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DIET-MEDIATED INTER-COLONIAL AGGRESSION IN THE FORMOSAN SUBTERRANEAN TERMITE *Coptotermes formosanus*

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Abstract—In most social insects, intercolonial and interspecific aggression are expressions of territoriality. In termites, cuticular hydrocarbons (CHCs) have been extensively studied for their role in nestmate recognition and aggressive discrimination of nonnest-mates. More recently, molecular genetic techniques have made it possible to determine relatedness between colonies and to investigate the influence of genetics on aggression. In the Formosan subterranean termite, *Coptotermes formosanus*, however, the role of CHCs and genetic relatedness in inter-colony aggression has been ambiguous, suggesting the involvement of additional factors in nest-mate recognition. In this study we assess the range of aggression in this termite species and characterize the influence of genetic relatedness, CHC profiles and diet on aggression levels. We collected four colonies of *C. formosanus,* feeding either on bald cypress or birch, from three locations in Louisiana. Inter-colony aggression ranged from low to high. Differences in CHC profiles, as well as genetic distances between colonies determined by using microsatellite DNA markers, showed no significant correlation with aggression. However, termite diet (host tree) played a significant role in determining the level of aggression. Thus, two distantly related colonies, each feeding on different diets, showed high aggression that significantly diminished if they were fed on the same wood in the laboratory (spruce). Using headspace solid phase microextraction, we found three compounds from workers fed on birch that were absent in workers fed on spruce. Such diet-derived chemicals may be involved in the complex determination of nest-mate recognition in *C. formosanus*.

Key Words—Cuticular hydrocarbons, microsatellite DNA markers, agonism, termite.

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INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), was introduced into the continental United States in wooden cargo crates and pallets shipped from the Asian theater following World War II (La Fage, 1985). Since it was initially discovered, *C. formosanus* has spread to several southern states and causes over $1 billion per year in damage and control costs (Su and Scheffrahn, 1990). Investigations of this pest have primarily focused on methods of control such as chemical and baiting technologies. Although some studies have focused on biological issues, including aggression and colony interactions, results have been ambiguous.

Many social insects show territoriality in defending their resources from other insects (Wilson, 1971). Among termite species, agonistic behaviors include fighting, fleeing, or submission, and such behaviors occur between individuals from different colonies of the same or different species (Haverty and Thorne, 1989; Thorne and Haverty, 1991). Factors responsible for agonistic behavior in termites are not fully understood, especially in *C. formosanus*. Several factors that could be involved include cuticular hydrocarbons (CHCs), glandular secretions, or pheromones, as well as environmental factors (Su and Haverty, 1991).

It has generally been held that CHCs of insects play a role in interspecific and intraspecific recognition (Blomquist et al., 1987). There is a growing body of evidence suggesting that termites have species-specific mixtures of CHCs (Page et al., 2002). Also, a correlation between aggressive behavior and hydrocarbon phenotypes was reported among *Zootermopsis* spp. (Haverty and Thorne, 1989), and CHC phenotypes and their relationship to agonism have been extensively studied among species of *Reticulitermes* (Haverty et al., 1999; Delphia et al., 2003). Additionally, genetic components have been implicated in inter-colonial recognition, as is the case in *Microcerotermes arboreus* (Adams, 1991) and *Schedorhinotermes lamanianus* (Husseneder et al., 1998).

Like other termite species, *C. formosanus* also exhibits inter-colony agonism, but the behavior is not always consistent. In a study of *C. formosanus* from cypress trees in Lake Charles, LA, no agonism was observed between different colonies (Delaplane, 1991). The author suggested that high genetic relatedness may have caused the lack of agonism. Aggression between colonies of Hawaiian *C. formosanus* was apparently not correlated with inter-colonial relatedness, i.e., CHC data did not correlate with agonistic behavior (Su and Haverty, 1991). CHCs have been characterized from numerous colonies of *C. formosanus*, and statistically significant quantitative differences have been found between workers and soldiers, between colonies, and for different sampling months (Haverty et al., 1996). A series of alternative mechanisms were listed for nest-mate recognition in *C. formosanus* (Su and Haverty, 1991), collectively called the "multiple stimulus hypothesis," which essentially states that recognition may depend on a complex

set of behaviors and stimuli that act together in determining nest-mate recognition (Thorne and Haverty, 1991).

In the present study, after identifying colonies of *C. formosanus* that exhibited either strong agonism or no agonism, we investigated factors that might be responsible for the induction of the observable agonistic behavior. Such factors included cuticular hydrocarbon profiles, genetic relatedness of colonies, and diet.

METHODS AND MATERIALS

Termites. Four colonies of *C. formosanus* were collected to determine the range of inter-colonial aggression. Two of these, located approximately 430 meters apart, were collected from bald cypress, *Taxodium distichum*, trees in City Park located in New Orleans, LA, and designated CP-2 and CP-3. The third colony was collected from a river birch, *Betula nigra*, tree on the University of New Orleans campus and named UNO. A fourth colony was collected from a bald cypress tree in Sam Houston State Park in Lake Charles, LA (approximately 320 km west of New Orleans) and named LC. With the exception of the LC colony (which was maintained on blocks of pine, *Pinus* sp., wood in the laboratory for approximately, 6 mo before use), and those termites subsequently used in determining the effect of diet (maintained on either spruce, *Picea* sp., or birch, *Betula* sp., wood for three months), termites were collected directly from their host trees immediately prior to use in aggression assays, chemical analysis, or genetic tests.

Aggression Assays. For short-term (5 min) assays, a Whatman No. 4 filter paper (90 mm diam) placed in a 100 × 15 mm Petri dish (Becton Dickinson, Franklin Lakes, NJ) was sprayed with a fluorescent solution [1.5 g Blue Luminous Powder (BioQuip, Gardena, CA) suspended in 500 ml distilled water] and dried in an oven at 60°C. The filter paper was moistened with 1 ml distilled water and ten workers of at least third instar and one soldier from a test colony were allowed to walk in the dish for 1 min to ensure sufficient acquisition of the powder on their appendages and abdomen. The marked termites were then released into a 50 × 9 mm Petri dish (Becton Dickinson) with a 42.5 mm diam Whatman No. 4 filter paper moistened with 200 μl distilled water. Ten unmarked workers and one soldier from either a different colony or the same colony (control) were then released into the same Petri dish.

The dish was illuminated using a 45 cm fluorescent blacklight and placed under a color CCD camera (Emcal Scientific, Poway, CA) to distinguish between marked and unmarked termites. Behavior was recorded for 5 min. using a Panasonic AG-6740 time-lapse video recorder. All possible colony pairings and controls were replicated ten times. The colonies were alternately marked and unmarked resulting in five marked and unmarked trials for each colony. In a second experiment, to determine the effect of marking on aggression, 10 replicates

consisting of ten unmarked workers and one soldier paired with 10 marked workers and one soldier from the same colony served as control pairings.

The number of aggressive behaviors were observed and recorded for each pairing. Aggressive encounters included only those in which attempts at inflicting damage upon another individual were noted. That is, mandibles were open and a lunging action was made toward another individual of the opposite colony, or workers chased individuals from a different colony in an attempt to inflict bites. Behavior was not considered aggressive if mandibles were simply open without aggressive maneuvers. The mean and standard error of the number of aggressive behaviors occurring within 5 min were calculated for each set of pairings. For 24 hr assays, (long-term aggression assays), colony pairings used in short-term tests were removed from beneath the CCD camera and placed in an incubator maintained at 28 ± 1°C, 80% RH and constant darkness. After 24 hr, the total number of dead individuals was recorded and converted to percentage mortality. Average and standard error for the percentage mortality were calculated for each colony pairing.

Genetic Analyses. Workers from each test colony were preserved in 95% ethanol and shipped to the University of Hawaii, Honolulu, HI for DNA extraction. Individual DNA was extracted and purified from up to 20 workers per colony using the DNeasy Kit (Qiagen, Inc., Chatsworth, CA). Purified DNA was then shipped to Dr Edward Vargo, North Carolina State University, for genetic analyses using microsatellite DNA markers (Vargo and Henderson, 2000; Husseneder et al., 2003; Vargo, 2003). To describe the social organization and differentiation of the colonies, eight microsatellite loci were scored using a subset of markers developed by Vargo and Henderson (2000): Cf 10-4, Cf 10-5, Cf 12-4, Cf 8-4, Cf 4:1A2-4, Cf 4-10, Cf 4-9A, Cf 4-4. A group of individuals (16.8 ± 4.3, mean ∀ SE) were genotyped per locus and colony according to published protocols (Vargo and Henderson, 2000).

Cuticular Hydrocarbon Profiling. Through preliminary GC-MS analysis, 0.232 g (approximately 50 CP-3 workers) was determined to be an ideal weight for extraction. Consequently, 0.232 g of workers (37–72 workers depending on colony) from each colony were placed in 5 ml glass vials capped with Teflon inserts. Workers were extracted in 2.0 ml of hexane (J. T. Baker, Phillipsburg, NJ) for 10 min, and the extract was filtered through a 0.45 μm PTFE filter using a 1.0 ml glass syringe. The eluate was collected in a 2 ml vial, evaporated under nitrogen to 100 μl, and transferred to a glass insert contained in an autosampler vial. Analyses for hydrocarbons were carried out as reported by Bland et al. (2001). Briefly, 2 μl of each extract were analyzed by GC-MS using an Hewlett-Packard 6890 Series GC System with a 5973 Mass Selective detector and connected to an Hewlett-Packard Kayak XA computer interface. The inlet temperature was set at 250°C, and samples were injected in splitless mode. Chromatograms were run with a constant flow of 1 ml/min of ultrapure helium gas. A DB-5 column

(30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA) was used with temperature programming from 60°C with a 1 min hold, to 300°C at 10°C/min with a final 10 min hold. Previously determined hydrocarbon profiles (Bland et al., 2001) were used to identify CHCs in all chromatograms.

Effect of Diet on Aggression. UNO and CP-3 termites were collected from their respective colonies. Workers and soldiers from UNO were placed on birch (UNO-birch) or spruce (UNO-spruce) wood blocks, and workers and soldiers from CP-3 were placed on spruce (CP-3-spruce) wood blocks for three months. Three sets of assays were performed. In one group, UNO-birch was compared with UNO-spruce. The second group included UNO-birch vs. CP-3-spruce, and the third group included UNO-spruce vs. CP-3-spruce. For each assay, ten workers and one soldier, marked or unmarked, from each colony were tested for mortality over a period of 24 hr as described above. Each pairing was replicated five times, and mortality was recorded after 24 hr.

Solid Phase Microextraction (SPME) Analysis. Because previous hexane extractions showed no significant qualitative differences in CHC composition among colonies, a different method of chemical analysis, solid phase microextraction, was performed (Bland et al., 2001). Workers (0.037 g) from each sample of UNO-birch, UNO-spruce, and CP-3 spruce were placed into separate vials with a septum. Five types of SPME fibers [100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB), 70 μm Carbowax, 50/30 DVB/Carboxen/ PDMS, 75 μm Carboxen-PDMS] used in this experiment were obtained from Supelco (St Louis, MO). Each SPME fiber was inserted into the vial at a point 1 cm above the termites. The vial, with the SPME fiber, was placed in a sand filled heating block maintained at 120°C for 60 min. SPME samples were analyzed by GC-MS as described above. Mass spectra were recorded from 40 to 750 m/z. Compounds of interest were identified by mass spectral matches with Wiley database entries and retention time and by mass spectral comparison with authentic samples obtained from Sigma–Aldrich (St Louis, MO).

Statistical Analyses. General descriptive statistics of the colony genetic structure, such as numbers of alleles per locus, number of private alleles, and allele frequencies were calculated for each colony and locus using the program GDA (Lewis and Zaykin, 2000). To assess genetic differentiation between colonies, we first searched for private alleles, i.e., alleles that are exclusively found in one of the colonies and not in others. Second, we used Fisher's exact test of genotype frequency differentiation among colonies using GENEPOP (Raymond and Rousset, 1995). Third, genetic distances (F_{ST}) were calculated between colonies using the methods of Weir and Cockerham (1984) as implemented in FSTAT (Goudet, 1995). Colonies were treated as subpopulations (Thorne et al., 1999; Bulmer et al., 2001). To assess the significance of the F-statistics, 95% confidence intervals (CI) were constructed by bootstrapping over loci. F-values were

considered significantly different from zero if their confidence intervals did not span zero.

To determine statistically significant differences in aggression and mortality among pairings of colonies, a one-way analysis of variance (ANOVA) and a Tukey's honestly significant difference (HSD) test were performed on the data using SPSS 11.0 statistical analysis software (SPSS Inc., Chicago, IL) Tests were performed using a significance level of $\alpha = 0.05$. Pairwise F_{ST} values were used as genetic distances for analysis of correlation between short-term aggression levels and mortality after 24 hr using a Mantel test (Mantel, 1967).

RESULTS

Aggression Assays. Short term aggression assays (Figure 1A) showed aggression resulting in injury between individuals from all colonies collected from the field except between pairings of CP-2 and CP-3. No aggressive behaviors were observed in any of the pairings from the same colony (control). The highest numbers of short term aggressive behaviors occurred in UNO vs. CP-3 (50 $\pm$ 3.8) and UNO vs. LC (42 $\pm$ 3.0). Moderate aggression was observed in UNO vs. CP-2 (28 $\pm$ 2.3), CP-3 vs. LC (20.1 $\pm$ 2.5), and CP-2 vs. LC (13.1 $\pm$ 1.3). There was essentially no aggression observed in CP-3 vs. CP-2 (0.20 $\pm$ 0.13). ANOVA indicated that, overall there was a significant effect of colony pairing on aggression levels ($P < 0.001$). *Post hoc* tests using Tukey's HSD test showed that the only pairings not significantly different in levels of short term aggression were UNO vs. CP-3 and UNO vs. LC ($P = 0.212$); UNO vs. CP-2 and CP-3 vs. LC ($P = 0.223$), and CP-3 vs. LC and CP-2 vs. LC ($P = 0.348$). However, all pairings were significantly different from the control pairings, except for the pairing of CP-3 vs. CP-2 ($P = 0.166$).

Although both CP-2 vs. LC and CP-3 vs. LC showed only moderate short term aggressive behaviors, these pairings had the greatest mortality after 24 hr (Figure 1B). UNO vs. CP-3, which had the greatest number of short term aggressive encounters, also had the third greatest percentage mortality. Although, UNO vs. CP-2 showed an average of 14 fewer aggressive encounters than UNO vs. LC in short term assays, both pairings had approximately the same average percent mortality after 24 hr (57.8% and 60.5%, respectively).

Overall, there was a significant effect of colony pairing on long-term aggression ($P < 0.001$). However, some individual group pairings including UNO vs. CP-2 and UNO vs. LC ($P = 0.999$); CP-3 vs. LC and CP-2 vs. LC ($P = 0.999$); CP-2 vs. LC and UNO vs. CP-3 ($P = 0.999$), and CP-3 vs. LC and UNO vs. CP-3 ($P = 0.991$) were not significantly different in levels of mortality. All colony pairings had significant differences in levels of mortality vs. controls except for CP-3 vs. CP-2, which had P-values of 0.112 vs. UNO and CP-2 controls and 0.053 for CP-3 and LC controls.

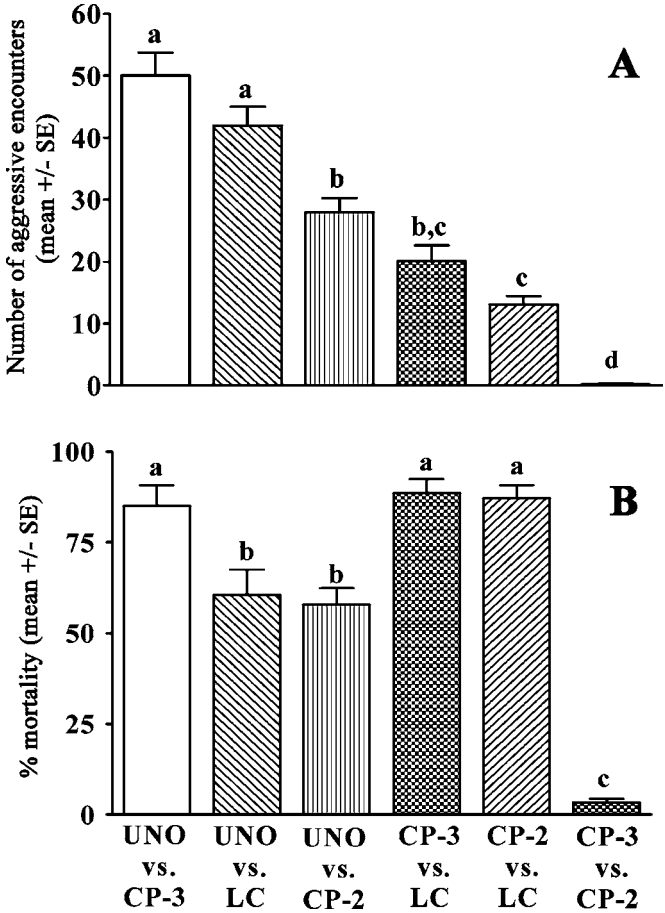

FIG. 1. Results from aggression assays among individuals of four test colonies of *C. formosanus*. (A) Short-term aggressive encounters over 5 min. (B). Mortality after 24 hr. Mean ± SE. Means with the same letters are not significantly different from each other at $\alpha = 0.05$ ($N = 10$).

Genetic Analyses. All eight microsatellite loci were polymorphic with three to five alleles per locus and allele frequencies ranging from 0.01 to 0.54 across all colonies. Microsatellite analysis showed that the four colonies were genetically distinct. First, private alleles were found in each of the colonies, distinguishing UNO, LC (both with private alleles at three different loci), CP-2, and CP-3 (both with different private alleles at the same locus). Second, Fisher's exact test confirmed that the four colonies were significantly differentiated at all loci ($P < 0.001$, $\chi^2 = \infty$, d.f. = 16). Third, F_{ST}, a measure of genetic contrast

between the colonies, was significantly different from zero ($F_{ST} = 0.38$, 95% CI = 0.30–0.46, nonoverlapping with zero). Pairwise F_{ST}-values ranged from 0.24 to 0.48. Neither short-term levels of aggression ($r = 0.2314$, $P = 0.659$) nor long-term levels of aggression (i.e., mortality) ($r = 0.449$, $P = 0.371$) were significantly correlated with genetic distance.

Chemical Profiles from Worker Extracts. Analysis of hexane extracts of workers sampled from the four colonies showed no qualitative differences between colonies, but did show quantitative differences in the amounts of some CHCs (Figure 2). LC and CP-2 had greater amounts of several CHCs compared to UNO and CP-3, with the exception of peak #2 (9-,11-,13-methylpentacosane), of which UNO had the greatest amount. CP-2 and LC had greater amounts of all the straight chain *n*-alkanes observed and identified in the chromatogram. The straight chain *n*-alkanes include peaks #1 (pentacosane), #5 (hexacosane), and #8 (heptacosane). The greatest differences in *n*-alkane hydrocarbon quantities can be seen in peaks #12 (octacosane) and #14 (nonacosane). These chromatograms show that there were greater amounts of octacosane and nonacosane present in samples from CP-2 and LC than in the other two colonies. CP-2 and CP-3 showed observable differences in CHCs based upon peaks 1, 5, 8, 12, 14, and unidentified peak 18.

Effect of Diet on Aggression. When workers from previously aggressive colonies were fed the same diet for 3 months, aggression between the colonies was significantly reduced. From the results (Figure 3), it was concluded that diet had a significant influence on inter-colonial aggression in *C. formosanus*. There was no significant difference in aggression between UNO-spruce vs. CP-3-spruce and UNO-birch vs. UNO-spruce ($P = 0.567$). When UNO- birch was paired against CP-3-spruce, mortality was over 90%, which is similar to levels of aggression seen in previous aggression assays involving UNO and CP-3. In this assay, when both UNO and CP-3 colonies were fed spruce wood, the intense levels of aggression towards each other greatly diminished. However, if diet alone were enough to manipulate aggression then the UNO-birch, should have been aggressive towards UNO-spruce. This was not the case in the pairing of UNO-spruce vs. UNO-birch, suggesting that although diet appears to influence intercolonial aggression there must be other factors involved that affect intra-colonial aggression.

SPME Analysis. Five different fibers were used for SPME analysis of the groups of termites from CP-3 fed on spruce and groups of termites from UNO fed on spruce or birch. Results indicated that in all analyses, three compounds, eluting between 5 and 15 min, were found to be in either substantially greater amounts or completely unique in the UNO-birch samples (Figure 4). Based upon the retention times and mass spectra, identification was made of each of the three compounds as dimethyltrisulfide (1), 2-aminoacetophenone (2), and 8-heptadecene (3). Mass spectra of the three authentic compounds after SPME, matched the compounds

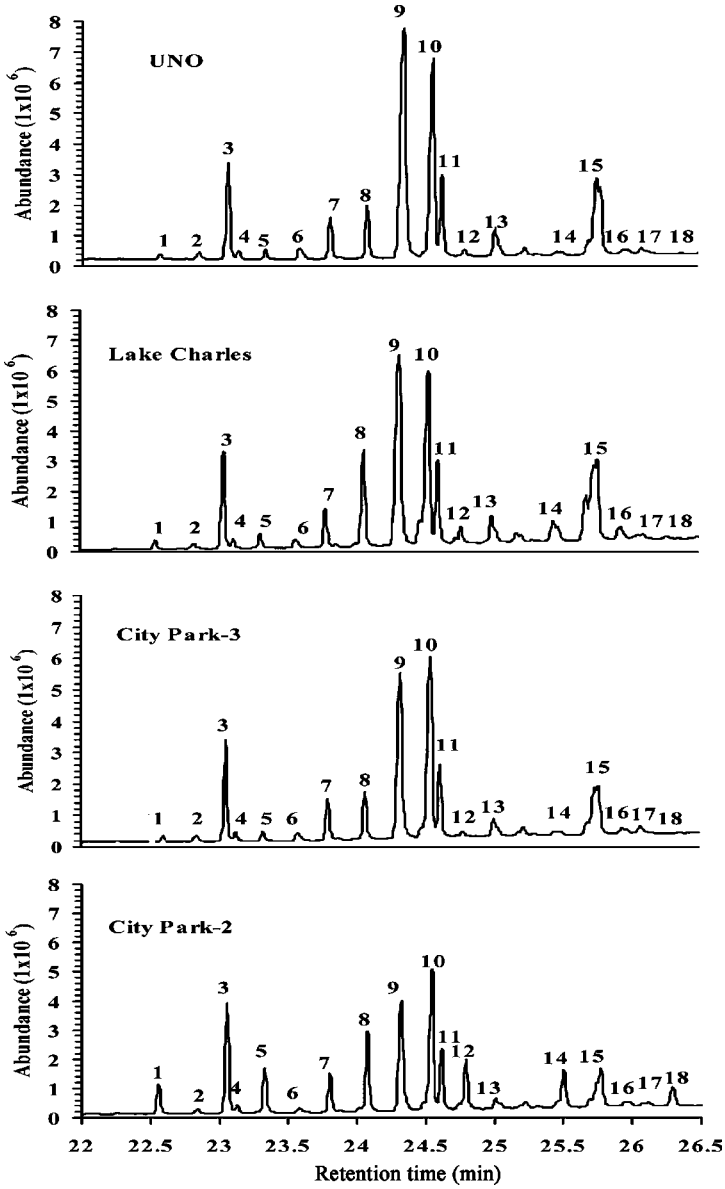

FIG. 2. Chromatograms of CHCs from workers of four test colonies of *C. formosanus*. No qualitative differences in CHCs were found. Quantitative differences in peaks 1,5,8,12,14, and 18 between CP-2 and CP-3 did not correlate with aggression.

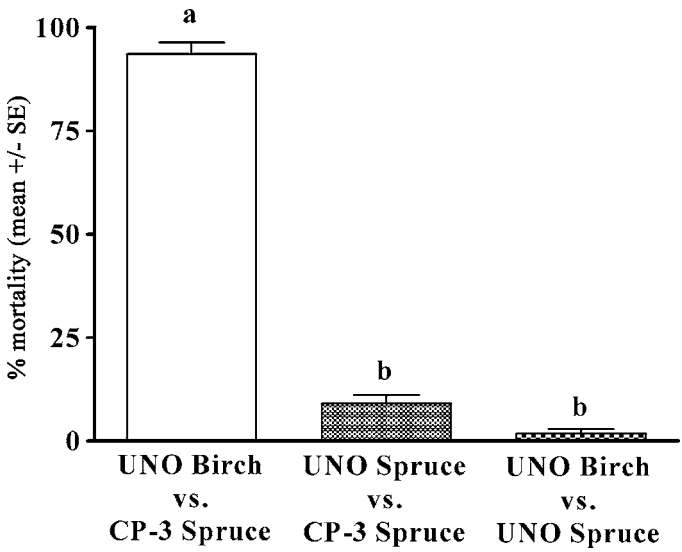

FIG. 3. Mortality after 24 hr as a result of aggression between two colonies of *C. formosanus* fed on spruce or birch woods. Mean ± SE. Bars with the same letters are not significantly different from each other at $\alpha = 0.05 (N = 5)$.

identified from the termites. Retention times and mass spectra were essentially the same for each compound across all five types of fibers used. No qualitative differences in hydrocarbon profiles were noted using different fiber types.

DISCUSSION

Intraspecific or intercolonial aggression has been studied in numerous genera of termites including *Hodotermes*, *Trinervitermes*, *Amitermes*, *Reticulitermes*, and *Zootermopsis* (Shelton and Grace, 1996 and references therein). Previous investigations involving *C. formosanus* showed that intercolonial pairings from Lake Charles, LA did not evoke significant aggression, and individuals could not accurately discriminate nest-mates from nonnest-mates (Delaplane, 1991). It was also reported that colonies of *C. formosanus* from Hallandale, FL showed no aggression towards individuals from other colonies from the same area. However, colonies located on the University of Hawaii campus were able to discriminate nest-mates from nonnest-mates in some pairings, and were variable in their levels of inter-colonial aggression (Su and Haverty, 1991). We also observed varying degrees of aggression in all paired combinations among the four test colonies, except for pairings between CP-2 and CP-3.

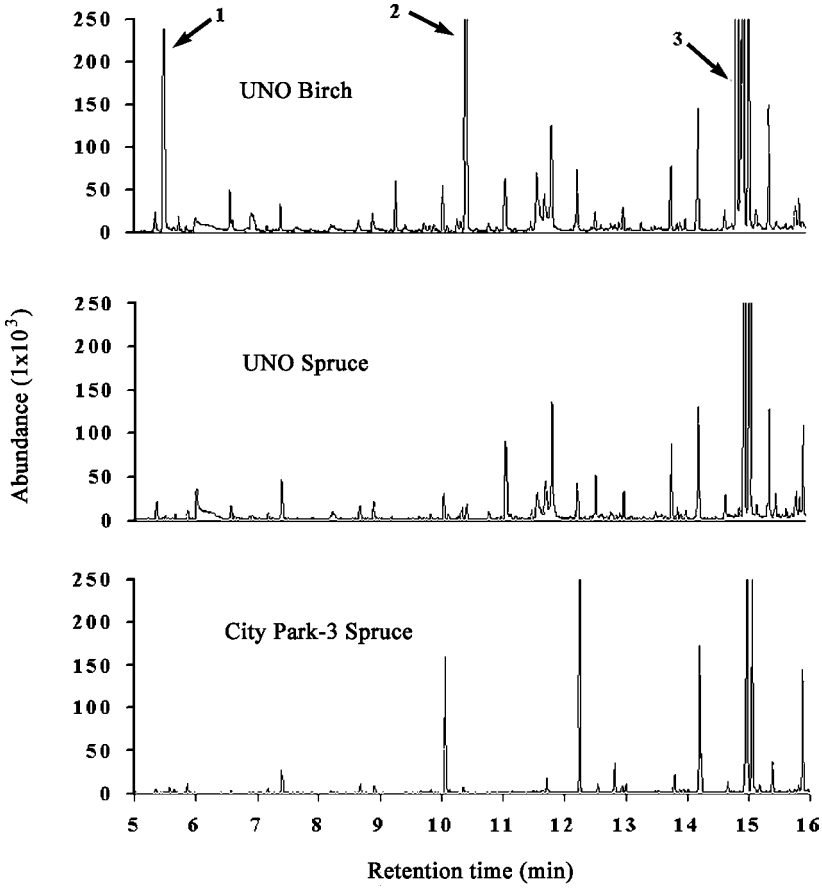

FIG. 4. Chromatograms of compounds obtained by headspace solid-phase microextraction of workers from two colonies of *C. formosanus* workers fed on spruce or birch wood. Three compounds [dimethyltrisulfide (peak 1), 2-aminoacetophenone (peak 2), and 8-heptadecene (peak 3)] associated with the UNO colony fed on birch were either absent or highly reduced in the other two colony/diet combinations.

Aggression could not be accurately described by short-term observation alone. For example, CP-2 and LC pairings resulted in only moderate short-term aggression. However, they displayed a high level of mortality with 87.1 ± 3.6% of individuals dead after 24 hr. Mortality after 24 hr ranged from 58% to 90% in five of the six pairings, whereas it was <4% in CP-2 vs. CP-3. Although no aggression was encountered between the two samples from City Park, microsatellite DNA analyses showed that all four colonies were genetically distinct.

Neither short-term levels of aggression nor mortality were significantly correlated with genetic distance. In the same way, using multilocus DNA fingerprinting, no correlation between genetic similarity and aggression was found in termites from 12 collection sites in Hawaii (Husseneder and Grace, 2001). Similarly, in an earlier study, differences in mitochondrial DNA of different colonies of *C. formosanus* could not be linked with agonism (Broughton and Grace, 1994). Thus, in contrast to other termite species (Adams, 1991), genetic traits cannot fully explain intercolonial patterns of aggression in *C. formosanus*.

Visual observation of the CHC profiles of each colony revealed that the CHC profiles did not appear to correlate with aggression. For example, although some quantitative differences were observed among CHC profiles of the workers from the four colonies if aggression in *C. formosanus* was correlated with quantitative differences in CHC profiles, then both CP-3 and UNO should act aggressively towards CP-2 and LC. However, CP-2 and CP-3, with clear quantitative differences in CHC profiles, were not aggressive to each other in behavioral assays. We did note some quantitative differences in CHCs between pairs of aggressive colonies. As example, the LC colony which showed high level of aggression towards all other colonies had higher amounts of straight chain *n*-alkanes (Figure 2, peaks 1, 5, and 8). Similarly, for UNO vs. CP-3, which exhibited high short-term aggression and mortality, the ratio between peaks 9 and 10 was reversed. Because CHC profiles in *C. formosanus* vary between castes within a colony (Haverty et al., 1996), it has been suggested that it is unlikely that CHCs would be used by this species as the sole cue of nest-mate recognition (Matsuura, 2001).

In contrast, experiments performed on numerous other species of social insects have shown that aggression is correlated with different CHC profiles. In the paper wasp, *Polistes dominulus*, it was reported that methyl-branched alkanes and alkenes were used as the chemical signature for nest-mate recognition (Dani et al., 2001). In dampwood termites, *Zootermopsis* spp., when individuals from different colonies containing different CHC phenotypes were paired, they typically showed high levels of aggression (Haverty and Thorne, 1989). On the other hand, pairings between termites of the same CHC phenotype showed consistently low levels of aggression or even no aggression. In *Reticulitermes* spp., pairing of individuals from different colonies with the same CHC phenotype resulted in little immediate aggression, but showed high mortality after 24 hr, whereas pairing of individuals from different CHC phenotypes resulted in immediate aggression in most pairings and approached 99% mortality after 24 hr (Haverty et al., 1999; Delphia et al., 2003). These authors concluded that *Reticulitermes* could differentiate CHC phenotypes and recognize nest-mates and nonnest-mates even within a CHC phenotype.

Our behavioral tests, which were designed to analyze the effect of diet on colony aggression, showed clear differences in the levels of aggression. When

UNO (originally from birch) and CP-3 (originally from cypress) were both fed on spruce, aggression was significantly reduced compared to the intense aggression observed previously between these two colonies. Few studies have focused on the influence of diet on aggression in social insects. Liang and Silverman (2000) determined that in the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae), hydrocarbons acquired from the feeding on insect prey are used in nest-mate recognition. Subsequently, Silverman and Liang (2001) determined that when colony fragments were separated for a period of time and provided prey that possessed distinct CHC profiles, these fragments were no longer able to reintegrate into the colony due to the high levels of aggression. These results indicated that diet could influence not only inter-colonial aggression, but also intra-colonial aggression. Although there was a limited number of pairings in our study, the results suggest that diet may influence inter-colonial aggression in *C. formosanus*, but not intra-colonial aggression. Similarly, when *S. lamanianus* workers were kept on nest materials from other colonies inter-colonial aggression diminished. However, it was not possible to induce intra-colonial aggression by placing workers on alien nest material (Husseneder et al., 1997). In listing a series of alternative mechanisms for nest-mate recognition in *C. formosanus*, collectively known as the "multiple stimulus hypothesis" (Haverty and Thorne, 1989), one of these mechanisms was suggested to be based on volatile digestive components (Thorne and Haverty, 1991). In *Reticulitermes speratus,* it has been suggested that differential intestinal bacterial composition (perhaps due to feeding on different foods) leads to production of colony-specific chemical cues responsible for nest-mate recognition (Matsuura, 2001).

The results of the SPME analyses indicate that a change in diet qualitatively altered the chemical signature of the termites in our study. Three compounds (dimethyltrisulfide, 2-aminoacetophenone, and 8-heptadecene) were found in groups of termites fed on birch but absent or in significantly lower amounts in groups fed on spruce. At this time, we are not sure if these chemicals were acquired from the host wood, were produced within the termite by the action of gut fauna, or were produced as a result of subjecting the termites to high temperature. When the termites were removed from birch and placed on spruce, the characteristic chemical signature changed. This may explain why individuals from CP-3 (cypress) and UNO (birch) placed together showed intense aggression, and when later they were reared on the same diet (spruce), aggression was greatly diminished. Thus, diet appears to be an important factor influencing intercolonial aggression in *C. formosanus*.

Although feeding on the same diet could reduce intercolonial aggression, feeding on different diets could not induce aggression within the same colony. Thus, when UNO-birch was paired with UNO-spruce, there was no aggression and no resulting mortality, even after 24 hr. Some genetic or other predisposition must exist which limits colony members from attacking one another, even when

they feed on different food sources. It appears that there must be an intricate process involving a hierarchy of cues that regulates nest-mate recognition in *C. formosanus*. This hierarchy may also explain why individuals from the UNO colony did not show intra-colonial aggression when reared on different diets. If they detect dissimilar diet-derived volatile components, they may then rely on a second level of recognition in which they verify endogenous genetically based colony-specific cues. This might represent a mechanism to maintain territoriality and, at the same time, lead to resource partitioning between different colonies of a termite species.

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