

Agonistic Behavior Correlated with Hydrocarbon Phenotypes in Dampwood Termites, *Zootermopsis* (Isoptera: Termopsidae)

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The dampwood termite genus Zootermopsis Emerson contains three recognized species with four distinct and consistent hydrocarbon phenotypes. Agonistic behaviors among nonreproductive insects from colonies of the same and different hydrocarbon phenotypes were observed in the laboratory. Various combinations of soldier versus nymphs, pseudergate versus pseudergate, and soldier versus soldier encounters were used in experimental trials. Soldiers or pseudergates seldom attack individuals of the same hydrocarbon phenotype. Z. angusticollis (Hagen) (phenotype II) is typically aggressive toward phenotype III of Z. nevadensis (Hagen) but not always aggressive against phenotype I of Z. nevadensis. The variation in response is dependent on which castes are placed in the bioassay arena: soldier versus soldier bouts result in consistent aggression, while pseudergate versus pseudergate or soldier versus nymphs contacts do not. Both pseudergates and soldiers of Z. laticeps (Banks) (phenotype IV) respond agonistically toward the other three phenotypes: Z. angusticollis (II) and Z. nevadensis (I and III). Although hydrocarbon phenotypes I and III, both Z. nevadensis, are morphologically indistinguishable, agonistic behavioral responses between phenotype I and phenotype III are not equivalent to I versus I or III versus III behavioral responses. The I versus III engagements, regardless of the castes involved, display a greater proportion of avoidance and aggressive responses than I or III intraphenotype encounters. We interpret the lack of avoidance or aggressive behavior within each of the two phenotypes of Z. nevadensis and the significant avoidance and aggressive behavior between phenotypes as definite evidence of discrimination between

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disparate hydrocarbon phenotypes. These agonistic bioassays along with data on distinct hydrocarbon patterns and geographic distributions serve as the basis for creating two subspecies of Z. nevadensis: Z. n. nevadensis (Hagen) and Z. n. nuttingi Haverty and Thorne, ssp. nov.

KEY WORDS: chemotaxonomy; aggression; kin recognition; kin discrimination; *Zootermopsis*; Isoptera; species interactions; cuticular hydrocarbons.

INTRODUCTION

Agonistic behavior involves social interactions between individuals, including fighting, fleeing, and submitting. Termites show a wide range of agonistic behaviors when interacting with other termites from a different colony of the same or a different species. Aggressive encounters are often particularly dramatic because of defensive morphological modifications of the soldier caste and effective use of strong mandibles by the pseudergate or worker castes. Here we describe patterns of agonistic behavior observed in laboratory encounters between colonies within the North American dampwood termites, *Zootermopsis* (Hagen). We demonstrate that *Zootermopsis* termites discriminate between insects of like versus unlike hydrocarbon phenotypes, with the consistency and degree of the response dependent on the castes involved in a given trial.

The initial motivations for this series of experiments were two. First, Haverty *et al.* (1988b) discovered that *Zootermopsis* specimens collected over a broad geographic range (parts of Washington, Oregon, California, and Arizona) sort into four unique and clearly distinguishable hydrocarbon phenotypes (I–IV). At present, the genus has only three described species (reviewed by Emerson, 1933; Sumner, 1933; Weesner, 1970). *Z. angusticollis* (Hagen) always bears hydrocarbon phenotype II, *Z. laticeps* (Banks) corresponds to phenotype IV, and phenotypes I and III both fit the present morphological description of *Z. nevadensis* (Hagen) (Haverty *et al.*, 1988b). Never in the literature has a case been reported of a single species showing such dramatic qualitative differences in hydrocarbons as the two phenotypes of *Z. nevadensis* (I and III) (see Howard and Blomquist, 1982; Carlson and Bolten, 1984; Blomquist and Dillwith, 1985). For this reason, field-collected termite colonies were grouped by hydrocarbon phenotype in the behavioral bioassays described in this paper. We initiated these experiments to see if behavioral interactions within and between phenotypes might provide insightful information regarding whether or not termites show behaviors consistent with hydrocarbon patterns, perhaps enabling us to resolve further the species problem of *Z. nevadensis*.

The second impetus for the study was a set of observations by M. I. Haverty and others (Weesner, 1970) on nesting habits and colony foundation of field colonies of *Zootermopsis*. Single logs may be inhabited by more than one

colony, of the same or different species. Numerous nuptial pairs, groups of dewinged alates, or incipient colonies can be found under the bark of a single log or recently killed tree. These field observations, in combination with preliminary behavioral bioassays demonstrating that termites from different colonies often show no agonistic responses when placed together, led us to consider the possibility that *Zootermopsis* colonies of the same species inhabiting the same log may occasionally fuse in natural circumstances. To investigate this potential further, we initiated a set of experiments combining insects from different colonies.

Interspecific aggression is common in termite-termite interactions (Grassi and Sandias, 1896-1897; Nel, 1968; Clément, 1982; Springhetti and Amorelli, 1982; Thorne, 1982; Traniello and Beshers 1985; Adams and Levings, 1987). Intraspecific aggression (between colonies) has also been shown in a variety of other termites (Dudley and Beaumont, 1889; Andrews, 1911; Pickens, 1934; Nel, 1968; Howick and Creffield, 1980; Thorne, 1982; Levings and Adams, 1984; Clément, 1986; Jones, 1987; Adams and Levings, 1987; W. L. Nutting, personal communication; N.-Y. Su, personal communication; M. I. Haverty, unpublished observations). A few documented cases exist, however, where aggression between conspecific termites is absent, i.e., where individuals from different colonies can be mixed with little or no reaction (Clément, 1986; Jones, 1987; R. W. Howard, personal communication; J. K. Mauldin, personal communication; N.-Y. Su, personal communication; J. F. A. Traniello, personal communication).

Here we demonstrate that behavioral responses of soldiers, nymphs, and pseudergates of *Zootermopsis* placed in arenas with members of foreign colonies clearly segregate based on hydrocarbon phenotypes. Interphenotype agonism is consistently high; intraphenotype aggression is always low or non-existent. These results suggest that the termites themselves discriminate chemical cues correlated with, or possibly contained in, hydrocarbon patterns. This behavioral consistency provides further evidence that the taxonomy of the genus *Zootermopsis* should be reevaluated.

MATERIALS AND METHODS

The three experiments described below developed from casual observations made in the laboratory of M.I.H. in 1985, in which pseudergates or nymphs were combined with soldiers from different *Zootermopsis* colonies. Although we assumed that reactions between termites of different colonies would all be aggressive, very few of these preliminary encounters resulted in aggressive behavior. These observations led to further pairings of soldiers and pseudergates or nymphs from two colonies each belonging to hydrocarbon phenotypes I from the Eldorado National Forest, about 40 km SE of Placerville, California,

and phenotypes II and III from Pacific Grove, California. When a soldier was confronted with a pseudergate or nymph from the same or different colony of the same phenotype, aggressive behavior was rare. When phenotype I soldiers were presented pseudergates or nymphs of phenotype II or III, they rarely responded aggressively. Soldiers from phenotypes II and III always reacted aggressively toward pseudergates or nymphs of the other phenotype, whereas their reaction toward pseudergates and nymphs from phenotype I was mixed. These early, informal observations were our first indication that aggressive behavior might be correlated with hydrocarbon phenotypes and encouraged us to design more robust studies of intra- and interphenotypic agonistic behavior (Experiments 1-3).

Collection of Termites

All *Zootermopsis* colonies were collected by chopping or sawing into the natal log or stump and removing a large portion of the colony. Seldom was an entire colony collected, and the primary reproductives were rarely found. Nest material was thoroughly disaggregated in the field or returned to the Berkeley area for removal of the termites. Surviving termites were placed on small pieces of moist, natal wood and kept in one or more plastic nest boxes (either $3.5 \times 10.0 \times 14.0$ or $6.5 \times 12.0 \times 17.5$ cm) depending on the number of termites collected. Boxes containing these *Zootermopsis* colonies were kept in the laboratory in Berkeley, Calif., or Marblehead, Mass., at room temperature for up to 21 months before we initiated the agonistic bioassays. Wood of the original collection species was replenished approximately every 2 to 3 months.

Experiment 1

In this first set of agonistic encounters we used colonies only from the Eldorado National Forest and Pacific Grove. Prior to conducting Experiment 1 these colonies comprised our sole source of live *Zootermopsis* specimens. Concurrent to initiating Experiment 1 M.I.H. began to collect additional colonies of *Zootermopsis* from Oregon, California, and southeastern Arizona, to examine the geographical distribution of each hydrocarbon phenotype, and to increase significantly the number of geographically diverse samples for correlation of morphology and hydrocarbon phenotype. These collections also supplied additional, geographically diverse colonies for the remaining two agonistic behavior experiments. Since the first bioassays in Experiment 1 were admittedly insular, some expansion of the intraphenotype encounters was done later to extend the geographic distribution of the colonies used in these bioassays (Fig. 1).

These trials assessed the agonistic response of soldiers from one colony confronting nymphs from a second colony. Colonies had been cultured in the laboratory from 6 to 11 months. Tests were conducted pairing individual sol-

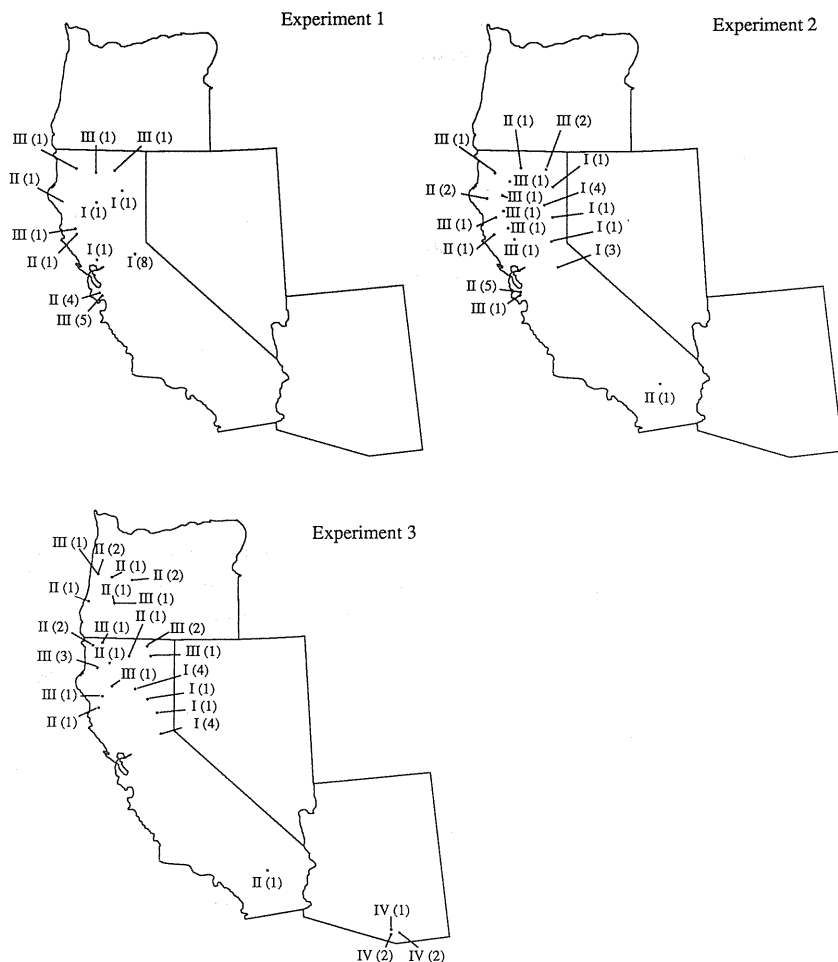


Fig. 1. Location of colonies used in three experiments to assess agonistic behavior among four hydrocarbon phenotypes of *Zootermopsis*. Roman numerals refer to the hydrocarbon phenotype of colonies used in one of three experiments. The numbers in parentheses are the number of colonies from a site that were used in an experiment.

diers from at least three colonies from each of the three hydrocarbon phenotypes with nymphs from each phenotype. The numbers of soldiers per colony and colonies per phenotype were not equal and were determined strictly on availability. The number of replicates or bouts varied with each combination. Soldiers encountered nymphs from a different colony of the same or different phenotype for the first time since being collected.

In this series of bioassays, the experimental design was slightly different from the fully randomized encounters used later in the pseudergate–pseudergate and soldier–soldier confrontations (Experiments 2 and 3). In Experiment 1, soldiers were removed from their nest boxes and individually placed in 5-cm-diameter, covered, plastic petri dishes lined with clean, moist filter paper. Within 30 s and after the soldier had become less agitated (ceased searching behavior or opening and closing of mandibles), three nymphs from a second colony (chosen at random from within a given phenotype) were gently dropped into the opposite end of the arena. Soldier response to the nymphs was monitored for 4 min and recorded as shown in Table I. After each encounter, termites and filter paper were removed and a new, moist paper was put in its place. No individual soldier or nymph was used in more than one encounter.

Experiment 2

This set of trials assessed the intracaste agonistic behavior of either pseudergates or soldiers from hydrocarbon phenotypes I, II, and III. Pseudergates were defined as individuals lacking wing buds and having head-capsule diameters greater than or equal to that of nymphs from the same colony (Miller, 1969). Colonies had been in culture in the laboratory from 3 to 12 months.

For both the pseudergate–pseudergate and the soldier–soldier interactions, 10 colonies from each phenotype were selected to be used in the experimental trials (Fig. 1). These colonies were chosen to represent as broad a geographic distribution as possible at the time the bioassays were conducted. Single pseudergates (or soldiers) from each of the two paired colonies were placed in opposite ends of the 5-cm-diameter, covered, plastic petri dishes which were lined with fresh, moist filter paper. Termites were placed in the petri dish (arena) within seconds of each other. We, therefore, avoided the possibility of one individual establishing a local odor or territory. Termites were observed for 2 min and agonistic responses were classified as shown in Table II. Termites were removed and filter papers were replaced after each trial.

The order of trials and colony pairings for the pseudergate–pseudergate

Table I. Classification of Behaviors of Soldiers of Hydrocarbon Phenotypes I, II, and III When Presented Nymphs of the Same or Different Phenotypes During 4-min Trials (Experiment 1)

Classification	Behaviors observed
Passive	No reaction or simply antennation
Discrimination	Open mandibles or false snapping directed toward the nymphs (no actual biting)
Aggression	Biting, always resulting in body puncture of at least one nymph

Table II. Classification of Behaviors of Pseudergates or Soldiers of Hydrocarbon Phenotypes I, II, III, and IV When Presented Like Castes of the Same or Different Phenotypes During 2-min Trials (Experiment 2)

Classification	Behaviors observed
Passive	No reaction, antennation or mutual grooming, or bumping with no aversion
Avoidance	Retreat or avoidance upon encountering other individual
Aggression	Attempted biting or actual brief bite, prolonged biting or chewing, "grappling," evisceration, crushing or severing head

interactions was determined using a randomly generated assignment of pairings of individuals (based on 10 colonies of each of the three hydrocarbon phenotypes). Individual pseudergates from a given colony would therefore be expected to be paired, at random, with an individual from a variety of other colonies and, occasionally, with another pseudergate from its own natal colony. No pseudergate was used in more than one encounter. Because the six combinations of phenotype pairings (I versus I, I versus II, . . . , III versus III) appeared at random on our computer-generated trial assignments, and we executed only the first half of the possible 600 trials, the number of trials per phenotype pairing is unequal across the six combinations.

Soldier-soldier pairings were also randomly assigned. If no more soldiers were available from a given colony, we substituted an individual from the next randomly selected colony in which a sufficient number of soldiers was present. No individual soldier was used in more than one trial. Random encounter assignments were made sequentially across the six phenotype pairs, so in this series of experiments sample sizes within phenotype pairings were initially equal. However, after 13 trials of each of the six phenotype pairings, we continued four more trials of phenotype I versus III and one more trial of phenotype II versus III. We had additional unused soldiers of those groups and mixed results which we hoped to clarify. Agonistic responses of the soldiers were classified as shown in Table II.

Experiment 3

This set of trials assessed the intracaste agonistic behavior of pseudergates or soldiers from hydrocarbon phenotypes I, II, III, and IV. These bioassays were not possible until *Z. laticeps*, phenotype IV, was collected in quantity. As in Experiment 2, colonies of phenotype I, II, and III were chosen to represent as broad a geographical distribution as possible (Fig. 1) and had been in culture

from 2 to 21 months. Procedures for behavioral observations of pseudergates were identical to those in Experiment 2 (Table II).

Prior to presenting phenotype IV pseudergates or soldiers with those from another phenotype, both castes from each of five colonies of *Z. laticeps* were marked on the frons with a unique color of paint so that they could be returned to their natal colony. In studies done in conjunction with Experiment 1, paint did not appear to affect behavior. Marked termites can easily be reincorporated into a colony and may live for over 1 year with their acrylic tattoos. Fifty-one random pairings of phenotype IV pseudergates were done to assess intraphenotype agonistic behavior. Since all but one of these resulted in no reaction, the insects were returned to their natal colonies after all 51 pairings were complete. Five like pairings were made with phenotype IV soldiers. All showed passive behavior. Thus many of the phenotype IV pseudergates and 5 of the 11 soldiers were not naive when matched against individuals of the other three phenotypes. This was done primarily to save the relatively rare *Z. laticeps* specimens.

For the pseudergate-pseudergate trials, individuals from 10 colonies from each of phenotypes I, II, and III were paired with pseudergates from five colonies of phenotype IV. Pairings were randomly assigned. Replications per phenotype IV colony were also not equal; the number of pseudergates available in each phenotype IV colony was unequal.

Soldier-soldier encounters were done slightly differently from those in Experiment 2. The number of available phenotype IV soldiers was limited (we had only 11). We were forced to use each soldier in as many encounters as possible (up to nine). Thus, phenotype IV soldiers were naive only for their first encounter; soldiers from the other phenotypes were always naive, i.e., they were used only once. Each phenotype IV soldier was randomly assigned a challenger from one of the other phenotypes. Each bout consisted of one 2-min round. Soldiers were observed until they became aggressive or the 2-min observation period ended. If soldiers began to attack, the fight was stopped and the phenotype IV soldiers were returned to the holding petri dish. Thus a phenotype IV soldier was not used again until the other 10 had had an engagement. In this way all soldiers had the opportunity to "cool down" between bouts. Soldiers were used until it was obvious they were handicapped from previous battles: one soldier had its head punctured in bout 5 and one lost two legs in bout 7. These were removed from further encounters. Results of encounters were classified as in Experiment 2 (Table II).

Statistical Analysis

Results of all three experiments were analyzed in the same manner. For each experiment or caste pairing within an experiment, the significance of the differences between the proportions of passive encounters within a phenotype

and between all combinations of interphenotype encounters was evaluated by tests of proportions [the critical-ratio test described by Fleiss (1981), p. 30]. Each comparison was considered significantly different if the z statistic exceeded 1.96. This implies a significance level of approximately 0.05.

RESULTS

Experiment 1

Agonistic behavior of soldiers varied greatly and was highly dependent on the hydrocarbon phenotype of the nymphs presented to them. Our observations are summarized in Table III and Fig. 2 and as follows.

(1) Soldiers seldom attack nymphs of the same phenotype from a different colony and never challenge nymphs from their natal colony. Nonpassive responses were common (14.7% of the encounters) within phenotype II, but were rare within phenotypes I and III (3.5 and 1.1%, respectively). Soldiers never fight or show discrimination behavior within a hydrocarbon phenotype when colonies are from the same geographic location. All discrimination or aggressive, intraphenotype responses were between colonies that were from distant geographic locations.

(2) Soldiers from phenotype II almost always attack nymphs from phenotype III but not phenotype I. Recall that phenotype II colonies are sympatric

Table III. Comparison of the Proportion of Passive Agonistic Encounters Between Soldiers and Nymphs Within Phenotypes (Intra) and Between Phenotypes (Inter) of *Zootermopsis* Phenotypes I, II, and III (Experiment 1)

Intraphenotype			Interphenotype			z statistic ^b
Bouts ^a	Total No.	Proportion passive	Bouts ^a	Total No.	Proportion passive	
I $\times$ I	85	0.965	I $\times$ II	104	0.567	8.13
I $\times$ I	85	0.965	II $\times$ I	47	0.830	2.32
I $\times$ I	85	0.965	I $\times$ III	100	0.270	14.28
I $\times$ I	85	0.965	III $\times$ I	55	0.764	3.32
II $\times$ II	68	0.853	I $\times$ II	104	0.567	4.41
II $\times$ II	68	0.853	II $\times$ I	47	0.830	0.33
II $\times$ II	68	0.853	II $\times$ III	53	0.000	19.88
II $\times$ II	68	0.853	III $\times$ II	45	0.044	15.36
III $\times$ III	92	0.989	I $\times$ III	100	0.270	15.72
III $\times$ III	92	0.989	III $\times$ I	55	0.764	3.86
III $\times$ III	92	0.989	II $\times$ III	53	0.000	90.31
III $\times$ III	92	0.989	III $\times$ II	45	0.044	29.09

^aThe first Roman numeral signifies the phenotype with the soldier; the second is the phenotype with the nymph.

^b $z = 1.96$ for $\alpha = 0.05$. For experimentwise $\alpha \leq 0.05$, $z = 2.64$, using the Bonferroni inequality.

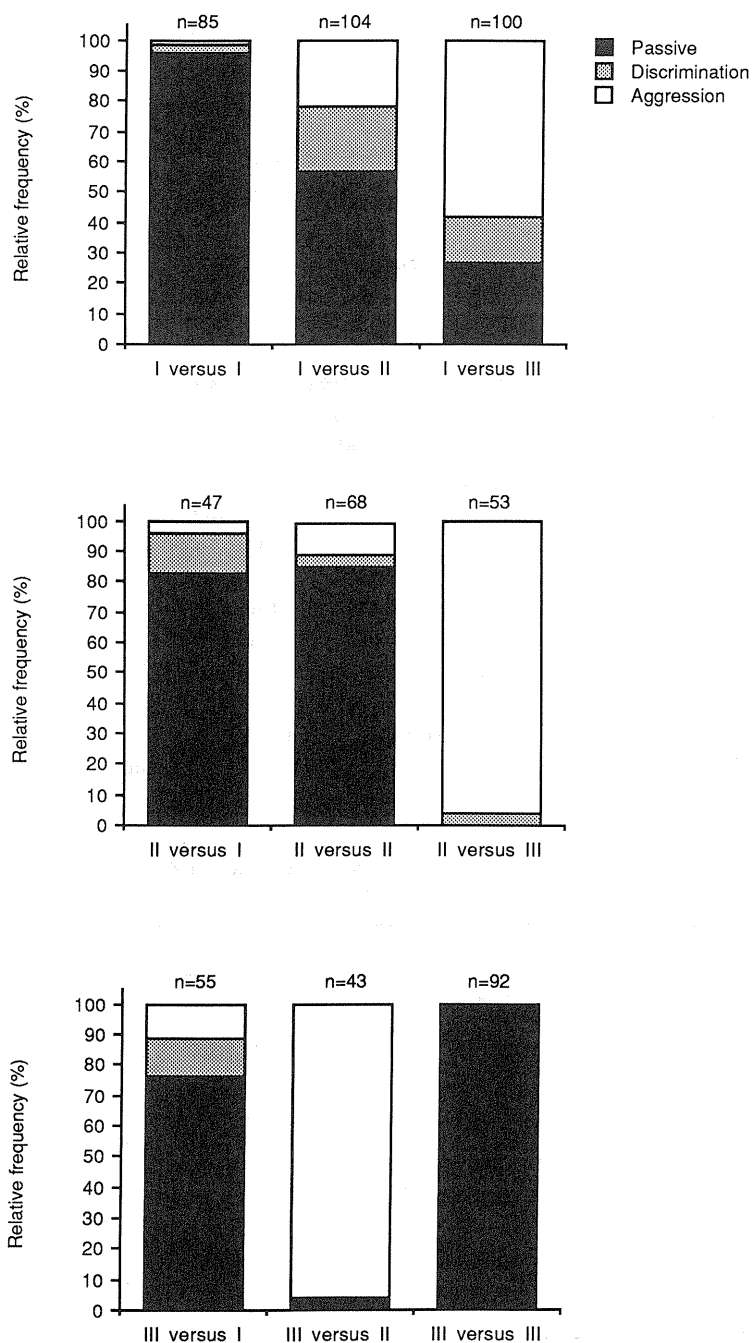


Fig. 2. Agonistic behavior between soldiers of hydrocarbon phenotypes I, II, and III (first Roman numeral) and nymphs of like or different phenotypes (second Roman numeral). The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, recognition without attack or discrimination, and aggression or biting (Experiment 1).

with phenotype III colonies, whereas the phenotype I colonies are from a site about 300 km away.

(3) Soldiers from phenotype III colonies always attack nymphs from phenotype II colonies but seldom attack nymphs from phenotype I colonies. Again, this may be related to the fact that phenotypes II and III are sympatric.

(4) A high percentage of the I versus III encounters (58%) resulted in an aggressive response, whereas the I versus II engagements resulted in a considerably lower percentage (22%) of aggressive responses.

(5) The III versus I, and especially the I versus III, responses are not equivalent to either the I versus I or the III versus III responses: the intraphenotype encounters display a significantly higher proportion of passive responses (Table III).

Experiment 2

Agonistic behavior in pseudergate-pseudergate trials was more variable and showed a broader spectrum of behaviors than did the soldier-soldier encounters. The former showed a full range of behaviors, whereas the latter exemplified almost exclusively the extremes: passive or aggressive behavior. Our observations are summarized in Table IV and Figs. 3 (for pseudergates) and 4 (for soldiers) and as follows.

Table IV. Comparison of the Proportion of Passive Agonistic Encounters Between Pseudergates or Between Soldiers Within Phenotypes (Intra) and Between Phenotypes (Inter) of *Zootermopsis* Phenotypes I, II, and III (Experiment 2)

Intraphenotype			Interphenotype			z statistic ^a
Bouts	Total No.	Proportion passive	Bouts	Total No.	Proportion passive	
Pseudergates						
I × I	50	0.980	I × II	57	0.439	7.88
I × I	50	0.980	I × III	50	0.660	4.58
II × II	54	0.963	I × II	57	0.439	7.43
II × II	54	0.963	II × III	45	0.133	14.62
III × III	42	1.000	I × III	50	0.660	5.07
III × III	42	1.000	II × III	45	0.133	17.13
Soldiers						
I × I	13	1.000	I × II	13	0.077	12.49
I × I	13	1.000	I × III	17	0.588	3.45
II × II	14	0.643	I × II	13	0.077	3.89
II × II	14	0.643	II × III	13	0.000	5.02
III × III	13	0.923	I × III	17	0.588	2.39
III × III	13	0.923	II × III	13	0.000	12.48

^a $z = 1.96$ for $\alpha = 0.05$. For experimentwise $\alpha \leq 0.05$, $z = 2.40$, for each caste, using the Bonferroni inequality.

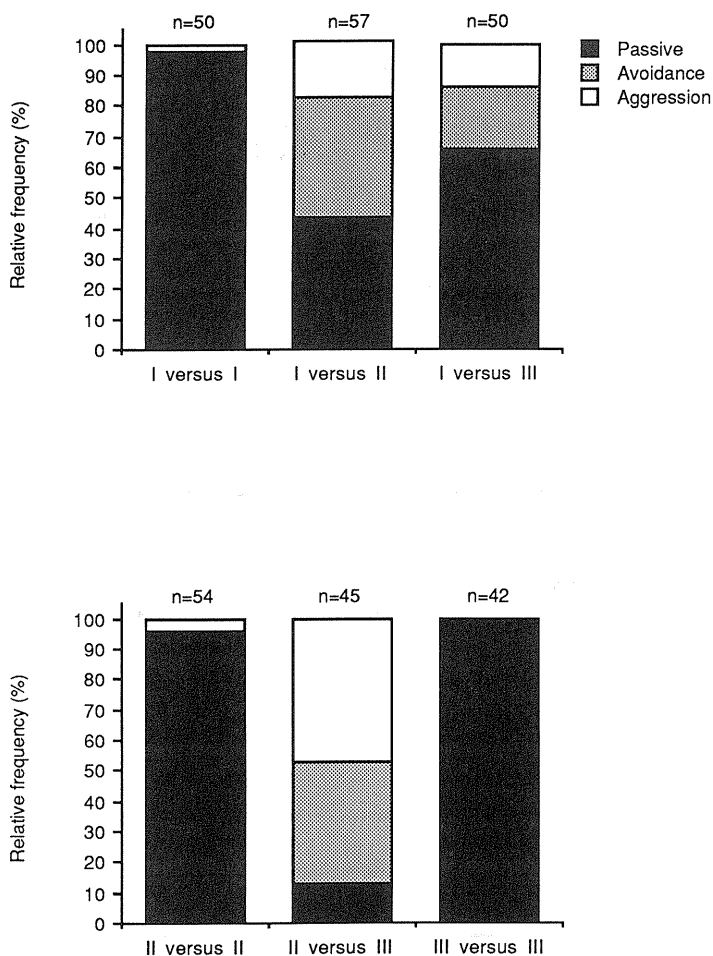


Fig. 3. Agonistic behavior between pseudergates of hydrocarbon phenotypes I, II, and III and like or different phenotypes. The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, avoidance, and aggression (Experiment 2).

(1) Pseudergates of the same phenotype seldom, if ever, avoid or react aggressively toward one another. There were exceptions, but we feel that these anomalies may have been accidental. Often when termites were placed into the petri dish, they were “on alert” and aggressively challenged the introduced individual. Seldom was this behavior continued beyond the first few seconds of the bout unless the response was truly aggressive. We always tried to place the

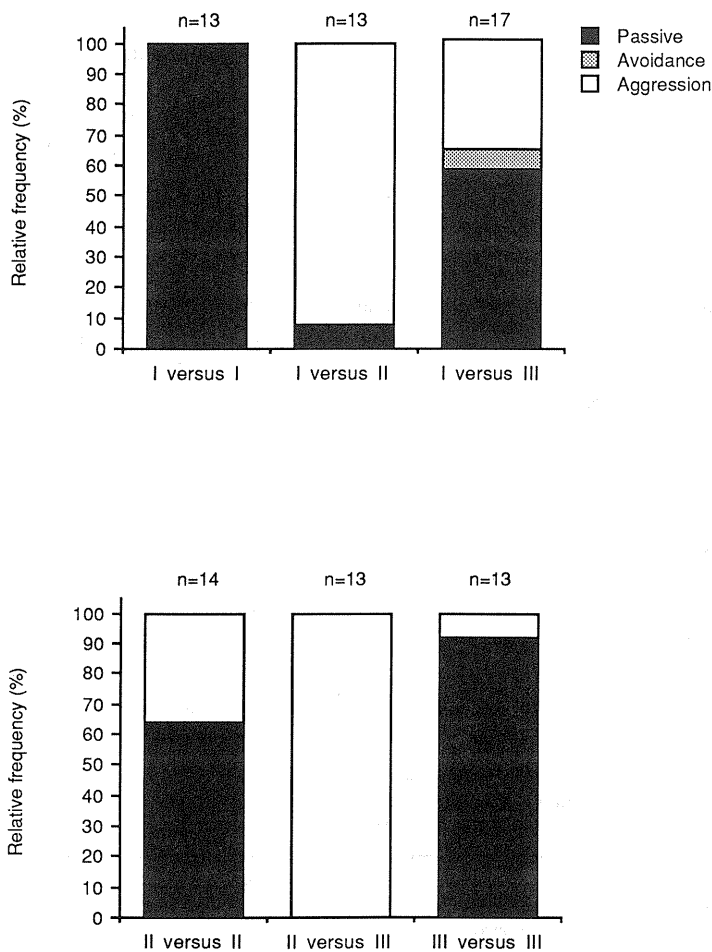


Fig. 4. Agonistic behavior between soldiers of hydrocarbon phenotypes I, II, and III and like or different phenotypes. The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, avoidance, and aggression (Experiment 2).

second individual in the opposite side of the petri dish. There was never any intraphenotype avoidance behavior.

(2) Phenotype II pseudergates reacted negatively to phenotypes I and III pseudergates at least 56% of the time.

(3) Pseudergates from phenotypes I and III were obviously able to discriminate (avoidance or aggregation) the opposite phenotype 34% of the time.

(4) Soldiers in phenotypes I and III seldom fight soldiers from the same phenotype. Only one intraphenotype aggressive response was observed in I and III in 26 trials.

(5) Soldiers in phenotype II fought soldiers from the same phenotype in 5 of 14 trials. We saw no clear geographical or host-wood trends with these five aggressive responses.

(6) Soldiers from phenotype II always fought soldiers from phenotype I or III, with one exception (I versus II).

(7) Soldiers from phenotypes I and III reacted negatively (avoidance or aggression) toward the other phenotype in 41.2% of the encounters.

(8) Soldier versus soldier encounters always resulted in either a passive or an aggressive response, with one exception (I versus III).

(9) All intraphenotype encounters had a significantly greater proportion of passive responses than any paired interphenotype encounters.

Experiment 3

Agonistic behaviors observed in these trials parallel those seen in Experiment 2. The emphasis here is exclusively on phenotype IV. Our observations are summarized in Table V and Fig. 5 and as follows.

(1) Neither pseudergates nor soldiers from phenotype IV react negatively toward individuals of the same caste and phenotype. We observed only one exception in which there was a mild avoidance response by one pseudergate.

Table V. Comparison of the Proportion of Passive Agonistic Encounters Between Pseudergates or Between Soldiers Within Phenotypes (Intra) and Between Phenotypes (Inter) of *Zootermopsis* Phenotypes I, II, III, and IV (Experiment 3)

Intraphenotype			Interphenotype			z statistic ^a
Bouts	Total No.	Proportion passive	Bouts	Total No.	Proportion passive	
Pseudergates						
IV × IV	51	0.980	I × IV	56	0.589	5.70
IV × IV	51	0.980	II × IV	58	0.069	23.62
IV × IV	51	0.980	III × IV	57	0.333	9.89
Soldiers						
IV × IV	5	1.000	I × IV	33	0.364	7.59
IV × IV	5	1.000	II × IV	26	0.000	
IV × IV	5	1.000	III × IV	35	0.086	19.29

^a $z = 1.96$ for $\alpha = 0.05$. For experimentwise $\alpha \leq 0.05$ for each case, $z = 2.13$, using the Bonferroni inequality.

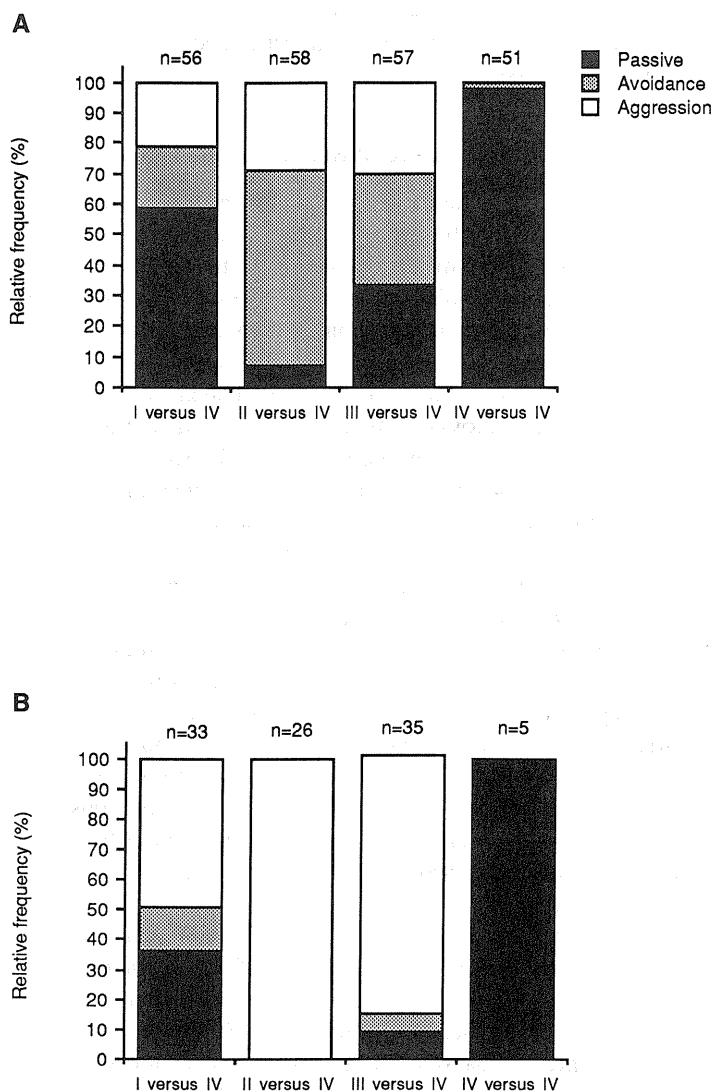


Fig. 5. Agonistic behavior between pseudergates (A) or soldiers (B) of hydrocarbon phenotype IV (*Z. laticeps*) and like castes of hydrocarbon phenotypes I, II, III, and IV. The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, avoidance, and aggression (Experiment 3).

(2) Pseudergates and soldiers from phenotype IV often reacted negatively toward like castes from phenotypes I, II, and III. These negative agonistic responses appear to be most pronounced against phenotype II and at least pronounced against phenotype I.

DISCUSSION

From these sets of experiments some overall trends are apparent. In no case did we see aggression in these bioassays between members of the same colony. Furthermore, individuals from different colonies of the same phenotype or species rarely react negatively (avoidance or aggression) toward one another. The one minor exception to this generalization is encounters involving soldiers of phenotype II. Lack of aggressive behavior between colonies of the same species or phenotype is inconsistent with most of the published literature, which suggests that nonreproductives from different colonies within a termite species usually react aggressively toward one another. Thus far we have seen only one published report which describes lack of aggressive behavior within a species: *Reticulitermes santonensis* (Clément, 1986). Colleagues have, however, noticed a lack of aggressive behavior between colonies of *Reticulitermes flavipes* (Kollar) (R. W. Howard, J. K. Mauldin, and J. F. A. Traniello, personal communication), *R. virginicus* (Banks) (R. W. Howard, personal communication), and *Coptotermes formosanus* Shiraki (N.-Y. Su, personal communication).

With all interphenotype combinations we saw a significant proportion of avoidance and aggressive interactions. In the soldier versus soldier and pseudergate versus pseudergate trials (Experiments 2 and 3), agonistic responses are most pronounced in phenotype II bouts with all other phenotypes. This pattern was not as dramatic in the soldier versus nymph combinations between *Z. angusticollis* and *Z. nevadensis* (phenotype I from the Sierra Nevada), but the *Z. angusticollis* colonies we used in Experiment 1 were from a very limited geographic area (see Fig. 1).

Although we currently have no way of morphologically distinguishing insects within the two hydrocarbon phenotypes of *Z. nevadensis* (Thorne and Haverty, 1989), observations of agonistic behavior between these two phenotypes consistently yield a sizable proportion of avoidance or aggressive responses. We interpret the passive, intraphenotype behavior within each of the two hydrocarbon phenotypes of *Z. nevadensis* and the significant negative and aggressive behavior between phenotypes as clear evidence of phenotype recognition. Discrimination within phenotypes may also be taking place, but it is seldom manifested in visible avoidance or aggressive behavior in the context of these assays.

Passive discrimination and aggressive discrimination are behaviors that can be logically explained by defense of the colony or competition for resources.

The avoidance or fleeing behaviors without any aggression by the other termite are more difficult to explain. We have seen no reference to this in the literature: most investigators concentrate on passive or aggressive behavior. Perhaps fleeing would be followed by an alarm response and would result in aggressive behavior by soldiers, pseudergates, or nymphs in the retreating termites' colony. The value of avoidance or fleeing behaviors might be better demonstrated in group encounters.

The agonistic bioassays discussed in this paper were conducted in the laboratory in artificial arenas. Single individuals were used in confrontations, obviously out of their natural social context or nest habitat. Results among trials may be compared, but we are uncertain of agonistic behavior patterns between colonies encountering one another in the field. Many of the encounters, for example, *Z. laticeps* versus all other *Zootermopsis* species or hydrocarbon phenotypes, are not even possible under natural conditions due to geographic isolation and host differences. The evaluation time used in these experiments was short (2 or 4 min). Results might have changed if termite groups were left together for hours or days as has been done in other aggression bioassays (Clément, 1986).

Several authors have noted a moderated aggressive response of termites from laboratory colonies (Nel, 1968; Clément, 1978). Colonies used in our experiments had been kept in the laboratory for varying periods before the trials (from several weeks up to 21 months), but some of the longest-kept colonies remained highly aggressive. Clément (1986) reported that changing the host wood of *Reticulitermes* colonies did not alter aggression. We did not control for colony diet: insects were reared on their own nest wood until it was consumed, then given either Monterey pine (Pacific Grove colonies), ponderosa pine (all other *Z. nevadensis* and *Z. angusticollis* colonies), or decayed willow (*Z. laticeps*). As part of laboratory colony maintenance, several of our *Zootermopsis* colonies were chilled briefly to allow removal of alates from the nest boxes. Both Dropkin (1946) and Howick and Creffield (1980) noted a mild reduction of aggression in chilled colonies. Our *Zootermopsis* colonies were chilled weeks or months before the experimental trials, yet many of our colonies remained highly aggressive (in the right circumstances) despite chilling. Clément (1986) reported a seasonal variation in aggression in the European *Reticulitermes lucifugus* complex. We did not control for seasonal cycle in *Zootermopsis*, but in Experiments 2 and 3 all trials were conducted within the same week. Bouts in Experiment 1 occurred over a period of 2 months.

Within all *Zootermopsis* hydrocarbon phenotypes we observed considerable variance in agonistic response toward other phenotypes among and within colonies. This may be due to heterogeneity in response of different individuals within colonies and/or a potential population component. In encounters with local colonies we observed that soldier-nymph contacts of phenotype II versus

II within Pacific Grove colonies yielded no fights. Pacific Grove soldiers meeting nymphs from other geographic localities showed a higher incidence of fighting. Limited dispersal may mean that local populations tend to be composed of kin and that closely related termites are less likely to fight.

The interaction between insect hydrocarbon phenotypes and behavioral patterns is only beginning to receive attention (Obin, 1986). Bioassays to implicate hydrocarbons as elicitors of behavior are difficult to conduct. Alteration of the hydrocarbons on an insect's cuticle is likely to be fatal to the altered insect. Evaluating the response of an insect to extracted hydrocarbons of another insect rarely elicits normal behavior patterns (Howard *et al.*, 1982). Results presented here, however, show a clear and consistent correlation between hydrocarbon phenotype and agonistic behavior within the genus *Zootermopsis*, even within the morphological species *Z. nevadensis* which bears two hydrocarbon phenotypes. Intraphenotype avoidance or aggressive responses were very rare within *Zootermopsis*; interphenotypic aggression occurred commonly among all four phenotypes.

The geographic and habitat distribution of the four *Zootermopsis* phenotypes has also been studied (Haverty *et al.*, 1988a), and from the field data collected thus far, phenotypes I and III (*Z. nevadensis*) appear to show allopatric distributions. Phenotype I occurs almost exclusively in pine, in high and dry regions of the Sierra Nevada, the east side of the Coast Range adjacent to the Sacramento Valley, and the San Gabriel, San Bernadino, and San Jacinto Mountains in southern California. In contrast, phenotype III is found in logs or stumps of a wide variety of tree species, in moister habitats of the Coast Range and Cascade Mountains of California and Oregon.

On the basis of the hydrocarbon, behavioral, and geographic data we feel justified in designating two subspecies within the species *Z. nevadensis*. Phenotype I is that found at the type locality of *Z. nevadensis*, is called *Z. nevadensis* subsp. *nevadensis*, and is characterized by the presence of the isomeric mixture of 7,15- and 5,17-dimethylheneicosane in its cuticular hydrocarbons (Haverty *et al.*, 1988b). Phenotype III is named *Z. nevadensis* subsp. *nuttingi* Haverty and Thorne, ssp. nov. after our friend, colleague, and noted North American termite biologist, Professor William L. Nutting. This subspecies is easily separated from *Z. n. nevadensis* by the presence of 2-methyldocosane and *n*-tricosene and the absence of dimethyl alkanes in its cuticular hydrocarbons (Haverty *et al.*, 1988b). Upon further study we may feel justified in elevating *Z. nevadensis* subsp. *nuttingi* to full species status. Presently, no diagnostic morphological characters have been found to segregate the two subspecies, and preliminary isozyme work also shows considerable overlap within the phenotypes I and III complex (A. K. Korman, unpublished observations). Flight times of imagoes (as a potential temporal isolating mechanism) are not precisely known for field colonies of either phenotype I or phenotype III. However,

Weesner (1970) describes the flights of *Z. nevadensis* as occurring from June or July in the Sierra Nevada and from August to September in the coastal mountains. Clarification of these differences in flight times as a reproductive isolating mechanism might help to resolve the species question.

We have successfully interbred phenotypes I and III alates in the laboratory, at least to the point of getting viable eggs, larvae, and small soldiers. However, incipient colony hybrids between phenotypes I and II and phenotypes III and II are also possible in the laboratory. These breeding results are complicated by artificial laboratory conditions and by the uncertainty as to whether winged females removed from their natal colony are truly virgins or if they might be inseminated by a colony mate before we introduce them to a male of another phenotype. We have never seen hybrid hydrocarbon patterns from field collections (Haverty *et al.*, unpublished observations) as has been demonstrated for *Solenopsis* species (Vander Meer *et al.*, 1986). Analysis of the combined cuticular hydrocarbons of the F_1 of these hybrids has shown intermediate profiles, with diagnostic hydrocarbons of each phenotype present. The origin of these hydrocarbons, i.e., self-produced or obtained from contact with their "parents," is not known at this time. The laboratory mating experiments neither clarify nor dismiss the notion that the four hydrocarbon phenotypes may represent four distinct species of *Zootermopsis*. We suspect that where *Zootermopsis* species are sympatric, agonistic behavior between alates in their initial encounter is an isolating mechanism (Weesner, 1970), whereas flight time, geography, and/or habitat reproductively isolate the two subspecies of *Z. nevadensis*.

Our observations of four distinct hydrocarbon phenotypes in *Zootermopsis* are corroborated by the agonistic behavior reported in this study. The aggressive interphenotype and passive intraphenotype behaviors support the notion that each phenotype represents a separate biological grouping. Aggression between colonies of two species in the same tree or log, as occurs in coastal California (Weesner, 1970), is easily explained by competition for limited resources (food and habitat of the host wood). Still perplexing, however, is the remarkably consistent lack of intercolony, intraphenotype aggression which apparently would permit intermingling of soldiers, pseudergates, and nymphs from two or more colonies inhabiting the same log. If local colonies of a species of *Zootermopsis* are closely related, perhaps the benefits of a larger combined work force outweigh the costs of defending individual colony territories. Both colonies would share wood and contribute to production of sibling or closely related alates; neither would suffer the expense of initial or continued aggression.

Indications are, however, that complete colony fusion within a *Zootermopsis* phenotype is unlikely. The studies reported in this paper have involved only the nonreproductive castes. Preliminary observations suggest that there is considerable intraphenotype aggression toward functional reproductives (but not

alates) from different colonies. Functional reproductives, primary or supplementary, male or female, are aggressively attacked and totally consumed within 30 min when placed in an alien colony of the same phenotype. Thus while soldiers, pseudergates, and nymphs from different colonies within the same phenotype may intermingle passively, functional reproductives from one or both colonies may be killed. Studies to investigate fusion of *Zootermopsis* colonies and the associated agonistic interactions and reproductive dynamics are in progress.

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REFERENCES

- Adams, E. S., and Levings, S. C. (1987). Territory size and population limits in mangrove termites. *J. Anim. Ecol.* **56**: 1609-1081.
- Andrews, E. A. (1911). Observations on termites in Jamaica. *J. Anim. Behav.* **1**: 193-228.
- Blomquist, G. J., and Dillwith, J. W. (1985). Cuticular Lipids. In Kerkut, G. A., and Gilbert, L. I. (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Pergamon Press, Oxford, Vol. 3, pp. 117-154.
- Carlson, D. A., and Bolten, A. B. (1984). Identification of Africanized and European honey bees, using extracted hydrocarbons. *Bull. Entomol. Soc. Am.* **30**: 32-35.
- Clément, J. L. (1978). L'agression inter et intraspecific des especes française du genre *Reticulitermes* (Isoptère). *C.R. Acad. Sci. Paris* **286**: 351-354.
- Clément, J. L. (1982). Signaux de contact responsables de l'agression inter spécifique des Termites du genre *Reticulitermes* (Isoptera). *C.R. Acad. Sci. Paris* **294**: 635-638.
- Clément, J. L. (1986). Open and closed societies in *Reticulitermes* termites (Isoptera, Rhinotermitidae): Geographic and seasonal variations. *Sociobiology* **11**: 311-323.

- Dropkin, V. H. (1946). The use of mixed colonies of termites in the study of host-symbiont relations. *J. Parasit.* **32**: 247-251.
- Dudley, P. H., and Beaumont, J. (1889). Observations on the termites, or white-ants of the Isthmus of Panama. *Trans. N.Y. Acad. Sci.* **8**: 85-114.
- Emerson, A. E. (1933). A revision of the genera of fossil and recent Termopsinae (Isoptera). *Univ. Calif. Publ. Entomol.* **6**: 165-196.
- Fleiss, J. L. (1981). *Statistical Methods for Rates and Proportions*, 2nd ed., John Wiley and Sons, New York.
- Grassi, B., and Sandias, A. (1896-1897). The constitution and development of the society of termites: Observations on their habits: With appendices on the parasitic protozoa of Termitidae, and on the Embiidae. *Q. J. Microsc. Sci.* **39**: 245-322; **40**: 1-82.
- Haverty, M. I., Page, M., and Nelson, L. J. (1988a). Geographic distribution of hydrocarbon phenotypes of the North American dampwood termites., *Zootermopsis*. *Environ. Entomol.* (in preparation).
- Haverty, M. I., Page, M., Nelson, L. J., and Blomquist, G. J. (1988b). Cuticular hydrocarbons of the dampwood termites, *Zootermopsis*: Intra- and intercolony variation and potential as taxonomic characters. *J. Chem. Ecol.* **14**: 1035-1057.
- Howard, R. W., and Blomquist, G. J. (1982). Chemical ecology and biochemistry of insect hydrocarbons. *Annu. Rev. Entomol.* **27**: 149-172.
- Howard, R. W., McDaniel, C. A., Nelson, R. N., Blomquist, G. J., Gelbaum, L. T., and Zalkow, L. H. (1982). Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species- and caste-recognition cues. *J. Chem. Ecol.* **8**: 1227-1239.
- Howick, C. D., and Creffield, J. W. (1980). Intraspecific antagonism in *Coptotermes acinaciformis* (Froggatt) (Isoptera: Rhinotermitidae). *Bull. Entomol. Res.* **70**: 17-23.
- Jones, S. C. (1987). *Foraging Party and Territory Size of the Desert Subterranean Termite Heterotermes aureus (Snyder) in a Sonoran Desert Grassland*, Ph.D. thesis, University of Arizona, Tucson.
- Levings, S. C., and Adams, E. S. (1984). Intra- and interspecific territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian mangrove forest. *J. Anim. Ecol.* **53**: 705-714.
- Miller, E. M. (1969). Caste differentiation in the lower termites. In Krishna, K., and Weesner, F. M. (eds.), *Biology of Termites*, Academic Press, New York and London, Vol. I, pp. 283-310.
- Nel, J. J. C. (1968). Aggressive behaviour of the harvester termites *Hodotermes mossambicus* (Hagen) and *Trinervitermes trinervoides* (Sjöstedt). *Insectes Soc.* **15**: 145-156.
- Obin, M. S. (1986). Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Effect of environment and role of cuticular hydrocarbons. *J. Chem. Ecol.* **12**: 1965-1975.
- Pickens, A. L. (1934). The biology and economic significance of the western subterranean termite, *Reticulitermes hesperus*. In Kofoid, C. A. (ed.), *Termites and Termite Control*, University of California Press, Berkeley, pp. 157-183.
- Springhetti, A., and Amorelli, M. (1982). Competitive behavior between two species of Isoptera: *Kaloterms flavicollis* and *Reticulitermes lucifugus*. *Sociobiology* **7**: 155-164.
- Sumner, E. C. (1933). The species of the termite genus *Zootermopsis* Emerson (= *Termopsis* Hagen). *Univ. Calif. Publ. Entomol.* **6**: 197-230.
- Thorne, B. L. (1982). Termite-termite interactions: Workers as an agonistic caste. *Psyche* **89**: 133-150.
- Thorne, B. L., and Haverty, M. I. (1989). Accurate identification of *Zootermopsis* species (Isoptera: Termopsidae) based on a mandiblar character of non-soldier castes. *Ann. Entomol. Soc. Am.* **82**: 262-266.
- Traniello, J. F. A. and Beshers, S. (1985). Species-specific alarm/recruitment responses in a Neotropical termite. *Naturwissenschaften* **72**: 491-492.
- Vander Meer, R. K., Lofgren, C. S., and Alvarez, F. M. (1986). Hybridization in fire ants: Biochemical and behavioral evidence. *Fla. Entomol.* **68**: 501-506.
- Weesner, F. M. (1970). Termites of the Nearctic Region. In Krishna, K., and Weesner, F. M. (eds.), *Biology of Termites*, Academic Press, New York and London, Vol. II, pp. 477-525.

