1 M	57.3*	11.5	51.3	−3.9	54.7	8.6
0.5 M	50.7	0	49.3	−8.1	50.7	1.4
Uric acid						
Controls	51.3		52.7		51.3	
0.001 M	52.0	1.3	52.7	0	49.3	−4.1
0.005 M	52.7	2.7	53.3	1.1	52.7	2.7
0.01 M	51.3	0	54.7	3.7	51.3	0
0.05 M	49.3	−4.1	48.7	−8.2	58.0*	11.6
0.1 M	50.7	−1.2	52.7	0	50.0	−2.6
0.5 M	51.3	0	55.3	4.7	50.7	−1.2

Control, acetone-only disc; *M*, molar; *REA*, relative efficacy of attraction; *, significant difference ($P < 0.05$) compared to 50%. Males do not feed, thus, are not included. Maximum standard error was ±2.9%. Each proportion is based on assays of 150 ticks, 5 ticks per assay

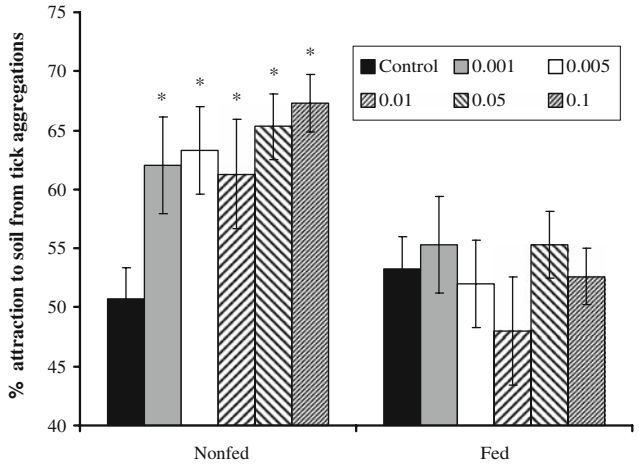

Fig. 2 Responses of non-fed and fed nymphs of *I. uriae* to soil extracts (g soil/ml) taken from sites near tick clusters. * significant difference ($P < 0.05$) compared to 50% attraction

presence of water. The response of non-fed nymphs to penguin guano was 5–7% higher than to pure guanine, Fig. 3 and Table 1 (ANOVA; $P < 0.05$). Penguin guano caused more non-fed nymphs to express assembly behavior

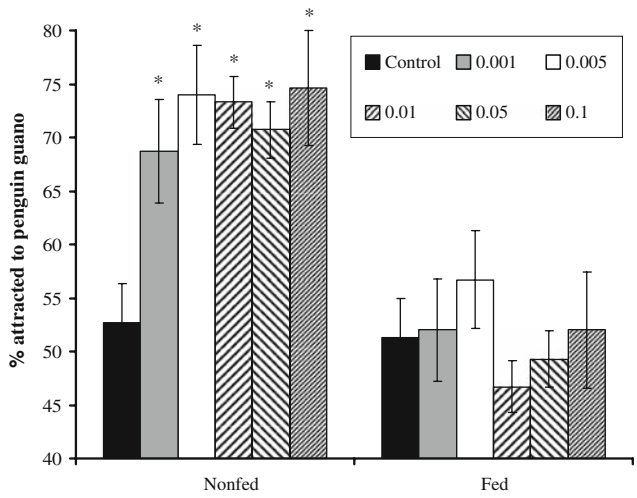

Fig. 3 Responses of non-fed and fed nymphs of *I. uriae* to extracts of penguin guano (g guano/ml). *, significant difference compared to 50% attraction ($P < 0.05$)

than the natural assembly pheromone present in tick excreta collected on filter paper (Table 1) or extracts from soil samples where mixed stages of ticks were aggregated (Fig. 2) (ANOVA; $P < 0.05$). Fed nymphs, however, were not responsive to penguin guano; the response was indistinguishable from that of the controls (Fig. 3). Likewise, uric acid did not elicit a response (Table 2; ANOVA; $P > 0.05$). Again, settling time was more rapid and crawling activity was more pronounced at 25°C than at 4°C. We conclude that non-fed ticks are highly responsive to penguin guano and uric acid, the chief component of penguin guano, while fed ticks are not attracted to these chemicals.

Discussion

Assembly pheromone (tick excreta, guanine)

Guanine and other components of excreta from *I. uriae* function as an assembly pheromone that promotes formation of the large aggregations of this tick species that we have observed under rocks near Adelie penguin rookeries (Fig. 1) on the Antarctic Peninsula. Not only do these aggregation sites provide access to mates, but they identify microhabitats that have proven to be suitable environments, meeting the temperature and humidity requirements needed for tick survival. To meet the tick's requirements for water balance, such sites must exceed 93% RH, the critical equilibrium humidity documented in this species (Benoit et al. 2007).

Our consistent set of behavioral, chemical and field observations of *I. uriae* meet the definition of assembly (Allan and Sonenshine 2002), i.e. ticks cease crawling, retract their legs, and remain immobile for as long as a day

(arrestment plus retention) when contacting the pheromone present in tick excreta or in response to guanine alone (Sonenshine 1991). Assembly pheromones characteristically have low volatility and a long residual life; thus, it is not an attractant used for long range detection. The response is chemotactic, requiring physical contact to elicit a response. Our experiments indicate that all non-fed stages respond to the pheromone, and all stages produce it, features that are both typical of tick assembly pheromones. In this case, guanine, the insoluble and non-volatile excretory product produced by all life stages, appears to be a major component of the assembly pheromone. Likely, guanine is not the sole active component, thus the natural pheromone is likely to be a multi-component blend consisting of multiple nitrogenous compounds (Gothe 1987; Allan and Sonenshine 2002). REA values are in the 20–40 range for good tick attractants, such as 2,6-dichlorophenol, *o*-nitrophenol, and methyl salicylate (Norval et al. 1991); REAs for the natural assembly pheromone, guanine and uric acid, are at the lower limit of this range, thus underscoring their poor ability as attractants and suggesting that these chemicals function only for retention, which has been noted for *Amblyomma americanum* in response to uric acid (Yoder et al. 2003). Higher concentrations of guanine or uric acid were not successful in increasing the response. Interestingly, fed stages were not responsive to the assembly pheromone for at least a few days after feeding. This response is lost immediately after feeding, and it may be associated with the natural dispersal that follows a feeding bout, where locating areas with proper moisture and temperature are more important than remaining in a site where other ticks are present.

Kairomone (penguin excreta, uric acid)

Ixodes uriae exhibits stronger behavioral responses to the kairomones emanating from the penguins than to its assembly pheromone, as has also been noted in other ticks (Petney and Bull 1981). Indeed, there is an approximately 10% increase in responsiveness by all non-fed stages of *I. uriae* to penguin excreta compared to tick excreta (assembly pheromone). Based on their structural similarities, uric acid and guanine might be expected to elicit a similar behavior (Allan and Sonenshine 2002). The fact that uric acid is a major component of bird excreta, including excreta from penguins (Lindeboom 1984), suggests that uric acid is the most active component of penguin guano. Uric acid has also been reported to be an important host cue for other tick species including the lone star tick, *A. americanum* (Yoder et al. 2003, 2008). Yet, it is also clear that uric acid is not the only active component of the kairomone because penguin guano was more attractive than uric acid alone.

Like the response to assembly pheromone, the tick's response to the uric acid-based kairomone is switched off after drop-off from the host. This seems logical since they no longer need to locate a host, and it may suggest that the same receptor is used for both the arrestment response to guanine (assembly pheromone) and the kairomone response because both are turned off immediately after feeding, and the two chemicals are closely related. Birds are well known tick predators, particularly on the fed stages of ticks (Samish and Rehacek 1999), thus switching off the receptor when a host is no longer needed, coupled with rapid crawling activity of fed stages, permits fed ticks to quickly escape zones of potential predation after they drop from the host. Fed stages then migrate to moist reprieves suitable for molting or oviposition, but the precise location of the tick aggregation site is partially dictated by the duration of the wandering period. The immature ticks will not again be prepared to aggregate until the wandering period has been completed and the ticks are once again responsive to the assembly pheromone. The fed females never regain responsiveness, thus we assume that the physical features of the habitat, rather than the assembly pheromone, dictate that off-host location. While many fed females do indeed return to off-host sites occupied by other ticks, we have also observed single, fed females under rocks in isolated locations, suggesting that this stage is important in dispersal. This is possible since *I. uriae* mates before blood feeding (McCoy and Tirard 2002).

In summary, a similar guanine/uric acid system functions in *I. uriae* as both an assembly pheromone and a kairomone for locating penguin hosts. The tick excreta, rich in guanine, is present in high abundance in the aggregation sites and functions as the assembly pheromone. The penguin guano, rich in uric acid, is used by the ticks to identify penguin nesting sites. We suggest the loss of sensitivity, following feeding, enables the ticks to escape the rookeries to avoid possible predation and facilitates dispersal to both previously-used aggregation sites or to new off-host sites.

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