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SENSORY BEHAVIOR AND ELECTRON MICROSCOPY OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE).

The University of Oklahoma, Ph.D., 1977
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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SENSORY BEHAVIOR AND ELECTRON MICROSCOPY OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

WILBURN LYNN LAWS

Norman, Oklahoma

1977

SENSORY BEHAVIOR AND ELECTRON MICROSCOPY OF THE

COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI

(ACARINA: RAPHIGNATHIDAE)

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TABLE OF CONTENTS

Page

Acknowledgements iii

List of tables and appendices vii

List of illustrations viii

General introduction 1

Paper I. Responses of the Cockroach Parasite Pimeliaphilus cunliffei (Acarina: Raphignathidae) to Odors of the American Cockroach

Abstract 6

Introduction 7

Materials and Methods 8

Results 12

Discussion 15

References Cited 19

Paper II. Temperature Preference of the Cockroach Parasite Pimeliaphilus cunliffei (Acarina: Raphignathidae) After Acclimation to three Different Temperatures

Abstract 21

Introduction 22

Materials and Methods 23

Page

Results 24

Discussion 29

References Cited 33

Paper III. Response of the Cockroach Parasite Pimeliaphilus cunliffei (Acarina: Raphignathidae) to Overhead Light

Abstract 35

Introduction 36

Materials and Methods 37

Results 39

Discussion 44

References Cited 48

Paper IV. Description of Copulating and Feeding Behavior of Pimeliaphilus cunliffei (Acarina: Raphignathidae)

Abstract 50

Introduction 51

Copulating Behavior 51

Feeding Behavior 53

References Cited 55

Paper V. Electron Micrographs of Pimeliaphilus cunliffei (Acarina: Raphignathidae)

Abstract 56

Introduction 57

Materials and Methods 58

Results and Discussion 58

References Cited 69

Page

Summary and General Conclusions 71

Appendices 73

LIST OF TABLES AND APPENDICES

TABLE		PAGE
Paper I		
1	Numbers and percentages of acclimated individuals responding to temperature within a temperature gradient	25
Paper II		
1	Number of responses to light and dark in a two choice chamber	40
2	Number of individuals moving and resting in a lighted test chamber	42
3	Number of responses to top, side, and bottom of test chamber	43
APPENDIX		
1	Data for the two-Choice Contact Test	73
2	Data from the Y-Tube Test with vacuum	74
3	Data from Y-Tube Test without vacuum	75
4	Data from Y-Tube Test with plunger	76
5	Data from Two-Choice Distance test (morning)	77
6	Data from Two-Choice Distance test (evening)	78

LIST OF ILLUSTRATIONS

FIGURE		PAGE
Paper II		
1	Percentages of individuals of three populations responding to temperature within a temperature gradient	26
Paper IV		
1	Electron micrograph, dorsal view of the male mite . .	62
2	Electron micrograph of right palp of female	62
3A	Electron micrograph of right leg of female mite . . .	64
3B	Electron micrograph of coeloconicae	64
3C	Electron micrograph of sensory pit	64
4A	Electron micrograph of female's genital opening . . .	66
4B	Electron micrograph aedeagus	66
5A	Electron micrograph of the right eye of female mite .	68
5B	Electron micrograph of the right eye of male mite . .	68

SENSORY BEHAVIOR AND ELECTRON MICROSCOPY OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

GENERAL INTRODUCTION

Pimeliaphilus cunliffei (Jack) 1961 has had a controversial taxonomic history. Trägårdh (1905) found a mite on a beetle in Egypt and named it P. podapolipophagus placing it in the family Raphignathidae, all members of which are arthropod parasites. Hirst (1917) placed this genus in the family Pterygosomidae, because of the similarity of Pimeliaphilus to Hirstiella, which is a member of that family. Vitzthum (1943) however, returned the genus to the family Raphignathidae, again because of its parasitic relationship with arthropods. Cunliffe (1952) briefly discussed the biology of P. podapolipophagus which he described as a cockroach parasite and placed it in the family Pterygosomidae as did Baker and Wharton (1952). Finally, Jack (1961) reviewed the genus and on the basis of behavioral and morphological characteristics, decided that Pimeliaphilus should be in Raphignathidae. Jack, with the agreement of Trägårdh, changed the scientific name of the cockroach mite from Pimeliaphilus podapolipophagus to Pimeliaphilus cunliffei, based on the morophological dissimilarities between these two mites.

Although this study is not taxonomic, this review indicates a need for further knowledge of the species placed in the genus Pimeliaphilus. There are approximately thirteen species in this genus (Jack 1961, Newell and Ryckman 1966). Most of the studies on these genera have been descriptive (Hirst 1917, Jack 1961, Newell and Ryckman 1966, Vitzthum 1943) with very little attention being given to their biology and host relationships. Pimeliaphilus typically parasitizes insects, primarily Triatominae (Beer 1960, Jack 1961, Newell and Ryckman 1966); however, one species, P. tenuipes has been reported on a gecko, Gonatodes albogularis. Two species, P. rapax (Beer 1960) and P. isometri (Cunliffe 1949), have been found on scorpions. The mite used in this study, Pimeliaphilus cunliffei, is known to parasitize three species of cockroaches; Blattella germanica, Blatta orientalis, and Periplaneta americana, (Field et al. 1966). Field et al. (1966) demonstrated that in sufficient numbers this mite could decimate a roach culture in a relatively short time. There are several species of mites which parasitize cockroaches (Roth and Willis 1960), but no others seem to have the impact of P. cunliffei. For example, Schaefer and Peckham (1968) found the cockroach Gromphadorhina portentosa to be infected with a laelaptid mite, Androlaelaps sp., however, Gromphadorhina did not appear to be harmed by the relationship.

To understand an organism one must know how that organism relates to its environment, how it selects a proper habitat, and how it obtains food. In a parasitic relationship all ramifications of habitat selection and food acquisition are interrelated with the general activities of the

host. In regard to this relationship, Camin (1963) described three types of ectoparasites: Group I, a "permanent" type ectoparasite, spends its entire life cycle on its host; Group II, the nidicole or nest-parasite, is on the host only while taking a meal; and Group III, is similar to Group II, but feeding less frequently and wandering off of the host. Camin (1953) further characterized nidicolous mites as having well developed locomotor and sensory organs, but responding to stimuli in a rather stereotyped manner. Camin also stated that even though there is much known about this type of parasite, relatively little work has been done on their sensory behavior. With this in mind, and in view of the possibility that P. cunliffei, a nidicolous mite, might be used as a biological control agent for cockroaches (Field et al. 1966), I have investigated the biology, behavior, and host relationships of the cockroach mite.

This thesis will be divided into five parts. First, tests are described which determine whether the mites can detect cockroach odor by distance and/or contact chemoreceptors. Second, tests are described that determine whether or not P. cunliffei can be acclimated and how acclimation affects their temperature preference in a temperature gradient. The third describes responses of fed and unfed mites to an overhead light in a two-choice chamber. The fourth section gives data on feeding and copulatory behavior. The fifth section consists of electronmicrographs of anatomical features which will be used to support the other four parts. The first three sections are prepared in the style of the Journal of Environmental Entomology. The fate of the fourth and fifth sections is still undecided.

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RESPONSES OF THE COCKROACH PARASITE
PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)
TO ODORS OF THE AMERICAN COCKROACH

Abstract

Four tests were used to determine the ability of P. cunliffei (Jack) to respond to cockroach odors. The spot test allowed the mites to run free, demonstrating their searching behavior. The two-choice contact test forced mites to make a choice between odor and non-odor sides of a two choice chamber. They significantly chose the odor side. The Y-tube test kept the animals away from the stimulus while giving them a choice of odor or non-odor. The mites' positive response in the tube was significant in the presence of an air current, but not so in the absence of a current. A plunger used in the Y-tube resulted in a significantly positive response from the mites. The two-choice distance test, which allowed the mites to come close to the odor without touching it, showed significantly positive responses from the mites. These tests indicated that contact and distance chemoreceptors are present, however, contact chemoreceptors appear to be the primary receptors utilized.

RESPONSES OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE) TO ODORS OF THE AMERICAN COCKROACH

W. Lynn Laws

The literature is replete with work on chemoreception among insects, but scanty on such works amoung the Acarina (Frings and Frings 1949, Hodgson 1958, Slifer 1970). Most of the work on the Acarina has been confined to the parasitic members, such as the mite Androlaelaps sp., which Schaefer and Peckham (1968) found to be positively attracted by the odor of its host, the Madagascar Hissing Cockroach Gromphadorhina portentosa, and the tick Ixodes ricinus, which Lees (1948) found to be responsive to sheep via contact receptors. Camin (1953) indicated that the snake mite, Ophionyssus natricis was attracted to the odor of fresh snake blood and to live snakes. The odor of the house fly, Musca domestica has been found to attract the mite Macrocheles muscaedomesticae (Jali and Rodrigues 1970). Coons and Axtell (1973) located possible chemoreceptors on Tarsus I of this fly parasite. Plant material has also been found to be an attractant, as reported in Cone and Prusynski's (1972) work with the Two-spotted Spider Mite, Tetranychus urticae.

Pimeliaphilus cunliffei, which parasitizes the American Cockroach,

Periplaneta americana is a nidicolous parasite and by Camin's (1963) description of this type of parasite, would be confronted by and respond to odors produced by its host. Odor is used by a number of species of cockroaches both as a defense mechanism and as an attractant (Wharton et al. 1954, Guthrie and Tindall 1968). Berthold and Wilson (1967) found that the odors left in the harborages by cockroaches had a strong influence on their selection of resting sites. Cornwell (1968) stated that approximately 83 percent of cockroaches returned to previously occupied quarters, primarily in response to odor. Since P. cunliffei is a nest parasite, any odor left in a harborage could influence its behavior. These mites spend a great deal of time off their host and any stimulus which could facilitate their locating a suitable food source would be advantageous. With this adaptive feature in mind I designed experiments to determine if P. cunliffei could detect the odor of its host and if this detection was via contact or distance chemoreceptors.

Materials and Methods

The mites were cultured in one-gallon glass jars with the American Cockroach, Periplaneta americana as the food source. The cockroaches were fed dry Purina Dog Chow and were given water in cotton-stoppered vials. As the cockroaches died, live roaches were added to the culture jars. Mite free cockroach colonies which provided the odor for the tests were maintained separately. Test mites were deprived of food for 1-7 days and will henceforth be called hungry. Mites were tested in groups of 10 or 20 at the same environmental conditions as they were cultured. The four tests described are: Spot Test, Two-Choice Test, Y-Tube Test, and the Two-Choice Distance Test.

Spot Test

The objective of this test was to establish some behavioral pattern which could be used to determine if the mites detected cockroach odor. These observations provided qualitative rather than quantitative data and were not treated statistically.

A roach extract was prepared to provide the possible stimulus. Cockroaches were killed and ground up in ether, producing a very concentrated solution. The test arena was a standard glass Petri dish with filter paper covering the bottom. Several drops of the roach extract were placed in the center of the arena and allowed to dry. For controls drops of ether were placed on the filter paper and allowed to evaporate. This same area then formed the test spot. All other variables were the same. Ten hungry mites were placed in the arena and allowed to wander for 20 minutes. During this time their behavior was observed, particularly how they responded to the test spots and to the new environment.

Two-Choice Contact Test

The spot test permitted the mites to wander about without committing themselves, as was the intent of the study. However, to eliminate ambiguity which might result from misinterpretation of such behavior, a two-choice test was designed. This test forced the mites to make a choice while permitting them to come in contact with the odor on a rough substrate similar to their culture environment.

The test arena was a finger bowl divided in half. A cotton ball which had remained in a nonparasitized roach culture for 24 hours, was placed on one side of the bowl. A clean cotton ball was placed on the

opposite side. The controls were presented with two clean cotton balls. Twenty hungry mites were placed in the center of the arena and observed for 20 minutes. At the end of this time a count was made as to the number of individuals on each side. After each test the bowls were cleaned with alcohol and new cotton introduced. Chi square tests were used to test for significance of differences.

Y-Tube Test

This test was designed to allow the mite to make a choice, without coming in contact with the odor source, as they could in the previous studies. Thus it tested for distance rather than contact chemoreception.

Glass Y-tubes (4 mm ID) were used to provide a two-choice situation. One arm (4 cm long) of the Y-tube led to an isolated cockroach while the other (4 cm long) led to an empty vial. The neck of the Y-tube (15 mm long) was used as the starting chamber. One end of the starting chamber was covered with a piece of cloth which permitted air to move through but not the mites. Initially a reduced pressure line was connected to the starting chamber to insure that odor was pulled across the mites, which were 55 mm away from the odor source. Later, odors were allowed to diffuse without forced draft.

Ten hungry mites were placed in the starting chamber and allowed to move about for 20 minutes. Every 5 minutes a count was made of the number of mites still in the starting chamber (0), on the stimulus side (+), or on the blank side (-). After 12 replications, the air current was discontinued, because it may have interfered with the mite's behavior. The odor was then allowed to diffuse down the tube and the mites were tested in the same manner as previously described. The mites

still did not seem to be leaving the starting chamber sufficiently, so another series of tests was run in which a glass rod was inserted into the chamber, forcing the mites to move into the arms. After each test the tubing was cleaned with alcohol and allowed to dry. A Chi square test was used to determine significance of the results.

Two-Choice Distance Test

Even though the above test kept the mites from coming in contact with the odor, it gave them the chance to remain in the starting area. This experiment provided no such opportunity, because there was no third choice. They were either on one side or the other.

Ten plastic boxes (32 mm long X 32 mm wide X 15 mm high, including lid) were used as the test chambers. The boxes were divided in half diagonally and provided with a cloth floor. This gave the mites a rough substrate, similar to the cloth in the Y-tube test, and it also permitted them to walk freely above the odor without coming in contact with it.

A small 5 mm sponge cube was soaked in cockroach extract, allowed to dry, and placed in one corner of the test box. A clean cube which had been soaked in ether and dried, was placed in the opposite corner. Ten hungry mites were placed in the center of each test box and allowed to wander undisturbed for approximately 90 minutes. A count was made every 10 minutes of the number of mites on each side. The results were scored as (+) if the mites were on the side which had odor and (-) if they were on the side without odor. Two time periods were run, morning and evening, which corresponded with the inactive periods of their host. The time covered a range from about 0800 hours to 1100 hours and from approximately 1500 hours to 1800 hours.

Results

The Spot Test was designed to be used for general observation and, as previously stated, to determine if there was some behavioral pattern indicative of odor detection. This test did not reveal any specific indicator, but it did show their searching behavior. After being placed into a new environment the mites move about waving and touching the substrate with their front legs. I had envisioned the mites immediately orienting toward the odor and gathering around the spot, but this was not the case. After being placed in the Petri dish they scattered in all directions, stopping periodically to wave their front legs in the air. As the mites moved about, some stopped on the odor spot and felt around with their front legs and mouthparts, while others walked over the spot, not appearing to respond to the odor. This same behavior was also noted among the control group.

One peculiar type of searching behavior was discovered, which I termed: "wallowing behavior." When this was first observed, I thought that finally some indicator of odor recognition had been found. However, I also observed it in the control arenas and in the holding chamber where mites were placed in preparation for testing. The behavior is similar to that of a dog wallowing in something which it had just found, except the mite does not roll over on its back. The mites placed as much of their mouthparts and front legs on the substrate as possible and swept them back and forth, allowing the front legs to trail like limp rags. This behavior was neither observed in all individuals nor was it observed regularly in any one individual.

Two-Choice Contact Test (Data Appendix 1)

This study provided the mites with a rough substrate, similar to their culture jars, which the Spot test did not. There appeared to be an interaction between odor and texture, because mites crawled around on the cotton and if there was no odor, they moved on. The side with the odor provided the mites with two stimuli, odor and texture, which appeared to influence the mites to remain.

There were 840 observations with 538 responses to the (+) side and 302 to the (-) side ($X^2 = 66.3$, $P < 0.05$). There were 840 observations of the control group with 403 responses to side A and 437 responses to side B ($X^2 = 1.37$, $P > 0.05$). Therefore, randomness was rejected for the experimental group and accepted for the control group. The mites obviously could distinguish between the odor and non-odor sides. These first tests thus supported the hypothesis that P. cunliffei was able to detect some chemical stimulus produced by the host. However, the test for heterogeneity (controls-$X^2 = 162.41$, 41 df, $P < 0.05$; experimental-$X^2 = 111.90$, 41 df, $P < 0.05$) suggest an extraneous variable. The mites were coming in contact with the stimulus, so the stimulus could have been biasing the results.

Y-Tube Test

This test did not allow any contact with the stimulus, permitting the mites to respond only to the airborne odor. During the portion of the test in which a vacuum was used, there were 480 counts. Approximately one half, 242 responses, were to neither the (+) or the (-) side, but rather to the starting chamber (0). The mites which stayed in the chamber usually remained on the cloth wall which confined them.

Of the 238 which responded, 138 entered the (+) side and 100 entered the (-) side ($X^2 = 6.06$, $P < 0.05$). Of those that did move into the arms, significantly more moved into the arm with the odor of the host.

Since a test for heterogenity resulted in $X^2 = 75.55$, 47 df, $P < 0.05$, the vacuum was turned off and the apparatus left undisturbed, allowing the mites to respond to diffused odor. There were 420 counts; 230 were to the starting chamber (0), 101 to (+), and 89 to (-) side. As before, approximately one half of the individuals were not in either arm, but instead stayed in the starting chamber. Of those entering an arm, there was no significant difference ($X^2 = 0.75$, $P > 0.05$) between the (+) side and the (-) side, indicating that they were not responding to diffused odor. This time they were not significantly heterogeneous ($X^2 = 29.47$, 41 df, $P > 0.05$).

Two-Choice Distance Test (Data Appendix 5,6)

This test eliminated null responses. There were 1000 observations in each of the morning and evening test periods. In morning tests, there were 573 positive responses and 427 negative responses ($X^2 = 21.32$, $P < 0.05$), at the evening test there were 561 positive responses and 439 negative responses ($X^2 = 14.88$, $P < 0.05$) and neither group was heterogeneous (morning-$X^2 = 123.09$, 99 df, $P > 0.05$; evening-$X^2 = 103.92$, 99 df, $P > 0.05$). A comparison of the two periods showed $X^2 = 0.25$, $P > 0.05$, indicating that there were no differences between the two groups. When the test is considered in its entirety, there were a total of 1134 positive responses and 866 negative responses ($X^2 = 35.91$, $P < 0.05$) again indicating that Pimeliaphilus cunliffei can detect odor of its cockroach host and respond positively to it.

Discussion

One might assume that, because these mites are nidicolous parasites, they would exhibit well defined behavioral patterns and would respond with vigor to the stimuli. After terminating the first phase of the study (Spot Test). I realized that my subjects were going to be somewhat inconsistent.

During the Spot Test, these mites were continuously searching and testing the environment with their mouthparts and front legs, indicating that there were receptor sites on these structures. This is consistent with Farish and Axtell's work with Macrocheles muscaedomesticae (1966). They found olfactory receptors on the mite's tarsus I, and contact chemoreceptors on the mite's palps. The "wallowing behavior" demonstrated by the cockroach mite suggested also that the receptor sites are on the legs. If the location was at the tip of the tarsus there would be no need to get as much of the leg on the substrate as they did. Jack (1961) and Newell and Ryckman (1966) reported a solenidon on the dorsal side of tarsus I ostensibly used as an olfactory receptor. There are differences of opinion between these two authors as to the shape of this seta, thus there could be differences of opinion as to its function. If,however, the solenidon is truly an olfactory receptor, this would explain the "wallowing behavior." The mite would be attempting to get the receptor in contact with the stimulus.

This phase of the study did not provide a behavioral pattern indicative of odor detection, but rather it demonstrated how these mites search. They wave their front legs in the air suggesting the use of distance chemoreceptors and they touch and drag their legs on the

substrate suggesting the use of contact chemoreceptors. Leg waving may have another function. It may be a means for making sure the contact chemoreceptors come in contact with something, rather than utilizing distance chemoreceptors. This behavior is analogous to someone's groping around in the dark or a blind person tapping the ground with his cane.

The intent of the Distance Contact Test was to determine if the mites were groping or if they were responding to a distant stimulus. However, as indicated in the results, mites wander about before coming in contact with the scented and unscented cotton balls. Upon having reached an unscented cotton ball, the mites left and moved about. They remained after reaching the scented side. Thus mites were usually found on the stimulus side at the time of inventory. Since the mites moved from one area to the next, distance chemoreception is indicated. After the mites got into the cotton, contact chemoreceptors were probably used. The mites could have been restless however, and since there was no odor to come in contact with on the clean side, they moved on. This experiment therefore substantiated the presence of contact chemoreceptors and hinted at distance chemoreceptors.

The Y-Tube Test was designed to eliminate any effect contact might have had in the above test. Since the mites were unable to come in contact with the odor source, any response would have to be a result of stimulation of distance chemoreceptors. As seen in the results the mites were positively attracted to the odor when a vacuum was used. However, removal of the vacuum resulted in the mites not responding

significantly to the stimulus. The test for heterogeneity suggested that the vacuum may have influenced the results. The individuals tested with an air current were heterogeneous while those tested in the diffused air were not. Distance from the odor source may have contributed to their negative response. The odor may not have diffused down the tube or it may have been so diluted that the mites were unable to detect it. This response indicates that the receptors may be relatively insensitive and thusly not too useful at any real distance. When mites are wandering around in the presence of roaches, they wave their front legs as usual, but orient toward the roach only after coming within a few millimeters of the insect. This again suggest the insensitivity of their distance chemoreceptors. These mites also seem to be attracted by material which allows them to hold on. The cloth confining them in the starting chamber provided such a stimulus and may have biased the results. However, if only those individuals which chose either the positive or negative side are considered, then distance chemoreceptors are indicated as was substantiated in the test with a glass plunger.

The Two-Choice Distance Test brought the mites closer to the odor source and provided them with a rough substrate which they seemed to prefer. The mites therefore, had only to make a choice as to whether to respond positively or neutrally to the odor of their host. This arrangement made the data easy to quantify, and as the results show the mites responded positively indicating the presence of distance chemoreceptors.

The intent of this study was not to demonstrate the site of

chemoreception, but rather to determine if chemoreceptors active in host finding were present. The data substantiate the hypothesis that distance and contact chemoreceptors are present, and I believe also that the mite's behavior indicates a possible location for the contact chemoreceptors: the solenidon on tarsus I described by Newell and Ryckman (1966).

To summarize, P. cunliffei must be near the source of the cockroach odor for detection. In such circumstances, the mite responds positively to the odor of its host. This behavior is appropriate, especially since Berthold and Wilson (1967) found that cockroaches preferred narrow crevices. Consequently, the mites would always be in close proximity to their host and not have to seek great distances for a suitable food source. Since the mites would always be inundated by odor, they would have no need for highly sensitive distance chemoreceptors. Contact chemoreceptors would be more advantageous.

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I wish to express my utmost gratitude to the members of my committee: Drs. Hubert W. Frings, Harley P. Brown, Charles C. Carpenter, Cluff E. Hopla and Frank J. Sonleitner, for critically reviewing the manuscript.

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TEMPERATURE PREFERENCE OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE) AFTER ACCLIMATION TO THREE DIFFERENT TEMPERATURES

Abstract

Three populations of P. cunliffei, populations "A", "B", and "C", were acclimated at three different temperatures, 33°, 22°, and 15°C respectively. Their temperature preference was determined in a temperature gradient ranging from 13°C to 35°C. The mean preferred temperature of population "A" was 26.05°C, "B" was 24.56°C, and "C" was 24.52°C. Population "C" was the most variable while population "A" the least variable. There were no significant differences between the variability of "B" and "A" and there were no significant differences between the mean preferred temperatures of "B" and "C".

TEMPERATURE PREFERENCE OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE) AFTER ACCLIMATION TO THREE DIFFERENT TEMPERATURES.

W. Lynn Laws

Temperature is an environmental stiumlus that continuously confronts an organism. It can affect behavior, such as egg laying, development, feeding activity, and in the case of parasites, response to the host (Lees 1948, Camin 1953, Mori 1961, Perttunen 1958, Wharton and Kanungo 1962, Cross 1964, Singh et al. 1967, McClanahan 1968, Burnett 1970). Temperature also can have ecological importance, as described by Wallwork (1966) who found that the temperature preference of free living mites is related to their habitat: a West African species of oribatid mite preferred higher temperatures than a North American species.

The preferred temperature of P. cunliffei has not been studied. The only study of temperature relationship for this species was that by Cunliffe (1952), who determined the temperature dependence of egg-hatching time. He found that eggs require 6-11 days at 26.64°C.

In an attempt to fill in this gap in the knowledge about P. cunliffei's response to environmental stimuli, I conducted an

investigation to determine the mites' temperature preference, the effect of acclimation, and the relationship between the mites' preferred temperature and the preferred temperature of its host, Periplaneta americana.

Materials and Methods

Six hundred mites were used to make 1205 observations. Mites used in this study were acclimated at three different temperatures: 15°, 22°, 33°C, for approximately one year. They were cultured in one-gallon glass jars with the American Cockroach as a food source. The cockroaches were fed dry Purina Dog Chow and were given water in cotton stoppered glass vials. The cultures were kept in temperature chambers on a normal light-dark cycle commensurate with the season.

A temperature gradient was established in a plastic tube (75 mm long, 9 mm ID) cut longitudinally in half. The bottom half of the tube was marked off into twelve equal sections. A thermistor probe was inserted in the center of each section, thus allowing each section to be monitored continuously. The top half, used as a cover, had three access holes for entrance of the mites. The cold end was chilled by a variable cold plate (Thermoelectrics Unlimited Inc. Model SK12). The warm end was heated by a covered lamp placed against the chamber. Temperatures were monitored with a 12 channel Yellowstone tele-thermometer. All tests were conducted with the same general ambient conditions otherwise.

The testing procedure was as follows. After the gradient had reached equilibrium, ranging from 13° to 35°C, 10 randomly selected

mites were removed from one of the three temperature chambers and placed in the gradient. Every 5 minutes for 20 minutes a count was made of the number of mites located in each of the 12 sections. Each of the 12 sections in the gradient varied slightly in recorded temperature. This resulted in 18 temperature groups to which the mites responded (table 1). At the end of 20 minutes, the mites were removed and the chamber cleaned with alcohol. This testing procedure was the same for all the mites from the three temperature chambers. Their searching behavior was also observed during the testing period. Controls were treated the same as the experimental groups except that there was no temperature gradient offered.

The statistical tests were Kruskal-Wallis's rank sum correlation, the Bartlett test of homogeneity of variance, and the "F" test. Percentages were plotted for comparison.

Results

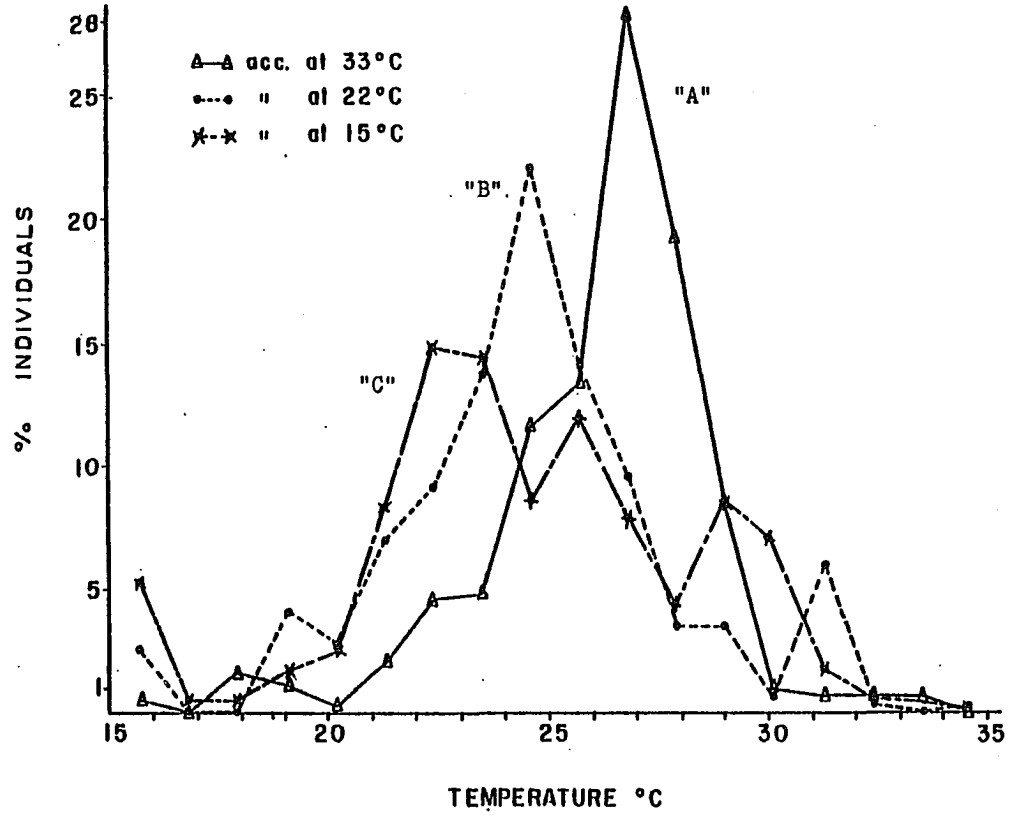
Populations of P. cunliffei maintained at three different temperatures: 33°, 22°, and 15°C, hereafter are referred to as populations "A", "B", and "C" respectively. Table 1 presents numbers of individuals of each of these groups selecting temperatures ranging from 13°C to 35°C. The range was spread at approximately 1 degree intervals resulting in 18 groups (table 1). The resulting mean temperature for each group was: "A" = 26.09°C, "B" = 24.56°C, "C" = 24.52°C.

The number of responses at each temperature for each population was converted to percentages and plotted (Fig. 1). Inspection of the curves indicates that there is a tendency for the mites to select temperatures near the temperatures to which they were acclimated. However, they do not appear to have homogeneous variances. To test for the

Table 1. Numbers and percentages of acclimated individuals responding to temperatures within a temperature gradient.

	ACCLIMATION TEMP. °C					
	33°		22°		15°	
Temp. °C	no.	%	no.	%	no.	%
15.5 - 16.1	2	0.4	8	2.5	25	5.3
16.7 - 17.2	0	0	0	0	2	0.4
17.8 - 18.3	7	1.6	0	0	2	0.4
18.9 - 19.4	5	1.1	13	4.1	8	1.7
20.0 - 20.5	1	0.2	9	2.8	12	2.5
21.1 - 21.6	9	2.0	22	7.0	39	8.4
22.2 - 22.8	20	4.6	29	9.2	69	14.8
23.3 - 23.9	21	4.8	43	13.7	67	14.4
24.4 - 25.0	50	11.6	69	22.1	40	8.6
25.5 - 26.1	58	13.4	44	14.1	56	12.0
26.6 - 27.2	128	28.5	30	9.6	37	7.9
27.8 - 28.3	83	19.2	11	3.5	20	4.3
28.9 - 29.4	37	8.5	11	3.5	40	8.6
30.0 - 30.5	4	0.9	2	0.6	33	7.1
31.1 - 31.6	3	0.7	19	6.0	8	1.7
32.2 - 32.7	3	0.7	1	0.3	3	0.6
33.3 - 33.9	3	0.7	0	0	2	0.4
34.4 - 35.0	0	0	1	0.3	1	0.2

Figure 1. Percentages of individuals of three populations, "A", "B", and "C", responding to temperatures within a temperature gradient.


Δ—Δ acc. at 33°C
•---• " at 22°C
✱-✱ " at 15°C
"A"
"B"
"C"
% INDIVIDUALS
TEMPERATURE °C
28
25
20
15
10
5
1
15
20
25
30
35

validity of this assumption five analyses were run (Sokal and Rohlf 1969); Bartlett's test for homogeneity of variances, Kruskal-Wallis's rank sum correlation, F max test, t-test, and an approximate test of equality of means when the variances are heterogeneous. The Bartlett's test resulted in a $X^2 = 65.77$ (X^2 0.001 (2) = 13.81). The s^2 for populations "A" = 6.50, "B" = 10.38, and "C" = 14.20. Thus, the null hypothesis of homogeneity of variances may be rejected. The F max test was used to determine which groups had significant difference between their variances. Using the s^2 values, population "A" was compared with population "B", F = 1.59; "A" with "C", F = 2.18; "B" with "C", F = 1.36. All three were significant at the 0.01 level. Even though all comparisons were significant there was less difference between "C" and "B" than between either "B" or "C" and "A". This would indicate that even though these three populations are different there is similarity between the individuals acclimated at the lower temperatures.

The Kruskal-Wallis's test indicated that the samples were taken from populations with different means ($X^2 = 73.34$), but it did not reveal where the differences in means were located. Since the differences between the populations may have been in their mean temperature preference, I also tested for equality of means, resulting in an F = 123.88 which was found to be significant at the 0.001 level. This test however, did not tell where the differences were. A close look at the mean temperatures would cause one to recognize intuitively that there was very little difference between population "B" and "C", therefore, the difference must be caused by population "A". A t-test

was used to verify this assumption ($t\ 0.001\ (\infty) = 3.291$): "A" vs "B", $t = 6.98$ and "B" vs "C", $t = 0.15$. Even though this is an a posteriori situation, "A" was compared with "C" ($t = 7.40$), primarly because there was no other appropriate test to use. Thus there is a significant difference of mean temperatures between "A" and "B" and between "A" and "C". There is no significant difference between populations "B" and "C".

In summary, these tests show that the populations are heterogeneous. They are all variable, with population "C" being the most variable, and population "A" being the least variable. They tend to respond in greater numbers in the direction of the temperatures at which they were acclimated and the mean temperatures of "B" and "C" are similar while population "A's" mean temperature is dissimilar from the other two.

Discussion

Animals tend to select environmental conditions which are advantageous to them. This preference, in regard to temperature, may be described as a specific temperature or a broad range of temperatures, all of which may be modified by previous experiences (Gromysz-Kalkowska 1970, Prosser 1974). If an animal has been cultured at a constant temperature in a temperature chamber and has made physiological and behavioral adjustments to the culturing temperature, the animal is said to be acclimated. If the animal is allowed to adapt to a changing natural environment, it is said to be acclimatized (Folk 1974, Prosser 1974). Therefore, acclimation is an adjustment to an artifical environment. The stock of Pimeliaphilus cunliffei used in my studies

has been cultured for many years in the laboratory, an artificial environment. Therefore, the term acclimation rather than acclimatization is appropriate here.

Figure 1 indicates the three populations of mites made adjustments in their temperature preference relative to their acclimating temperatures. These adjustments, however, placed the mean temperature preference higher than the acclimating temperatures for populations "B" and "C" and lower for population "A". Thus there appears to be an adjustment of the populations toward an optimum range, between 22°C and 27°C. Cunliffe (1952) found P. cunliffei's eggs hatched faster at 90°- 95° F than at 80° F, however, this hatching temperature may not be the optimum temperature. Cunliffe also stated that the mite's life cycle "covers a period of from 28-32 days under laboratory conditions, depending upon the temperature." Even though he did not specify the temperatures which regulated the mite's life cycle, the statement does imply an optimum which may be within their preferred range.

There appears to be a difference between the ability of the populations to become acclimated. Statistical analyses of the data reveal that there is a greater variability amoung the individuals acclimated at lower temperature, than those acclimated at the higher temperature. This variability of population "C" indicates less commitment to a specific range. Keeping mites at 15°C for approximately one year may have altered their metabolism to the point of minimizing their ability to become strongly acclimated. One possible reason for the variability and reduced capacity for acclimation is that 15°C maybe

approaching their lower limit of thermal tolerance. This concept is substantiated in their selection of a mean temperature 9.5 degrees higher than their acclimating temperature. Population "A" may have been acclimated near the upper limit of thermal tolerance. However, they were less variable in their response than population "C" and they selected a mean temperature 6.9 degrees below their acclimating temperature. Population "B" on the other hand, did not show much variability and they selected a mean temperature only 2.5 degrees above their acclimating temperature. These data indicate that P. cunliffei can be acclimated, but only within a narrow range.

Even though these mites are capable of adapting to different temperatures, their preference and possible optimum range falls within the range of their host, Periplaneta americana, which prefers 24°-33°C (Cornwell 1968). Other cockroaches, such as Blattella germanica and Blatta orientalis are frequently parasitized by this mite and have similar preferences. This similarity indicates that temperature would not be a limiting factor in determining which of the three species of cockroaches would be most likely parasitized. Any discrimination by P. cunliffei would most likely be due to some other biological or physical factor.

This mite has been a source of trouble with researchers attempting to culture cockroaches, because in sufficient number P. cunliffei can decimate a cockroach culture in a few months (Field et al. 1966). New culturing methods may help abate this problem of parasitism. However, as seen in this study, culturing cockroaches at different temperatures

would have no impact on the mites, as they would be able to adjust concomitantly and continue to thrive.

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RESPONSE OF THE COCKROACH PARASITE

PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

TO OVERHEAD LIGHT

Abstract

Fed and unfed mites were tested beneath a cool fluorescent lamp which provided 40 foot candles. The mites were tested in a two choice chamber which allowed them to move in a three dimensional plane. This apparatus permitted the simultaneous demonstration of kinesis and taxis. The results indicate that Pimeliaphilus cunliffei is photonegative and that the strength of this negative reaction is reduced by hunger. Mites which were unfed moved more than those which were fed. Therefore, being photonegative and active when hungry, these mites are likely to come in contact with a suitable host.

RESPONSE OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE) TO OVERHEAD LIGHT

W. Lynn Laws

As indicated by Lees (1969), acarine behavior has been little studied. Most of the work on mites has been confined to taxonomy. However, of the few behavioral studies carried out, most have been on sensory reception. All organisms respond to environmental stimuli as they pursue their daily activities and light is one such stimulus. Mites' reactions to light have been found to be affected by the type of food and environment (Camin 1953, Darling 1969, George 1963, Lees 1948, Mori 1962). These studies revealed that in a parasitic relationship the host's condition, habits, habitat, and environmental preferences are reflected in the parasite's responses. The American Cockroach, Periplaneta americana, has been found to be photonegative, therefore, P. cunliffei would be expected to reflect this preference in its response.

Photoreception has been found in mites, expecially the spider mites (McEnroe 1969, Suski and Naegele 1963a, 1963b), studied primarily because of their economic importance. Although raphignathids also

possess economically important species, very little is known about the behavior of the members of this family.

This study is one of three designed to clarify the relationship between the mite, Pimeliaphilus cunliffei and its host, the American Cockroach, Periplaneta Americana. Since hunger has been found to affect acarine behavior (Camin 1953, Lees 1948, Suski and Naegle 1963b, Welsh 1930,1931) this study will also consider the effect of hunger on the mite's photoreception.

Photoresponse is usually described in terms of kinesis or taxis. Kinesis is an undirected reaction which allows an animal to reach its destination without being truly orientated. Taxis is a directed reaction (Markl 1974, Wigglesworth 1974). Wigglesworth further described klinokinesis as an undirected response in which the animal moves straight in a favorable environment, but upon making contact with an unfavorable environment begins to make turns or wanders. Based on these definitions an attempt was made to determine the method which these mites use in reacting to light.

Materials and methods

The mites were cultured in one-gallon glass jars, with the American Cockroach, Periplaneta americana as the food source. The cockroaches were fed dry Purina Dog Chow and given water in cotton-stoppered glass vials. Five hundred and sixty of the mites were tested, providing 1120 experimental observations and 1520 control observations.

The test arenas were four plastic boxes (32 mm long X 32 mm wide X

15 mm high). One half of the lid and the bottom half of each box was painted with a non-reflective black paint. This method allowed light to enter only one side of the box and not be reflected to other parts. A partition extended from the middle of the lid to within 2 mm of the bottom, separating the light side from the dark side. The gap at the bottom permitted the mites to move freely from one side to the other. Four similarly constructed boxes were used for the controls, except that in these the lids were left unpainted, permitting light to fall on both sides.

The light source was a cool fluorescent lamp which provided 40 foot candles of illumination over the entire test area. This intensity of light would be equivalent to a room properly illuminated for reading, such as found in a kitchen or laboratory, typical cockroach habitats. The light was monitored with a Winston foot-candle meter, and the temperature in the test area was monitored with a Beckman thermometer.

To test the photoresponse, 10 randomly selected mites were placed in each of the 4 test boxes. These mites were selected either from a fed or an unfed group. They were allowed to move freely within the apparatus for 20 minutes. Every 5 minutes a count was made of the number of mites on the light and dark sides. The positions of the individuals in the lighted side were recorded (e.g., top, bottom, side) and whether the mites were moving or not. At the end of 20 minutes the mites were removed, the boxes cleaned with alcohol, and 10 more mites tested. This procedure was continued for 28 replications. The boxes were placed so that each faced a different direction, thus eliminating possible other directional

orientation. The test animals were either nymphs or adults as larva are too small to observe properly. The same procedure was used for the controls, except that the test chamber was entirely dark. At the end of each recording time a subdued light was used to make a count of the numbers of individuals on the top, bottom, or sides. The data were analyzed by the Chi-square test.

Results

Data presented in tables 1-3 are divided into 2 sections, analysis within groups and analysis between groups. Total number of responses are given with appropriate chi-square and probability values for the fed and unfed groups. Probability was set at 0.05 with 1 df.

Table 1 reveals that of the 1120 responses 734 were to dark and 386 were to light ($\chi^2 = 108.12$, $P<0.05$) indicating that the mites are photonegative. There were more fed mites on the dark side than unfed, suggesting that the unfed mites were less committed in their preference, however, the differences were not significant ($\chi^2 = 3.55$, $P>0.05$). The controls distributed themselves rather evenly when they were not allowed a choice. This indicates that the reactions demonstrated by the experimental groups were not a result of side preference but rather a response to the presence of light. Chi-square values of both experimental and control groups indicate that the unfed individuals were less decisive in their selections, as supported by the test of independence of the experimental and control groups.

Table 2 presents data pertaining to activity of mites in the light. The fed group had significantly more individuals resting than moving

Table 1. — The data represent numbers of responses to light and dark (experimental) and the number of responses to ends of the test chamber (control). Both ends (A and B) of the controls were in light. Comparison between groups is a test of independence. Comparison within the groups is a goodness of fit.

experimental						
	within groups				between groups	
	dark	light	X^2	P	X^2	P
fed	382	178	74.31	<0.05	3.55	>0.05
unfed	352	208	37.02	<0.05		
total	734	386	108.12	<0.05		

controls						
	within groups				between groups	
	A	B	X^2	P	X^2	P
fed	262	298	2.00	>0.05	2.41	>0.05
unfed	288	272	0.34	>0.05		
total	550	570	0.35	>0.05		

($X^2 = 65.53$, $P < 0.05$), while the unfed group had the opposite condition, more were moving than resting ($X^2 = 6.26$, $P < 0.05$). When the two groups were compared to determine if hunger had an effect on activity, an appreciable difference was found ($X^2 = 60.47$, $P < 0.05$), indicating that hunger had an influence. Those that were fed were less active than those that were unfed. Comparison of the mites' activity in light and dark or subdued light revealed that in light their movement was uncertain and wandering, while in dark their movement was straight and rapid. In the light mites quest with the front legs, frequently changing their pace or stopping as they move about the chamber. As the mites moved from the light into the dark their pace became quicker and straighter. However, when going from the dark into the light they momentarily hesitated, referred to as "titubant reaction" by Ewer and Bursell (1950), and often ran along the light-dark interface. Those mites that continued into the light usually altered their pace, direction of movement, or both.

Table 3 presents data on individuals located at the top, bottom and sides of an illuminated test chamber. The results show more mites on the side than on either the top or bottom. These results are understandable, because as the mites move up and down they come in contact with the sides more frequently than either the top or bottom. I have no way of knowing which way mites on the sides were going, since I recorded only their location but not their direction of movement. Therefore, side responses recorded in table 3 will be considered as neutral and only top and bottom responses will be used in

Table 2.— The data represent numbers of individuals moving and resting in a lighted test chamber. Comparison between groups is a test of independence. Comparison within the groups is a goodness of fit.

	within groups				between groups	
	moving	resting	X^2	P	X^2	P
fed	35	143	65.53	< 0.05	60.47	< 0.05
unfed	122	86	6.26	< 0.05		

Table 3.— The data represent numbers of mites on top, side, and bottom of test chamber. The X^2 values are based on locations at top and bottom. Comparison between groups is a test of independence. Comparison within the groups is a goodness of fit.

experimental in light

	within groups					between groups	
	top	side	bottom	X^2	P	X^2	P
fed	36	73	69	6.11	< 0.05	4.47	< 0.05
unfed	54	89	65	0.58	> 0.05		

controls in dark

	within groups					between groups	
	top	side	bottom	X^2	P	X^2	P
fed	27	38	35	1.03	> 0.05	1.85	> 0.05
unfed	33	41	26	0.83	> 0.05		

analysis. The experimental group, which were observed in the light, had a total of 90 individuals on the top that is, nearer the light source and 134 individuals on the bottom. However, when these two totals are broken down the fed individuals demonstrated a significant difference between their responses ($X^2 = 6.11$, $P < 0.05$), while the unfed individuals did not show such discrepancy ($X^2 = 0.58$, $P > 0.05$). A comparison between the groups further reveals that being fed or unfed influences their reaction ($X^2 = 4.47$, $P > 0.05$). The controls which were allowed to wander in the dark distributed themselves rather evenly. There were more unfed mites on the top than on the bottom. However, none of the differences were significant.

In summary, P. cunliffei tended to be photonegative. The strength of their reaction was dependent upon their state of hunger. They were more active when unfed than fed, and all tended to move down, away from the light source.

Discussion

The design of this experiment permitted P. cunliffei to demonstrate both types of reaction mentioned earlier, kinesis and taxis. Most experiments are designed to allow an animal to move in only two dimensions, thus negating any interaction which could be allowed in a three dimensional design. A three dimensional apparatus permits the animal to demonstrate more than one type of reaction simultaneously. When investigating an animal's reaction to light the researcher will usually investigate either directional or nondirectional response, but not both together. This study has shown that both can be investigated

simultaneously.

The primary intent was to establish whether P. cunliffei was photopositive or photonegative and if this reaction was altered by starvation. They are in fact photonegative, whether fed or unfed, as shown by the fact that there were significantly more individuals on the dark side than on the light side (table 1). There are no significant differences between the photoreactions of the two groups, indicating that hunger has no appreciable effect on overall choice. Hunger may have reduced the strength of reaction, but not significantly.

To a mite the size of P. cunliffei, 355 μ- 975 μ (Cunliffe 1952), the distance from the top of the test chamber to the bottom would be relatively long. With light as an overhead stimulus, any mite at the top of the lighted chamber could be considered as responding positively to light and any mite on the bottom could be considered as responding negatively to light, providing they were not reacting to gravity. Controls which were allowed to wander freely in darkness dispersed themselves randomly (table 3), indicating that gravity was not a decisive factor in dispersion. The experimental fed group, however, had significantly more responses to the bottom than to the top (table 2), indicating tactic behavior because the mites had to move toward the light to get to the top and away from the light to get to the bottom, a directed action. The experimental unfed group had more on the bottom than on the top, but the difference was not significant. These data indicate, therefore, that P. cunliffei is phototactically negative. However, as in the light vs dark section, hunger may have reduced the

strength of the negative reaction. This type response is not surprising, as Suski and Näegel (1963) demonstrated that Tetranychus urticae modified its photoresponse after having been fed: some reversed their reaction and some of the mites became neutral in their reaction.

Observations on the mite's behavior in light and dark suggest that klinokinesis is also responsible for the mite's selection of the dark. They move faster and straighter in the dark than in the light, one of the criteria of Markl's (1974) and Wigglesworth's (1974) definition of klinokinesis. They also exhibited a "titubant reaction" (Ewer and Bursell 1950) which would cause the mites to remain in the darkness. Klinokinesis is usually present in animals with one receptor, however as Camin (1953) noted it can be demonstrated in animals with two photoreceptors. Based on this information therefore, it appears that this mite's photonegative response is due to both a kinesis and a taxis and that there is an interaction between their response and hunger.

If hunger reduces the strength of the mite's photoresponse, how is this accomplished? One possible way may have been in their movement. There were significantly more fed mites resting than moving and more unfed mites moving than resting. Hunger has been found to cause mites to disperse (Suski and Naegele 1963). Therefore dispersion, as a result of movement may have been responsible for the lack of significance in the differences between the top and bottom responses of the unfed mites. This effect would have been reflected in the mite's phototactic behavior.

By being photonegative, the mite moves to a suitable environment

and then, by increasing its movement when hungry, is more likely to find food. I reported in a previous paper (Laws 1977) that P. cunliffei is able to detect the host's odor, but has difficulty orientating toward the host unless it is near. Being active would offset the weakness in the mite's ability to orient. Since the cockroaches spend a great deal of time inactive in their harborages, the mites' ability to smell probably is not as important as their being in the right place at the right time. Being photonegative and motile when hungry would fulfill this requirement.

Acknowledgement

I wish to express my utmost gratitude to the members of my committee: Drs. Hubert W. Frings, Harley P. Brown, Charles C. Carpenter, Cluff E. Hopla and Frank J. Sonleitner, for critically reviewing the manuscript.

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DESCRIPTION OF COPULATING AND FEEDING BEHAVIOR
OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI
(ACARINA: RAPHIGNATHIDAE)

Abstract

Mites were observed with the aid of a dissecting microscope as they pursued their daily activity. Fifteen pair of mites were observed copulating. After contact the female becomes quiescent, raising her posterior end, which allows the male to crawl beneath her. She pulls him up with her hind legs permitting insemination.

Feeding behavior was noted after the mites had found a suitable host. They feed by locating a seta with their front legs and the base of the seta with their mouth parts. They then penetrate the exoskeleton at the base of a newly found seta with their chelicerae and feed on the roaches body fluids.

DESCRIPTION OF COPULATING AND FEEDING BEHAVIOR OF THE COCKROACH PARASITE PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

W. Lynn Laws

The only work on Pimeliaphilus cunliffei (Jack) 1961 which concerns biology and behavior is Cunliffe's (1952) work, in which the mite's parasitic way of life was confirmed, and the work of Field et al., (1966) who reported the destructive ability of the mite. Nothing has been recorded about their copulating and feeding behavior.

Behavioral observations are presented in this section to add another dimension to the knowledge about this mite and to the relationship between it and its host, Periplaneta americana. Observations were made with the use of a dissecting microscope.

Copulating behavior

Pimeliaphilus cunliffei is a sexually dimorphic mite. The adult female is longer and wider than the male and her genital opening is posterior and ventral. The male's genital opening is posterior and dorsal. This arrangement lends itself to a peculiar mode of copulating.

Before copulating, the mites move around questing with the front legs. This behavior was observed in all mites at all times, expecially

when they were placed in a new environment. As the mites move about they may come in contact with members of the same sex. This contact does not cause them to make any appreciable change in their behavior. However, upon contact with members of the opposite sex they hesitate, and if the female is receptive she becomes fixed in a raised position. The male moves around the female, touching her with his front legs. This movement does not appear to be directional but rather a searching movement. He eventually makes his way to her posterior, at which time she lowers her anterior end, causing her hind feet to leave the ground. The male then crawls beneath her, both facing the same direction. The female, which is now on top, grasps the male with her hind feet and pulls him up to her causing the genital openings to come in contact. The male's feet remain on the ground. They remain in the position for approximately 2-5 minutes after which they break loose and go their respective ways.

I observed this behavior in only 15 pairs, of the hundreds of mites I have watched. I never saw these mites attempt to copulate after my initial observation and I have no way of knowing whether copulation had taken place before my observation or not. Cunliffe (1952) stated that in the two or three week life span of an adult female mite she is capable of producing two or three batches of eggs. This would be an average of one batch per week. Since copulation was rarely observed by me and only three batches of eggs are produced in a mite's life time, it would appear that the mites may copulate only once before producing eggs. Therefore fertilization of all the eggs may have resulted from

one insemination. This could explain the infrequent observations of copulatory behavior.

Feeding behavior

Cunliffe (1952) established, through the use of radioactive tagged food, that P. cunliffei is truly parasitic on the cockroach and ingests the fluids of the host. Field et al. (1966) mentioned some general areas on the roach's body where the mites were thought to feed. Exact locations, however, were not described. Since this is a small, delicate mite, it would be expected to feed on soft parts of the cockroach. I therefore, made some observations on the feeding behavior of this mite.

Before feeding, the mite located its host, relying on chemoreception and motility to come in contact with a suitable food source. The mites continuously move about waving the front legs, allowing them to come in contact with a host. After locating a roach, a mite immediately crawls onto the newly found food. Normally the parasite does not begin to feed as soon as it gets on the roach, but rather it moves about checking the substrate. This searching may go on for 5 to 30 seconds after which it starts to feed.

Feeding consists of first locating a seta with the front legs, next placing the mouthparts on the seta and then working the way down to the base. At this time the mite moves the body slightly, as if it were penetrating the roach with its chelicerae. The mite then becomes motionless and fluids can be seen moving into the gut. The parasite may stay in this position for several minutes, then withdraw the mouthparts and move to another seta. I have seen some mites feed at one

location for four or five minutes and not feed again, where as other mites feed at three or four locations within the same amount of time. Mites are likely to feed at any location on the roach where there are setae. After feeding, the mites may spend considerable time on the roach, 25-30 minutes, just sitting or wandering around. Those that do so on exposed areas, are usually brushed off as the cockroach moves about in its quarters.

Cunliffe (1952) stated that 25 mites on a cockroach could cause the roach to succumb to parasitism --the insect falls over on its back in about an hour and then thrashes about for approximately five hours before dying. However, I have observed cockroaches with hundreds of mites on them to be active for a day or so. The cockroaches I observed had access to food and water, which may or may not have made a difference. Nothing was stated in Cunliffe's paper about the culturing method. Field et al. (1966) stated that they had seen more than 180 on a single cockroach, and suggested that Pimeliaphilus cunliffei could possible be used as a biological control agent. However, my observations indicate that to do an adequate job the population of mites would be of such magnitude that the walls would be red with mites. In a home this might pose more of a problem than the cockroaches.

Acknowledgement

I wish to express my utmost gratitude to the members of my committee: Drs. Hubert W. Frings, Harley P. Brown, Charles C. Carpenter, Cluff E. Hopla, and Frank J. Sonleitner, for critically reviewing the manuscript.

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SCANNING ELECTRON MICROSCOPY OF

PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

Abstract

Mites of the species P. cunliffei (Jack) were coated with gold while alive and after being critical-point dried. This allowed them to be photographed with a scanning electron microscope. Sensilla, on the mites' legs and palps which are possibly chemo- and tangoreceptive, were studied along with, eyes, aedeagus and female genital opening. Magnifications ranged from 200X to 10,000X.

SCANNING ELECTRON MICROSCOPY OF PIMELIAPHILUS CUNLIFFEI (ACARINA: RAPHIGNATHIDAE)

W. Lynn Laws

With the advent of the scanning electron microscope (SEM) new methods of investigation have become available to the acarologist. The increased magnification and versatility of the SEM have allowed the researcher to study structures in greater detail than was permitted with a light microscope (Axtell et al. 1973, Griffiths et al. 1971, Woolley 1970). Sensory physiologist are now able to locate receptor sites which were previously inferred (Axtell et al. 1973, Homsher and Sonenshine 1975).

Very little is known about the systematics or physiology of Pimeliaphilus cunliffei. There are differences of opinion where the species should be placed taxonomically, and prior to Laws (1977) nothing was known about the mite's reception and response to environmental stimuli. The objective of this paper is two-fold. First, I shall demonstrate possible receptor sites on the mite which could support observations found in Laws (1977) and secondly, I hope to demonstrate features which may aid in future taxonomic work.

Materials and Methods

Representives of P. cunliffei were selected from cultures and treated by two different methods. Some were coated with gold and viewed while still alive, while others were critical-point dried prior to being gold coated and viewed. Instruments used were the Coutes and Walter SEM, ISI TV Mini-SEM, and the JEOL JSM2 SEM. All mites were mounted on viewing plugs with the aid of double adhesive tape. Photographs were taken with a Polaroid camera.

Results and Discussion

Chaetotaxy of this mite, based on the morphology of the female, was described, using the light microscope by Jack (1961), Newell (1971a), (1971b), and Newell and Ryckman (1966). Figure 1 shows an overall view of the male which has not been previously described. The type of setae found on this mite, as with the female, are serrate except for specialized locations such as the palps. Figure 2 shows smooth solenidons on the palps. The behavior described in Laws (1977) suggests that these are tangoreceptors. The mites use their mouthparts to locate the base of a seta for feeding and test the substrate with them in searching behavior. Figure 2 also shows a sensillum with a companion seta (arrow "a") which has not been described before on this structure. These setae may be used as mechanoreceptors, as suggested by Bostanian and Morrison (1973) in their study of Tetranychus urticae.

Another structure which, until now, has remained undetected is a small sensillum coeloconicum, seen in Figure 3A at the tip of arrow "a". This structure, seen enlarged in Figure 3B, is located at the distal end

of tibia I on the dorsal side and does not extend beyond the exoskeleton. Searching behavior noted in Laws (1977) suggests that this seta is a chemoreceptor.

Figure 3A shows another depression on tarsus I, seen at the point of arrow "b". This depression is found on the tarsus of all legs and has a seta coming from the center. An enlargement of this seta (Fig. 3C) shows it to be serrate, similar to the rest of the setae on the legs. It is curious that a seemingly common seta would be found associated with this unique depression. This seta does not appear to undergo transposition as some setae seem to do when the mite molts into an adult (Newell 1971a). This is based on the fact that it is found on the adult and nymph at the same location. Figure 3A is a photograph of such a structure on the leg of an adult, while the photograph in Figure 3C is of the same type structure on the leg of a nymph. The other setae on tarsus I are basically as Jack (1961) described; however, they are not all smooth as indicated in his drawings. Only four dorsal setae on tarsus I are smooth solenidons (Newell 1971a). Bostanian and Morrison (1973) pictured contact chemoreceptors on the palps and legs of the Two-spotted Spider mite as smooth with longitudinal stripes. These smooth setae on P. cunliffei do not show the stripes, but resemble chemoreceptors otherwise.

In copulation the female is dorsal to the male (Laws 1977). Figure 4 shows how this behavior is possible. The genital opening of the female is posterior and ventral (Fig. 4A), while the aedeagus of the male is posterior and curved dorsally (Fig. 4B). The aedeagus is also seen in

Figure 1 at arrow "a". The setae on the ventral side of the female and on the dorsal side of the male may help align the two structures during copulation.

Figure 5 shows the shape of the eye. Even though this structure has not been demonstrated to be a light receptor it resembles the eyes of Tetranychus urticae which have been shown to be photoreceptors (Bostanian and Morrison 1973). The texture of the eye is different from that of the body, which may facilitate light reception. There are no large ridges like those found over the rest of the body (seen in Figure 5B near the eye), which could diffract incoming light. Figure 1 (arrow "b") shows the location of an eye in an overall view. Laws (1977) demonstrated this mite to be photonegative, showing that they do possess photoreceptors, and these structures seem most likely candidates.

Acknowledgement

I wish to express my utmost gratitude to the members of my committee: Drs. Hubert W. Frings, Harley P. Brown, Charles C. Carpenter, Cluff E. Hopla and Frank J. Sonleitner, for critically reviewing the manuscript. I also wish to thank Dr. Mary R. Whitmore for the effort she extended in taking the electron micrographs.

Figure 1. Dorsal view of male. Arrow "a" points to aedeagus. Arrow "b" points to the right eye, 200X.

Figure 2. Right palp of female, showing smooth solenidons. Arrow "a" points to companion seta, 9,000X.

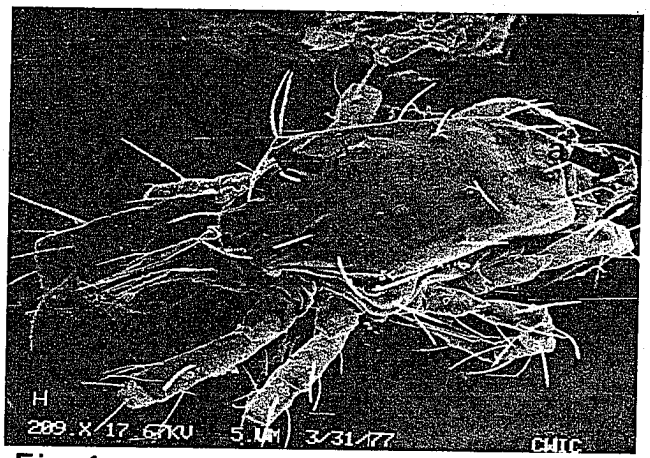


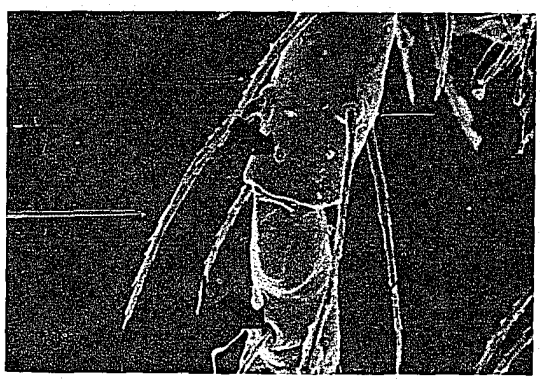
Fig.1

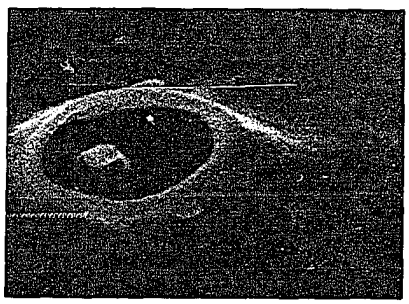


Fig. 2

Figure 3. A) Right leg of female, tibia and tarsus I. Arrow "a" points to sensillum coeloconicum on tibia I. Arrow "b" points to sensory pit on tarsus I with seta coming from center, 5,000X; B) Sensillum coeloconicum on tibia I, 7,000X; C) Sensory pit on tarsus I, 10,000X.

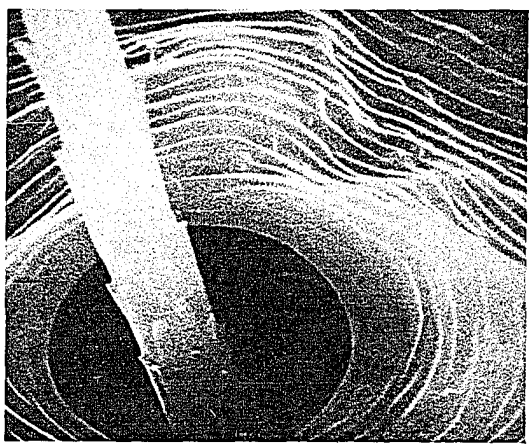
A.





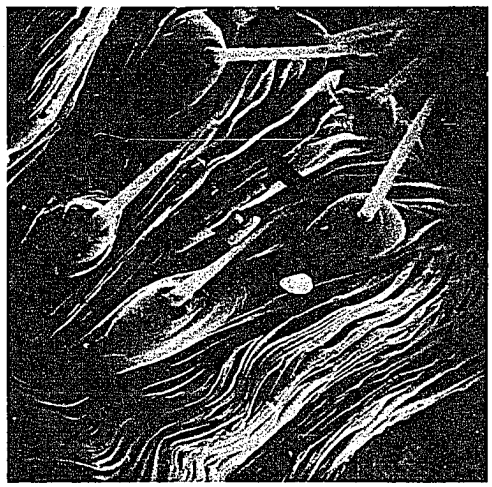
B.

C.

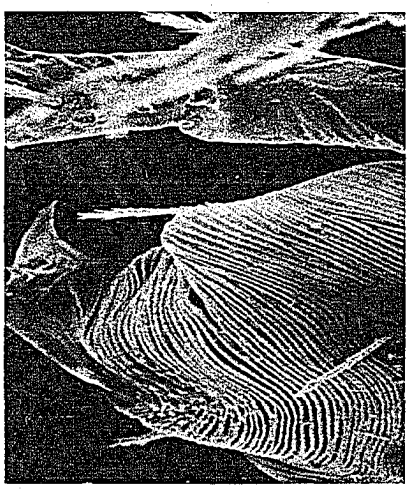


UMI

Figure 4. A) Female genital opening, as indicated by arrow, on ventral surface at posterior end. B) Posterior aedeagus curved dorsally, as indicated by arrow.



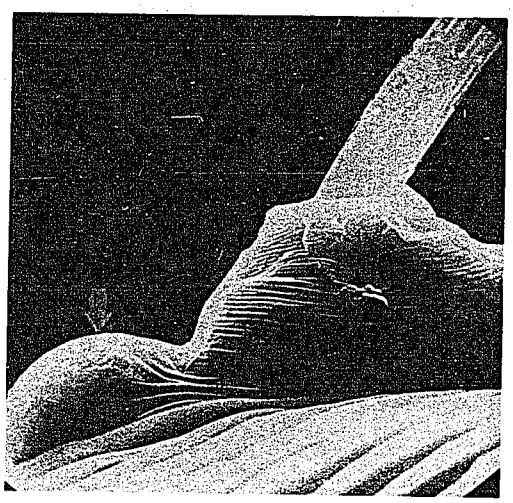
A.



B.

UMI

Figure 5. A) Right eye of female, indicated by arrow, at base of seta, 3000X; B) Right eye of male, indicated by arrow, 3000X.





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SUMMARY AND GENERAL CONCLUSIONS

This dissertation was divided into 5 parts: Part 1. Chemoreception was determined through the use of 4 methods: Spot Test, Two-Choice Contact Test, Y-Tube Test, and Two-Choice Distant test. Part 2. Temperature preferences of acclimated mites were determined through the use of a temperature gradient. Part 3. Photokinesis and Phototaxis were demonstrated through the use of a two-choice, light-dark, chamber. Part 4. Copulating and feeding behaviors were described with the use of a dissecting microscope. Part 5. Scanning Electron Micrographs were taken to show possible receptor locations on the mites and to provide taxonomic information for future studies.

General Conclusions

1. Pimeliaphilus cunliffei is able to detect odor via both distance and contact chemoreceptors. Their ability to use distance chemoreceptors appears to be minimal. Their primary means of detection seems to be via contact chemoreceptors. Under natural conditions, the mites are in quarters which are usually confined and highly concentrated with cockroach odors. Therefore, the mites are not required to move any great distance to come in contact with a roach, and since odor is all around, there would be nothing to orient toward. Thus, distance reception would not be as important as contact reception.

2. Their temperature preference can be altered slightly by acclimation which allows the mites to adapt to the temperature preference of the three species of cockroaches which they are known to parasitize. Their preferred temperature was found to be between 22°-27°C, well within their range of thermal tolerance.

3. This mite is phototactically and photokinetically negative, however, hunger reduces the strength of the negative reaction. When hungry, the animals become motile and consequently are more likely to find a host. The host is photonegative; thus, both host and parasite will be found in similar locations, increasing the likelihood of the mites' finding food.

4. The mites' behavior suggests that females copulate once and that the several batches of eggs which they produce are probably fertilized from the single insemination. As of now, however, there is no direct evidence to support this conclusion. Their behavior further shows that they feed at the bases of setae on the cockroach. This location provides them with a suitable place to penetrate with the chelicerae.

5. Scanning Electron Micrographs show that chemoreceptors may be located on tarsus I and that the eyes have a different texture than the rest of the body, which could allow light to penetrate.

6. Since the cockroaches are able to carry a heavy infestation of mites without damage, the mites would not seem very good agents of biological control. In cultures these mites may limit the host population, but under natural conditions it would be difficult to obtain a large enough concentration of mites to control the roach population.

Appendix 1.— Data for the two-Choice Contact test where (+) represents the odor side and (-) represents the non-odor side. The controls had no odor stimulus, they responded to either side "A" or side "B".

Experimental Group						Control Group					
+	-	+	-	+	-	A	B	A	B	A	B
17	3	12	8	10	10	9	11	10	10	8	12
13	7	2	18	16	4	2	18	12	8	7	13
15	5	18	2	15	5	15	5	11	9	12	8
12	8	10	10	5	15	7	13	14	6	13	7
15	5	17	3	16	4	13	7	6	14	13	7
14	6	15	5	12	8	15	5	6	14	10	10
11	9	17	3	2	18	6	14	1	19	18	2
13	7	17	3	14	6	17	3	12	8	14	6
11	9	17	3	11	9	5	15	3	17	12	8
15	5	15	5	12	8	9	11	14	6	10	10
10	10	7	13	18	2	3	17	7	13	16	4
12	8	9	11	9	11	5	15	6	14	8	12
12	8	14	6	17	3	16	4	15	5	3	17
13	7	12	8	16	4	12	8	2	18	6	14
183	97	182	98	173	107	134	146	119	161	150	130

df		x^2	
42	total	178.20	P<0.05
1	pooled	66.30	P<0.05
41	heterogeneity	111.90	P<0.05

df		x^2	
42	total	163.78	P<0.05
1	pooled	1.37	P>0.05
41	heterogeneity	162.41	P<0.05

Appendix 2.-- Data from the Y-Tube Test with the use of a vacuum. Responses recorded are to the odor side (+), to the blank side (-), and to the starting chamber (0).

Y-Tube With Vacuum

+	-	0	+	-	0	+	-	0	+	-	0
3	2	5	2	2	6	2	2	6	3	3	4
5	3	2	3	0	7	1	1	8	6	0	4
3	0	7	0	1	9	4	3	3	4	0	6
2	0	8	3	0	7	5	1	4	8	0	2
2	0	8	1	0	9	2	3	5	0	0	10
3	0	7	4	0	6	6	0	4	4	3	3
2	4	4	4	2	4	2	5	3	2	1	7
2	1	7	3	3	4	5	0	5	2	6	2
3	3	4	3	4	3	2	6	2	8	2	0
2	4	4	1	7	2	3	5	2	4	3	3
2	2	6	0	3	7	3	4	3	3	5	2
3	0	7	1	4	5	2	1	7	0	0	10
32	20	68	25	26	69	37	31	52	44	23	53

+ = 138

- = 100

0 = 242

X^2 for +, -, 0 scores

$X^2 = 67.55$

$P < 0.05$

df		X^2
48	total	81.61 $P<0.05$
1	pooled	6.06 $P<0.05$
47	heterogeneity	75.55 $P<0.05$

Appendix 3.— Data from the Y-Tube Test with diffused odor. Responses recorded are to the odor side (+), to the blank side (-), and to the starting chamber (0).

Y-Tube Without Vacuum

+	-	0	+	-	0	+	-	0
3	3	4	2	3	5	2	4	4
2	3	5	4	3	3	2	1	7
1	1	8	3	2	5	3	3	4
4	2	4	1	2	7	4	2	4
1	2	7	2	3	5	2	2	6
1	2	7	1	4	5	3	0	7
2	1	7	2	2	6	3	5	2
2	1	7	1	3	6	5	0	5
1	2	7	3	1	6	2	6	2
3	1	6	4	1	5	3	5	2
3	1	6	1	1	8	3	4	3
1	2	7	2	0	8	4	3	3
3	1	6	4	1	5	2	1	7
1	1	8	1	0	9	4	4	2
28	23	89	31	26	83	42	40	58

+ = 101	df		X^2	
- = 89	42	total	30.23	P>0.05
0 = 230	1	pooled	0.75	P>0.05
	41	heterogeneity	29.47	P>0.05

Appendix 4.— Data from the Y-Tube Test with plunger forcing mites to make a choice. Choices are: odor side (+), or blank side (-).

Y-Tube With Plunger

+	-	+	-	+	-	+	-
6	4	5	5	5	5	5	5
6	4	5	5	8	2	7	3
7	3	6	4	7	3	5	5
6	4	7	3	9	1	7	3
6	4	5	5	5	5	6	4
7	3	8	2	5	5	7	3
4	6	6	4	4	6	6	4
6	4	5	5	8	2	6	4
5	5	4	6	3	7	3	7
4	6	2	8	4	6	8	2
5	5	2	8	4	6	6	4
7	3	4	6	6	4	4	6
69	51	59	61	68	52	70	50

df		X^2	
48	total	52.00	P>0.05
1	pooled	5.63	P<0.05
47	heterogeneity	46.37	P>0.05

Appendix 5.— Data from the Two-Choice Distance Test run during morning hours. Choices are: odor side (+), or blank side (-).

Two-Choice Distance Test

Morning

+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
5	5	8	2	4	6	10	0	6	4	9	1	7	3	6	4	8	2	6	4
6	4	9	1	5	5	6	4	6	4	5	5	7	3	3	7	3	7	3	7
6	4	5	5	5	5	8	2	10	0	6	4	6	4	7	3	2	8	4	6
4	6	7	3	4	6	6	4	5	5	9	1	10	0	4	6	3	7	5	5
3	7	7	3	5	5	6	4	6	4	7	3	6	4	3	7	6	4	5	5
8	2	8	2	4	6	7	3	4	6	6	4	6	4	4	6	5	5	7	3
5	5	7	3	6	4	7	3	6	4	6	4	4	6	3	7	5	5	6	4
4	6	5	5	7	3	6	4	6	4	6	4	5	5	3	7	4	6	4	6
4	6	5	5	5	5	8	2	7	3	7	3	5	5	2	8	3	7	4	6
5	5	5	5	7	3	8	2	8	2	6	4	6	4	8	2	7	3	7	3
50	50	66	34	52	48	72	28	64	36	67	33	62	38	43	57	46	54	51	49

\+ = 573

\- = 427

df		x^2	
100	total	144.40	P>0.05
1	pooled	21.31	P<0.05
99	heterogeneity	123.09	P>0.05

Appendix 6.— Data from Two-Choice Distance Test run during evening hours. Choices are: odor side (+), or blank side (-).

Two-Choice Distance Test

Evening

+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
4	6	5	5	5	5	8	2	7	3	5	5	6	4	2	8	3	7	6	4
5	5	5	5	7	3	8	2	6	4	8	2	5	5	3	7	4	6	3	7
4	6	5	5	6	4	7	3	6	4	6	4	5	5	3	7	7	3	4	6
5	5	7	3	5	5	6	4	6	4	9	1	3	7	4	6	5	5	6	4
7	3	8	2	4	6	6	4	6	4	6	4	6	4	3	7	5	5	4	6
3	7	6	4	4	6	7	3	4	6	6	4	6	4	4	6	6	4	5	5
4	6	7	3	5	5	6	4	5	5	6	4	9	1	7	3	3	7	7	3
6	4	5	5	7	3	6	4	8	2	7	3	6	4	6	4	2	8	5	5
5	5	8	2	5	5	8	2	9	1	6	4	7	3	3	7	3	7	6	4
5	5	9	1	5	5	8	2	6	4	7	3	7	3	6	4	7	3	4	6
48	52	65	35	53	47	70	30	63	37	66	34	60	40	41	59	45	55	50	50

+ = 561

- = 439

df		x^2	
100	total	118.80	P>0.05
1	pooled	14.88	P<0.05
99	heterogeneity	103.92	P>0.05