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A preliminary investigation of cues used by mesostigmatic mites to locate their passalid beetle hosts

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Abstract

Four species of mesostigmatic mites associated with the passalid beetle *Odontotaenius disjunctus* (Illiger) (Coleoptera : Passalidae) were tested to define the cues used for host location. When placed in a free-choice disk olfactometer, mites were attracted to vials containing passalid beetles with which they had no physical contact. Mites were also attracted to vials from which a passalid beetle was removed just prior to testing. There was no significant difference between vials in control experiments when all were empty. Most importantly, mites were attracted to water washes of chloroform extracts taken from passalid beetle cuticle. Therefore chemical cues (kariomones) emanating from the host cuticle play an important role in the location of hosts by passalid mites. Using thin-layer chromatography, the attractant was found to be highly polar; however the specific chemicals in the attractant were not identified.

Introduction

Members of the family Passalidae are large, slow moving, subsocial beetles that inhabit decaying logs and seldom fly (Gray 1946; Pearse *et al.* 1936). Over 500 species of Passalidae have been described (Reyes-Castillo 1970), and, although most have not been examined for mites, over 200 species of mite associates from 24 families have been recovered from them (Hunter 1993). In fact the best known passalid species, *Odontotaenius disjunctus* (Illiger), an inhabitant of the eastern deciduous forest of the United States, is known to have a close association with sixteen species of mites representing 11 families (Delfinado and Baker 1975). For most of these mite species (hereafter referred to as "passalid mites"), the relationship is thought to be phoretic, however little is known concerning the biology of most. Pearse *et al.* (1936) considered parasitism a possibility in two species, and it has been demonstrated that females of one species (*Cosmolaelaps trifidus* (Pearse and Wharton)) cannot produce offspring if not associated with an adult host beetle (Mollin and Hunter 1964). In addition, all developmental instars of *Diarthrophallus quercus* (Pearse and Wharton) are found on adult beetles (Delfinado and Baker 1975). In the latter two cases a commensal relationship has been suggested with adult mites gaining nourishment from beetle salivary secretions (Mollin and Hunter 1964). It is possible that phoresy is but one of the relationships passalid mites have with their hosts and that further study will reveal other interactions as well. Whatever the relationship, it is apparent that the beetle host plays a significant role in the life histories

of the mite species. It is therefore important that mites have the ability to locate and maintain contact with their beetle hosts.

There exists at least some circumstantial evidence that chemical cues from host passalid beetles act as attractants to their mite associates. Hunter and Davis (1965) discovered that individuals of *Euzercon latus* (Banks) were only attracted to an artificial diet medium if it were mixed with an acetone wash from the host beetles, or if host beetles had recently been confined to the diet medium. They also found that, if given a choice in olfaction response experiments, *E. latus* preferred either live beetles or freshly chewed wood chips over beetle fecal material and non-chewed wood. Apparently mites did not distinguish between live beetles and wood chips freshly chewed by beetles. Mollin (1962) thoroughly washed passalid beetles using either distilled water or distilled water that contained detergent and then gave *Cosmolaelaps trifidus* (Pearse and Wharton) a choice between unwashed and washed beetles. Although mites chose unwashed beetles, filter paper soaked in the extracts did not attract mites. The present study is an attempt to better define the cues used by mesostigmatic passalid mites to locate their hosts.

Methods and Materials

Mite Culture

Beetles and their resident mites were collected from rotting logs in the College Woods on the campus of the College of William and Mary, Williamsburg, Virginia, and maintained in plastic shoe boxes containing moist wood from the extraction log. When not being used for experimentation, mites were maintained on their beetle hosts. Not all mite species associated with *O. disjunctus* were used in our study: some species are rare, some do not actively move about, some reside under the beetle's elytra or in other localities not readily accessible on live beetles, and others are too small. Investigations centered primarily around four mesostigmatic mite species that actively run about on the beetle's external surface and are therefore more easily collected: *Passalacarus sylvestris* Pearse and Wharton (Diplogyniidae), *Macrocheles tridentatus* Pearse and Wharton (Macrochelidae), *Diarthrophallus quercus* (Pearse and Wharton) (Diarthrophallidae) and *Hypoaspis disjuncta* Hunter and Yeh (Laelapidae). Since it is difficult to identify the exact species when moving mites from a beetle into an apparatus for experimentation, mites were lumped into two categories: large mites (*P. sylvestris*, *M. tridentatus*) and small mites (*D. quercus*, *H. disjuncta*).

Bioassays

Bioassays to quantify the attractiveness of mites to their beetle hosts were conducted using a free-choice disc olfactometer (Fig. 1A) fashioned after Krantz *et al.* (1991). The olfactometer was constructed by cutting four equally spaced holes, 2 cm in diameter, in the bottom of a 10 cm diameter plastic Petri dish. Each hole was 1 cm from the edge of the dish. A 44 mm mesh nylon screen was glued to the dish bottom and the inside walls of the dish coated with Fluon (DuPont), a liquid that dries to a low friction surface. Four 3 cm plastic screw-on vial lids, each containing a 2 cm diameter hole, were glued underneath the Petri dish such that the holes in the lids and the dish aligned. Plastic vials 5 cm tall that fit the lids could then be attached to hold beetles, other potential attractants, or left empty as controls. Vials were also easily removed for cleaning, and the entire olfactometer was washed with ethanol after each run to remove any residue or odors. The fine-mesh nylon screen on the test arena floor prevented mites from entering a vial as well as contact with a beetle, but allowed any odors from the vial to pass through. The nylon mesh also prevented a beetle placed in a vial from entering the test arena. Fluon coating the arena wall provided a low surface tension that hindered, but did not completely prevent, mites from climbing and thereby leaving the arena floor.

To remove the kariomone from the beetle cuticle for study, several different methods utilizing chloroform, ethanol, and water were attempted. The results of bioassays proved chloroform with a water wash to be the favored extraction method. A single live beetle was placed in a vial containing chloroform and a few drops of water and then shaken for ten minutes. The extract was then set aside for approximately two hours to allow the water containing the attractant chemicals to separate from the chloroform. The free-choice olfactometer used to bioassay cuticular extracts was much like the first, except there were only three holes in the arena (Fig. 1B). In addition, the vials were only

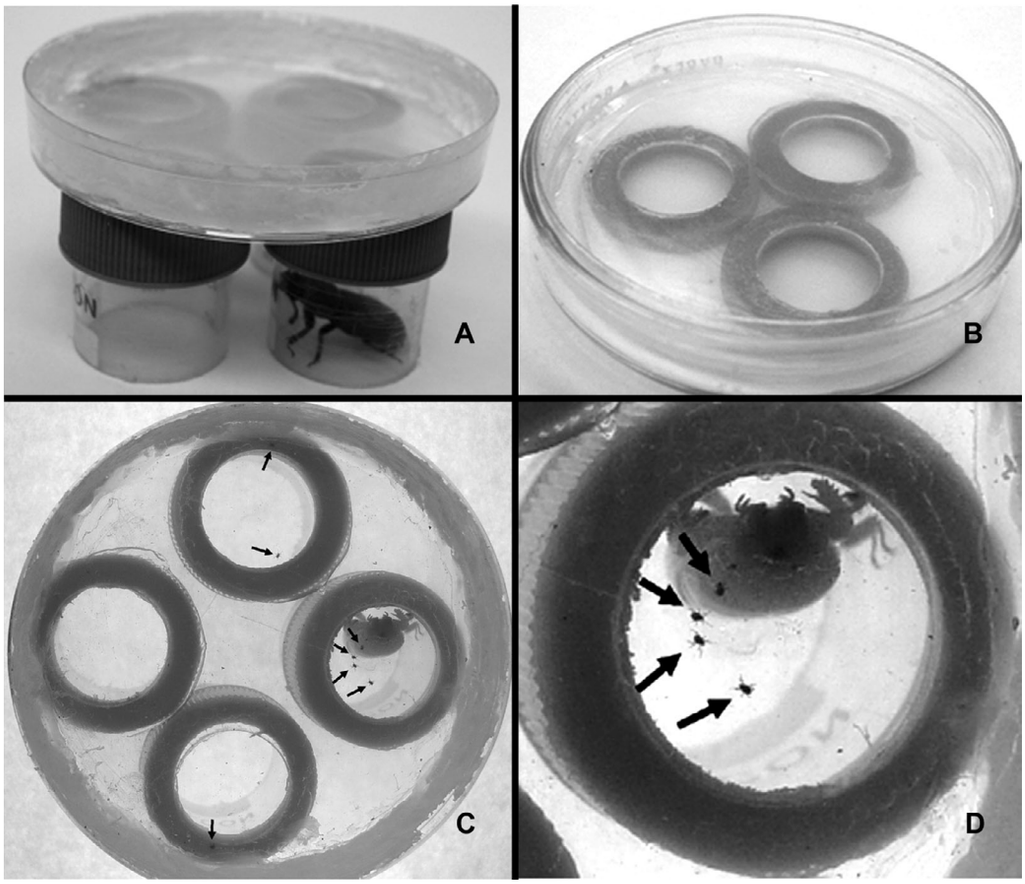

Figure 1. Apparatuses used to test mites for attraction to olfactory cues. 1A. Free-choice disk olfactometer used to test response to beetles and beetle odors. 1B. Free-choice disk olfactometer used to test response to cuticular extracts. 1C. Top view of olfactometer containing seven large mites during a run testing a vial containing a passalid beetle against empty (control) vials; arrows point to mites. 1D. Enlargement of vial in 1C illustrating mites above beetle.

4 mm tall so that the arena could lie very close to the Watman no. 1 filter paper on which the washes of extract were spotted. To test the attraction of mites to the cuticular extract, the water wash was spotted below one hole, chloroform (control) below another hole, and water (control) below the third hole.

Due to their size, small mites moved more slowly relative to large mites and therefore tended to cover less territory and remained nearer a vial containing a beetle or other attractant once discovered. They seldom left the arena floor or escaped. Large mites, on the other hand, proved to be more difficult research subjects. They moved more rapidly and therefore tended to venture farther from vials containing an attractant. They also more readily climbed the arena wall and escaped. Experiments and statistical testing therefore centered primarily on small mites, with fewer data collected for large mites. The data from large mites was used primarily for corroboration of the results obtained from small mites.

During a run, mites (usually between five and eight) of a single size category were brushed from a beetle into the middle of the arena. The arena was then videotaped for 30 minutes using a Sony VID-P100 video presentation stand and a Sony SLV-478 VHS VCR. Videotapes were recorded in SP mode for maximal resolution, and could be viewed multiple times to ensure accuracy of data. Viewing the videotape, a small mite was considered to have a positive response to a vial if it was directly over the opening, and a large mite was considered to have a positive response if it was within 5 mm of the opening. The maximum number

of mites simultaneously responding to each vial was determined for each one-minute interval, and these data in turn divided by the total number of mites to yield percentages. For example, in figure 1C there are seven mites; four (57.1%) above the vial containing the beetle, two (28.6%) above the upper vial, one (14.3%) above the lower vial, and none (0%) above the vial on the left. Calculating percentages was necessary since the number of mites in a given bioassay varied between runs. The percentages calculated for each vial were averaged, thereby yielding one independent variate per vial for each run. After a run, mites were allowed to reboard their beetle host and the beetle returned to its culture container. The entire apparatus was then washed with ethanol to eliminate any odors or other residues. Four or five runs, separated by time intervals and usually using different mites, were made for each of the following:

(1) Four empty vials to test the functionality of the olfactometer and to ascertain that mites did not show a bias toward any of the four vials.
(2) Three vials empty and a live passalid beetle in the fourth vial to test whether mites were attracted to passalid beetles with which they had no physical contact. The attractant could be chemical, vibratory, or both.
(3) Three vials empty and passalid beetle odor in the fourth vial. For this test a live passalid beetle was present in the vial for the five minutes immediately prior to a run. The only trace of the passalid beetle should be its chemical residues; this bioassay therefore tested for chemical cues and eliminated vibratory cues.
(4) One vial containing chloroform (control), one vial containing water (control), and one vial containing the water wash of the passalid cuticular extract.
(5) Three vials empty and the fourth vial containing a large unidentified carabid beetle to test for specificity of response to passalid beetles.

The variate used for statistical comparison was the average maximum number of mites per minute expressed as a percentage of the total number of mites used in the run. Bioassays without beetles (number 1 above), with beetles (number 2 above) and with extracts (number 4 above) were analyzed utilizing One-Way ANOVAs and Tukey post-hoc tests. Since there were only four replications, bioassays with a recently removed beetle (number 3 above) were analyzed with an independent-samples t-test. For these, values for the three unoccupied vials (control tubes) were averaged to create a single control value for each run. Since only two runs were conducted using carabid beetles (number 5 above) for both small mites and large mites, sample sizes were too small for analyses utilizing independent-samples t-tests. Data for the two runs were therefore combined and analyzed using paired-samples t-tests paired by minutes.

Chemical Analyses

Cuticular extracts were analyzed via non-preparative thin layer chromatography (TLC) plates (5x20 cm, 250 micron silica gel HLF), with solvents ranging from very nonpolar to very polar: hexanes and EtOAc (3:1, 1:1, 1:3); n-butanol, ethyl acetate, water, and aqueous ammonia (5:3:2:1); n-butanol, ethanol, and water (5:4:2, 5:4:3); 100% EtOAc; EtOAc, ethanol, and water (6:2:1, 12:2:1); and ethanol and EtOAc (1:4, 1:1). Plates were examined under UV light to detect molecules with fluorophores. Plates were then dipped in vanillin and heated to detect non-fluorescent molecules.

Results

Results from bioassays using small mites are given in Table 1. Mites demonstrated no affinity for any given vial when all were empty (blank), thereby indicating that the distribution of mites in a control test arena was more or less random. When a live passalid beetle was placed in one of the four vials, mites demonstrated a significant preference for the vial containing the beetle, but no difference between blank vials. Mites also demonstrated a significant preference for vials that contained a passalid beetle for the five-minute period immediately prior to testing. When mites were given a choice of the water wash containing passalid cuticular extracts, water (control) and chloroform (control), they demonstrated a significant attraction to the extract; there was no difference between the two controls. Bioassays similar to the above were conducted for large mites, but each had a limited number of

Table 1. Means, standard errors and sample sizes for the various experiments. Sample sizes for tests against carabid beetles are not given since a paired-samples t-test was used. For any given experiment, means with different superscript letters are significantly different at $\alpha = 0.05$.

	Mean	*Standard Error*	*Sample Size*
Control Run - All Vials Blank			
Vial 1	8.12[a]	0.769	5
Vial 2	9.67[a]	1.123	5
Vial 3	9.60[a]	1.732	5
Vial 4	12.11[a]	3.380	5
	$F_s = 0.674$	$p = 0.581$	
Three Blank Vials (Controls) vs Live Passalid Beetle in Vial			
Control	4.45[a]	1.914	5
Control	5.44[a]	1.803	5
Control	5.78[a]	1.698	5
Beetle Vial	54.84[b]	6.897	5
	$F_s = 42.9$	$p < 0.0005$	
Blank Vials (Control) vs Vial with Passalid Beetle Recently Removed			
Control (blank)	18.64[a]	6.589	4
Beetle Removed	47.82[b]	8.682	4
	$t_s = 2.68$	$p = 0.037$	
Control Vials vs Water-wash of Beetle Extract			
Chloroform	5.28[a]	2.587	4
Water	5.99[a]	1.050	4
Extract	39.59[b]	9.148	4
	$F_s = 12.61$	$p = 0.002$	
Blank Vials (Control) vs Carabid Beetle in Vial; Small Mites			
Control (Blank)	6.17	0.202	
Beetle	70.12	2.387	
	$t_s = 28.171$	$p < 0.0005$	
Blank Vials (Control) vs Carabid Beetle in Vial; Large Mites			
Control (blank)	33.09	1.096	
Beetle	37.41	2.363	
	$t_s = 1.875$	$p = 0.072$	

runs and were therefore not tested statistically. Visual analyses of the data, however, corroborated the results from small mites.

Small mites were strongly attracted to a vial containing a large, unidentified carabid beetle, whereas large mites were attracted, but only weakly.

When the cuticular extract was analyzed via TLC, a fluorescent spot appeared using the following solvents: 100% EtOAc, 1:4 EtOH: EtOAc, and 1:1 EtOH: EtOAc. The spot barely overlapped its starting position using 100% EtOAc, and overlapped the solvent front using 1:1 EtOH: EtOAc. Using a 1:4 ratio of EtOH to EtOAc resulted in an intermediately traveled spot. These results indicate that the polarity of the cuticular attractant approximates the polarity of this mixture and that it is a very polar substance.

Discussion

To date, the only studies investigating the role of olfaction in the location of passalid beetle hosts by mesostigmatic mite associates are those of Mollin (1962) and Hunter and Davis (1965). Mollin (1962), using a Y-maze apparatus, was unsuccessful in his attempts to determine if *C. trifidus* used chemical cues to locate hosts. He did, however, demonstrate that, when given a choice, *C. trifidus* preferred to board unwashed beetles over beetles washed with distilled water or washed with water containing a detergent. Attempts to attract mites to either of the wash residues were unsuccessful. Hunter and Davis (1965) gave *E. latus* mites a choice of live beetles, fresh beetle fecal pellets, freshly chewed wood chips, and non-chewed wood. Mites overwhelmingly preferred live beetles and freshly chewed wood chips, responding to the two in approximately equal proportions. They also found that mites were not attracted to an artificial diet medium unless it was mixed with an acetone wash from the beetles, or unless beetles had been recently confined to the diet medium. Since mites were attracted to the acetone rinse and since the rinse was only attractive for a short time, they concluded that the attractant could be washed off the beetle and that it was probably a volatile substance.

Results from our bioassays conclusively demonstrate that the four species of mites under study are attracted to their passalid beetle hosts by chemical cues emanating from the host cuticle. This kariomone is quite volatile as revealed by the fact that, although cuticular extracts are extremely attractive for the first several minutes, they rapidly loose their magnetism (Figure 2). Live beetles, on the other hand, continue to attract mites and appear

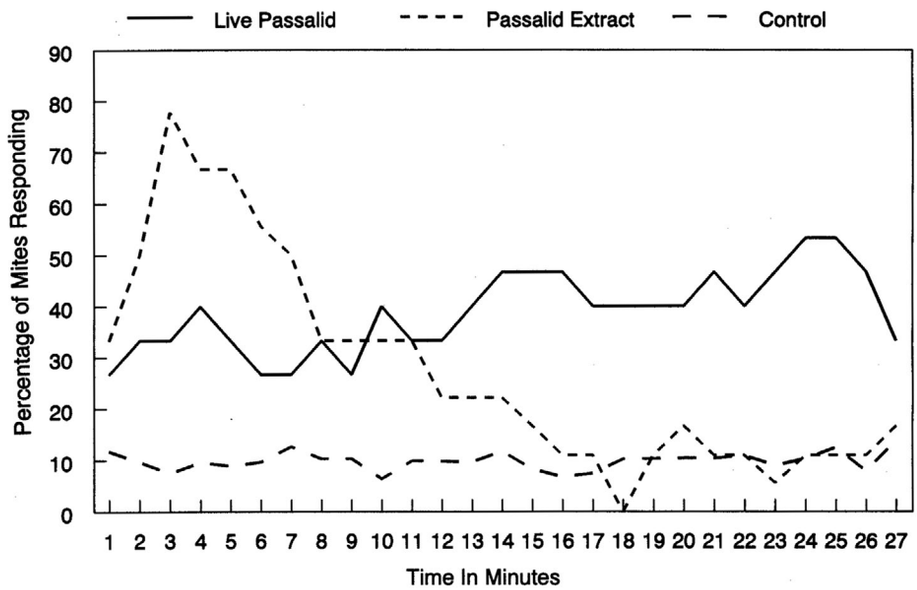

Figure 2. Mite responses to blank (empty) vials from bioassay 1, vials containing a live passalid beetles from bioassay 2, and vials containing passalid cuticular extract from bioassay 4. Data are plotted at one-minute intervals from start of experiment.

to even slightly increase in attractiveness as time progresses. Although our experiments do not rule out mite use of additional beetle cues such as vibration through the substrate or air (sound), we saw no evidence for the use of such cues during our investigations.

Our results implicating host kariomones as a primary cue are not surprising since other mite species have been shown to detect their hosts utilizing host odors; a few examples will suffice. Using disc olfactometer choice tests, Krantz and Mellott (1972) found that *Macrocheles mycotrupetes* and *M. peltorupetes* exhibit marked specificity to their natural scarab beetle hosts, and Krantz and Royce (1994) further established that *M. mycotrupetes* is able to chemically detect its host through at least eight cm of sand and burrow to it within a day. Dispersal stages of *Parasitellus fucorum*, an inhabitant of bumblebee nests that over-winters on queens, use chemical cues to actively discriminates between host species (Schwarz *et al.* 1996), as well as to distinguish between queen, drone, and worker castes (Huck *et al.* 1998).

Cuticular chemical cues are not always species specific, and a mite species therefore can be attracted to a non-host extract. Krantz *et al.* (1991) found that non-host extracts often provoked a positive response from species-specific phoretic mites in their free choice experiments. This happened even in the presence of extracts from preferred hosts, indicating that the kariomones produced by the host and the non-hosts are similar in chemical composition. According to Krantz (1991), the phoretic specificity of mites is based on two factors: "ecological isolation of the mite through adaptation to a particular beetle hypervolume [or environment], and perpetuation of the phoretic relationship through recognition of a chemical cue." In other words, the host and non-host species may have similar cuticular chemicals but do not share the same habitat. We too found attraction to a non-host species in our experiments. Although our unpublished observations indicated passalid mites were not attracted to species of june (scarab) beetles, they were attracted to a large, unidentified carabid beetle in our olfactometer tests. This indicates that the carabid beetle shares one or more cuticular chemical with passalid beetles. Carabid beetles, however, are adapted for running in open areas and would seldom, if ever, frequent the galleries constructed by passalid beetles inside decomposing logs

Arthropod cuticle is composed mainly of chitin and proteins, covered with a thin layer of wax. Extracts of the cuticle contain compounds from the waxy layer only, not the "insoluble macromolecular structure" of chitins and proteins (Stankiewicz *et al.* 1997). According to Stankiewicz *et al.* (1997), cuticular waxes "are easily extracted with organic solvents." Thus the attractants are usually waxes or lipids and therefore nonpolar molecules. Krantz *et al.* (1991) found the compounds involved in the attractants they studied to be lipids (nonpolar molecules) that were captured in a chloroform wash of water.

It was expected that the attractant in our extract would be polar rather than nonpolar, however, since it was captured in the water wash of chloroform. This expectation proved to be correct. TLC produced the best-resolved, intermediately traveled, spot when run in an 80% ethyl acetate, 20% ethanol solution that contained a few drops of acetic acid. The acetic acid was necessary to reduce streaking and thereby obtain a more resolved spot. The spot obtained was fluorescent and constituted the major component of the extract. Thus, the attractant is a polar compound with a fluorophore. The highly polar nature of the passalid-mite attractant is somewhat puzzling in that this polar attractant appears to be embedded in the nonpolar cuticular wax.

Preparative TLC was not attempted in our study, so no bioassays were made of the separated attractant. However, there were no other spots present in any of the TLC's across the range of polarities, so the spot obtained very likely represents the attractant. No attempt was made to determine the specific identity of the attractant.

The results reported herein conclusively demonstrate that kariomones emanating from host beetle cuticle serve as a primary cue for mite host location in the system we investigated. It is probable that they also function in host location by mites associated with other passalid species. Further research is necessary to determine the identity of the cuticular attractant in our study. In addition, further research is necessary to conclusively rule out vibratory cues.

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