Factors that Affect Aggression Among the Worker Caste of Reticulitermes spp. Subterranean Termites (Isoperta: Rhinotermitidae)

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Aggression was observed among both inter- and intraspecific combinations of four colonies of Reticulitermes flavipes (Kollar) and Reticulitermes hagenni Banks in laboratory assays each month for 4 consecutive months. Termites were observed to be more frequently aggressive toward colonies of a different species. Number of individuals that displayed aggression decreased over the study period, from April to July. There was a slight trend toward reduced aggressive behavior as termites were maintained in the laboratory for 3 months. Passive and aggressive individuals were identified and reexamined for display of aggressive or passive behavior toward nonestmates. Eighty-nine percent of previously aggressive termites displayed aggression a second time. Eighty-eight percent of previously passive termites were passive upon reexamination. Differences in head capsule size between passive and aggressive individuals provided no correlation between the presence of aggressiveness and the head capsule size in the worker caste.

KEY WORDS: Isoperta; Rhinotermitidae; Reticulitermes; polyethism; aggression.

INTRODUCTION

Agonism is defined as offensive and defensive responses between competing individuals (Klug, 1973). The presence of aggression and levels of agonistic behaviors can vary among termite species, colonies, and individuals (Thorne, 1982). Previous research with termite agonism indicates that not all pairings from particular colony combinations result in evidence of aggressive encounters.

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individuals also were tested for a correlation between the presence of aggression and the head capsule size. Size differences may indicate particular instars using the findings of Dyar (1890) and therefore be related to age of the worker. Significant size differences among passive and aggressive individuals could indicate age polyethism in the termite worker caste.

MATERIALS AND METHODS

Termite Collections

Termites were collected from field sites using the techniques of Forschler and Townsend (1996). Termites were removed from four termite monitors, representing four colonies, from Barnesville and Aldora in Lamar County, Georgia, on a monthly basis from March until June. The following field colonies collected from Barnesville were designated I, II, and III. Colony designate IV was collected from Aldora. Colonies I and II colonies were Reticulitermes flavipes (Kollar) colonies; II and IV were Reticulitermes hagani Banks colonies. Species determination was made using keys to the soldier and alate castes (Weesner, 1964; Scheffrahn and Su, 1994). We chose colonies of these two species because size difference in workers made identification of individuals possible when they were combined in bioassay. In addition, these colonies were used because they provided inter- and intraspecific colony combinations for testing. Termite colonies collected from each of the aforementioned monitors were maintained in separate 9-cm petri plates lined with moistened paper filter that were kept in an environment chamber at 24°C in total darkness. Individuals were tested within 2 weeks after being collected from field monitors for all tests except for the laboratory conditioning experiment.

Aggression Level Among Individual Workers Based on Monthly Collections

Assay arenas consisted of 6-cm petri plates, lined with filter paper moistened with 0.3 M distilled water. A pairing consisted of individuals from two colonies placed in the same arena simultaneously. A replication included three termites from each colony, for a total of six termites in a petri plate. Termites were collected prior to inclusion in bioassay by grasping the filter paper to which they were attached and removing them by striking the grasped hand with the other, free hand. Termites would then fall about 10 cm into clean, separate plastic weigh boats. The weigh boats, which contained termites from different colonies, were then tipped over test arenas so that individuals could fall into the arena from a height of 5 cm. This was performed to avoid handling with forceps or aspirators that could cause injury. Three termites from each colony were added to test arenas simultaneously. Only those workers that appeared uninjured, with no
missing or injured appendages, were used in bioassay. Each petri plate (replicate) with six termites was observed for 1 h following introduction of termites. Individuals that displayed aggressive behavior such as opening mandibles quickly with the intent to bite (mandible flaring), or bitting, were immediately removed with forceps and placed in a separate 3.5-mm arena labeled "aggressive" along with replicate number and colony name. New individuals were added to arenas when aggressors were removed so that three individuals from each colony were present at all times. The clock was reset for 1 h when new individuals were added. If individuals received injuries due to an aggressive encounter, they were removed and discarded, and new individuals added. Termites that did not fight within the 1-h observation time were maintained in the original arena for 24 h. Individuals in arenas where termites were unharmed after 24 h were considered to be passive and were placed into a separate petri plate according to colony and replicate that were labeled "passive." After 24 h, any test arenas with injured individuals were presumed to have aggressors present. Individuals from these arenas were removed from the test because it was impossible to determine the aggressor(s). The aforementioned procedures were repeated for all six colony combinations for each monthly collection, for 4 consecutive months, and replicated 2 to 10 times, depending on the number of termites collected each month.

Four or five pairings among nestmates were tested to act as controls during each monthly collection. The number of termites tested varied according to the number that showed aggression and the number collected each month. ANOVA and LSD mean separation techniques were performed on the percentage aggressive individuals to determine which colonies generated the most frequent displays of aggression and to compare the overall aggression of a colony from month to month (SAS Institute, 1990).

**Aggression Among Laboratory-Conditioned Termite Workers**

Untreated termites collected from colonies I, III, and IV, over the 4-month period, were kept in separate 9-cm petri plates lined with moistened filter paper according to collection date. Plates were maintained in an environmental chamber, in complete darkness, at 20 °C for 1 to 4 months. Distilled water and moistened filter paper were added when necessary throughout the maintenance period to provide for adequate food and moisture levels.

The steps to test for aggression, described in the previous section, were repeated for this experiment. Data taken from the previous section for these same colony combinations were compared to data from the laboratory conditioned termites taken at monthly intervals. Five arenas, consisting of six nestmates for each colony, were examined, per monthly collection, to provide controls. ANOVA and LSD mean separations of the number of aggressive individuals from each colony were performed for each laboratory conditioning time to compare the percentage of aggressive individuals among colony combinations that were newly collected to those conditioned through 4 months (SAS Institute, 1990).

**Recurring Behavior in Workers**

Those worker termites previously identified as passive and aggressive were used to test for recurrence of aggression among aggressive or passivity among passive individuals. These bioassays were performed at least 24 h, but no later than 48 h, after an individual's first interaction with a nonnestmate. An individual that was combined with termites from another colony (colony B) in the previous section was always retested with a member of colony B but never tested with the same individuals from the previous test. A replicate consisted of two termites, one from each colony, placed into arenas, using the procedure described previously, and observed for 0.5 h.

Arenas consisted of 17 × 14 × 2-mm (L × W × H) acetate rectangles taped to a 2.5 × 7.6-cm glass microscope slide. Tape was covered with 17 × 14-mm pieces of No. 1 Whatman filter paper, moistened with 0.05 ml of distilled water, and each arena was covered with a glass microscope slide cover slip. The following interactions were tested: both individuals previously aggressive; both individuals previously passive; one individual previously aggressive from colony A and one previously passive from colony B; or vice versa. The term "previously" is used to define the behavior of individuals tested as described in the preceding section. "Aggression Level Among Individual Workers Based on Monthly Collections." Five to ten replications of each interaction and colony combination were observed. A total of 161 previously aggressive termites and 259 previously passive termites was examined. Five pairings among two nestmates of each colony were tested during each collection time for controls. Following the test for recurring behavior, all individuals were preserved in 70% alcohol for later head capsule measurements.

An aggressive individual was defined as any individual that displayed mandible flaring and/or biting. Aggressive individuals were divided into two categories: instigators, or the first individuals to display aggression, and those that displayed aggression in response to being attacked. Passive individuals are defined as those that do not display mandible flaring and bitting behaviors. Numbers of aggressive and passive individuals per colony combination and instigators were recorded.

**Head Capsule Measurements**

Termites preserved in alcohol following the recurring behavior assays were used to take head capsule measurements. Each termite head was excised and placed under a dissecting microscope at a magnification of 38×. Mocha image analysis software was used to take consistent measurements (Jandel
RESULTS

Aggression Level Among Individual Workers Based on Monthly Collections

There was never an indication of aggressive behavior within the nestmate comparisons (controls) from any of the bioassays we conducted. Individuals most frequently displayed aggression, mandible flaring or biting, toward individuals from other species (Table I). Colony III was most frequently aggressive toward interspecific colonies, I and II. However, aggression toward an intraspecific colony, IV, was seen in 11% of the individuals tested. Forty-one and 37% of colony I termites fought with interspecific colonies, III and IV, respectively. None of the colony I termites observed displayed aggression toward colony II, an intraspecific colony. Colony II never displayed aggression toward colony I.

Table I. Comparison of the Mean Percentage of Aggressive Reticulitermes Termites Workers (±SE) by Source Colony from Bioassays Conducted Within 2 Weeks of Collection from the Field by Inter- and Intraspecific Competition, Listed by Colony

<table>
<thead>
<tr>
<th>Source colony (Species)*</th>
<th>Mean percentage aggressive ± SEP (±)*</th>
<th>I (Rf)</th>
<th>II (Rf)</th>
<th>III (Rh)</th>
<th>IV (Rf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>When paired with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (Rf)</td>
<td>0.0 ± 0 B</td>
<td>0.0 ± 0 B</td>
<td>33.5 ± 6.6 BC</td>
<td>37.0 ± 8.0 AB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[18]</td>
<td>[14]</td>
<td>[19]</td>
<td>[29]</td>
<td>[27]</td>
</tr>
<tr>
<td>II (Rf)</td>
<td>0.0 ± 0 B</td>
<td>0.0 ± 0 B</td>
<td>100.0 ± 0 A</td>
<td>51.8 ± 9.8 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[14]</td>
<td>[10]</td>
<td>[19]</td>
<td>[20]</td>
<td>[18]</td>
</tr>
<tr>
<td>III (Rh)</td>
<td>37.3 ± 7.4 A</td>
<td>100.0 ± 0 A</td>
<td>0.0 ± 0 D</td>
<td>14.0 ± 4.4 BC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[20]</td>
<td>[16]</td>
<td>[10]</td>
<td>[29]</td>
<td>[20]</td>
</tr>
<tr>
<td>IV (Rh)</td>
<td>41.4 ± 7.5 A</td>
<td>25.0 ± 8.7 B</td>
<td>11.0 ± 4.0 CD</td>
<td>0.0 ± 0.6 BC</td>
<td></td>
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<tr>
<td></td>
<td>[27]</td>
<td>[19]</td>
<td>[30]</td>
<td>[18]</td>
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</table>

*Ref. Reticulitermes flavipes Rf, Reticulitermes hagani.
*Means in the same column followed by the same letter are not significantly different (P = 0.05, LSD).
*Number of replicates.

Aggression to Worker Subterranean Termites

Table II. Comparison of the Mean Percentage of Reticulitermes Worker Termites Displaying Aggression When Combined with Nonnestmates by the Month Termites Were Collected from the Field and Placed in Bioassay Listed by Month and Colony

<table>
<thead>
<tr>
<th>Month collected</th>
<th>Mean percentage aggressive ± SEP (±)*</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>96.3 ± 3.7 A</td>
<td>(9)</td>
<td>(3)</td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td>May</td>
<td>51.6 ± 16.5 B</td>
<td>(20)</td>
<td>(12)</td>
<td>(10)</td>
<td>(20)</td>
</tr>
<tr>
<td>June</td>
<td>13.0 ± 3.8 C</td>
<td>(21)</td>
<td>(12)</td>
<td>(14)</td>
<td>(27)</td>
</tr>
<tr>
<td>July</td>
<td>10.7 ± 2.4 C</td>
<td>(20)</td>
<td>(12)</td>
<td>(14)</td>
<td>(27)</td>
</tr>
</tbody>
</table>

*Means in the same column followed by the same letter are not significantly different (P = 0.05, LSD).
*Number of replicates.
*No terms collected.

and always showed aggression toward colony III, but only 25% of their workers showed aggression toward colony IV. Workers from colony IV showed aggression to all colonies, most frequently colony II, an interspecific colony (Table I).

The level of aggression to nonnestmates, for all colonies, decreased significantly from May through June (Table II). Over the 4-month period the percentage of aggressive individuals from colony III fell from 9.11 to 10.7%; for colony I, from 96.3 to 10.7%; for colony II, from 52.2 to 8.6%; and for colony IV workers, from 100 to 17.2%. It is possible that the lower number of replicates tested in April for each group may have influenced the percentage of aggressive individuals. However, because the data show that greater than 90% of the individuals tested were aggressive in April, approximately 50% in May and less than 20% in June and July, across all groups tested, we believe that these tests indicate a real trend toward fewer aggressive individuals, not influenced by the number of replicates (Table II).

Aggression Among Laboratory Conditioned Worker Termites

Although termites were maintained under laboratory conditions for 4 months for these tests, the number of workers available for testing at 2 and 4 months allowed for testing only two of the possible six combinations. Therefore, only those months that provided all comparisons are listed in Table III. The results indicate a trend toward reduced percentage of aggressive individuals as the time in the laboratory increased (Table III). The percentage of aggressive individuals in the colony I and colony III combinations provided statisti-
Table III. Comparison of Various Termite Colony Combinations Showing the Mean Percentage of Aggressive Individuals from the Number of Termites Tested by Colony Combination and Length of Time that Termites Were Maintained in the Laboratory Prior to Testing

<table>
<thead>
<tr>
<th>Colony III vs Colony I</th>
<th>Colony IV vs Colony III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>Mean percentage aggressive (%)</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>0</td>
<td>33.5 ± 6.8 A</td>
</tr>
<tr>
<td>1</td>
<td>53.0 ± 3.2 A</td>
</tr>
<tr>
<td>3</td>
<td>75.8 ± 3.8 BC</td>
</tr>
</tbody>
</table>

*Means in the same column followed by the same letter are not significantly different (P = 0.05, LSD).

Data from these tests were combined across colony source and colony behavior type because we were interested in examining the initial aggressive or passive behaviors exhibited by individual termites when the two colonies would display aggressive behavior. The data from colony III were not included in the analysis because one of the two colonies was not observed. Because of the limited sample size, colony III was not included in the analysis.

Recurring Behavior in Workers

By the third week, the behavior of individuals in colony IV was significantly different from colony III. However, all combinations showed a trend toward increased aggressive behavior in colony IV, especially in colony III. All data indicate that maintaining colonies of aggressive behavior in the laboratory for up to 3 months may affect the percentage of aggressive individuals in the colony. In general, there was a significant increase in the percentage of aggressive individuals in colony IV compared to colony III.
displayed aggression during the recurrence bioassays only as a result of being attacked. No previously passive termite ever displayed aggression when paired with a previously passive nontester.

**Head Capsule Measurements**

There was considerable overlap among passive and aggressive individuals for each head capsule measurement taken for each colony as summarized in Table IV. Significant differences among passive and aggressive individuals were seldom seen (Table IV). Significant differences were predominantly seen in head length (Table IV). However, we believe that these significant statistical differences are of very little biological significance because overlap of head length measurements existed within every measurement category, making predictions based on Dyer's rule tenuous.

**DISCUSSION**

Displays of agonistic behavior are inexorably tied to questions of kin recognition. It is assumed that displays of aggressive behaviors between worker termites is predicated on discrimination of kin and nontoolkin. Our tests did not attempt to distinguish between more subtle behavioral indications of kin recognition but concentrated only on an overt display of nonkin recognition—aggression. This does not preclude that the worker termites we examined and labeled as nonaggressive did not recognize and distinguish between nestmates and nontestes. Our tests simply recorded those termites as nonaggressive. It is entirely possible that scoring bioassays of termite interactions by examining only indications of overt agonistic behaviors overlook more subtle behavioral displays of kin recognition. Therefore, we confine our comments on kin recognition between members of the worker caste in *Reticulitermes* as evidenced by displays of aggression.

Results from these trials were similar to other studies with Rhototermid termites in that aggression is seen predominantly in interspecific pairings and occasionally in intraspecific pairings (Polizzi and Forschler, 1997; Shelton and Grace, 1996; Clement, 1986). In our tests, the percentage of aggressive individuals steadily declined from month to month but aggressive individuals were present throughout the trials. In June and July we found a significantly lower percentage of aggressive individuals in all colonies compared to those same groups during the March bioassay. Although our sample of months was less than a full calendar year, it does agree with work conducted with several European Reticulitermes (Clement, 1986). Clement (1986) suggests that this seasonal aggressive behavior is influenced by genetic relatedness, caste proportions, and availability of food resources.

Other studies observed a decrease in aggression when colonies were main-
tained in the laboratory for from 9 months to 10 years (Net, 1968; Shelton and Grace, 1997). Although our time frame was considerably shorter, our results showed a possible trend toward decreased aggression as termites were maintained in the laboratory for 3 months. Shelton and Grace (1997) demonstrated a lack of agonism among Coptotermes formosanus Shiraki colonies that were placed in similar laboratory environments, although they were genetically different and their parental colonies were aggressive. They suggested that exogenous chemical cues from environmental conditioning could be acquired and be an important component in expressing aggressive behavior toward nonnestmates.

Their data also suggest that aggressive behaviors could be exacerbated by exogenous cues related to diet or undefined differences in available food resources between field and laboratory-maintained termites.

Our bioassay, retesting individuals for aggressiveness, demonstrated for the first time that aggression is expressed by certain Reticularitermes workers and is not a generic, group-related phenomenon. It was observed that not all termite workers fight and those that do are most likely to fight again. Very rarely (3 of 295 individuals, or 1%) did we see previously passive termites instigate battles and never did we see two previously passive nonnestmates display aggression toward one another. Previously passive termites most commonly displayed aggression only after being attacked. These studies demonstrate that all workers are capable of displaying aggression but some termites are more aggressive than others. These data also may indicate a division of labor within the worker caste concerning aggressive behavior.

We tested whether agonism could be an example of temporal polyethism assuming that head capsule size was related to termite worker age. However, termite worker head capsule measurements may not indicate age in Reticularitermes. We found no significant differences among head capsule cases of passive and aggressive individuals (Table IV). These results are similar to those of another study that attempted to distinguish scouts and harvesters of Reticularitermes flavipes, where overlapping size ranges were also observed (McMahan, 1979). Miller (1969) states that although Reticularitermes workers may molt regularly, after the ninth molt, changes in form are almost undetectable. It is possible that the majority of the individuals we tested had undergone the ninth molt, making head capsule measurements useless in determining age differences. Perhaps aggression in termite workers is governed by the age of termites. Rosengaus and Trianello (1993) explain that temporal polyethism is usually observed among higher termite species, which typically have large colony populations and high oviposition rates and usually collect food outside the nest. Lower termites, such as Reticularitermes, lack these traits that could encourage temporal polyethism (Rosengaus and Trianello, 1993).

It has been demonstrated, through these and other trials, that there is a variety of factors that can affect expression of aggression in worker termites: the colony, season, and testing conditions (Thorncr and Haverty, 1991). Su and Haverty (1991) proposed that recognition cues may be based on combinations of physical and chemical components such as cuticular components, exudates, volatiles, and behavioral and tactile differences. Shelton and Grace (1997) further confirm the possibility of the multiple stimulus hypothesis by demonstrating that more than one cue was probably required in the km recognition system and that, if all cues are not present, aggression may not occur. Studies have already examined cuticular hydrocarbons among several genera and equivocal data have always been observed where cuticular hydrocarbon phenotypes have and have not been correlated with aggression (Su and Haverty, 1991; Haverty and Thorne, 1989; Takamashi and Gassa, 1995).

However, exogenous cues alone do not explain our results that some individuals are predominately aggressive while others are not. Although early springtime seasonality may have influenced aggression in our tests, it was sometimes overridden when a termite was threatened, as indicated by the passive termites fighting in defense. This suggests that behavioral cues also may be significant. Clement (1981, 1982) has demonstrated the importance of greeting behaviors and variability found among different species of Reticularitermes preceding aggression. Greeting behaviors, as well as other behavioral elements, should be studied further to gain a better understanding of the association of the aforementioned cues on expression of passive and aggressive behaviors in individual worker termites. However, it also is possible that displays of aggression could be related to endogenous cues, possibly hormone levels. This could explain those observations of aggressive behavior associated with shifts in caste proportions and removal of individuals from the field into a laboratory situation. Clement (1997) states that to understand kin recognition in termites completely, one must have information concerning the genetics, ethology, and chemistry of termite societies. To this list we would add physiological influences on the expression of aggressive behaviors.

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