Agonistic Behavior and Cuticular Hydrocarbon Phenotypes of Colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California

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ABSTRACT *Reticulitermes* in northern California is supposed to be represented by *R. hesperus* Banks and *R. tibialis* Banks, yet at least 5 distinct cuticular hydrocarbon phenotypes have been characterized. Three hydrocarbon phenotypes from the Institute of Forest Genetics near Placerville, CA, and 3 cuticular hydrocarbon phenotypes from 2 separate sites in Marin County were used to characterize interactions of foraging groups or colonies at each site. Pairings from the same foraging group or different foraging groups of the same colony rarely resulted in immediate aggression and never resulted in high mortality. Pairings of workers from different foraging groups of the same cuticular hydrocarbon phenotype from either the Placerville or Marin sites resulted in few bouts (8 and 15%, respectively) with immediate aggression, but after 24 h, mortality was high in 56 and 81% of the bouts, respectively. Pairings from different cuticular hydrocarbon phenotypes resulted in immediate aggression 48.8 and 61.5% of the time, respectively; nearly all of these (>99%) resulted in high mortality after 24 h. These results suggest that these *Reticulitermes* recognize hydrocarbon phenotypes, and can differentiate colony mates and alien workers within a cuticular hydrocarbon phenotype. Because kin discrimination suggests genetic relatedness among individuals, this bioassay will be useful for determining the association of foraging groups in ecological studies of *Reticulitermes* colonies in northern California and indirectly may indicate relatedness among colonies of the same hydrocarbon phenotype.

KEY WORDS *Reticulitermes*, aggression, competition, fighting, kin recognition, subterranean termites

RESEARCH ON POPULATION dynamics of subterranean termites in North America, especially *Reticulitermes* species, has been severely neglected, largely because of the overwhelming success of the cyclodiene termiticides applied as a soil drench. There was never a serious need to study population dynamics from an applied perspective. Studies of the population size, foraging territories, or foraging periodicity have been conducted on *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks), and *R. hageni* Banks in the eastern United States (Howard et al. 1982a, Grace et al. 1989, Grace 1990, Su et al. 1993, Forschler and Townsend 1996) and *Reticulitermes hesperus* Banks in southern California (Haagsma and Rust 1995). However, until recently, similar information for *Reticulitermes* in northern California did not exist.

To measure the foraging territory of a colony and the size of the foraging population, most recent studies use mark–release–recapture methods. The development of dyes (Lai 1977) and other marking techniques (Forschler 1994) have greatly facilitated our ability to delimit foraging territories; however, estimates of subterranean termite populations must be interpreted with caution (Thorne et al. 1996).

Marking individual foraging groups in a community of *Reticulitermes* colonies is limited because only 3 satisfactory stains (Neutral Red, Sudan Red 7B, and Nile Blue A) are available (Su et al. 1991), and because adjacent foraging groups marked or stained with the same color may be confused. Agonistic behavior also has been used to determine the association of foraging groups in termites (Thorne and Haverty 1991). Agonistic behavior involves social interactions among individuals, including fighting, fleeing, and submitting (Haverty and Thorne 1989). Termites show a wide range of agonistic behaviors when interacting with other termites from a different colony of the same or a different species (Thorne and Haverty 1991, Shelton and Grace 1996). Interspecific aggression is prevalent among sympatric species; aggression among conspecific, sympatric colonies also is common. Reports of passive, intercolonial encounters are rare (Thorne and Haverty 1991).

Binder (1988) and Jones (1990), who investigated intraspecific agonism, used aggressive encounters to

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determine colony affiliation of foraging groups of *Heterotermes aureus* (Snyder). Jones (1990) corroborated colony determinations with mark–release–recapture studies in the field. Studies of intraspecific agonism in *Reticulitermes* reveal a complex suite of responses. These may be consistently aggressive as in the generally monogynous *Reticulitermes (l.) banyulensis* Clément (Clément 1980), always passive as in *R. santonensis* Feytaud (Clément 1986), mostly passive as in *R. flavipes* and *R. virginicus* (Grace 1996, Polizzi and Forschler 1998), or vary seasonally as with *R. (l.) grassei* Clément and *R. (l.) lucifugus* Rossi (Clément 1986).

At our field sites in northern California, only *R. hesperus* is supposed to be present (Pickens 1934a, b; Weesner 1970; Nutting 1990). We know that there are multiple cuticular hydrocarbon phenotypes of *Reticulitermes* at these sites and that they represent distinct taxa (Haverty and Nelson 1997). We suspected that we have multiple colonies of each phenotype at each site. Because of difficulties marking multiple colonies within the same area, we decided to assess interphenotype and intraphenotype agonism among workers from different foraging groups as a means of associating foraging groups of the same colony.

Materials and Methods

Termites. We used groups of *Reticulitermes* from 1 wildland location and 2 residential locations in northern California. The wildland site was in an arboretum at the Institute of Forest Genetics (IFG) near Placerville, CA. This site is ≈4 ha and is composed of a 50-yr-old plantation of mixed *Pinus* species. The residential sites were in Marin County: one each in Novato and Larkspur. The Novato site (St. Francis Church) consists of a single-family dwelling (the church rectory), the church, and extensive gardens, walks, and large trees on a 1-ha lot (Lewis et al. 1998). The Larkspur site is a single-family, 60-yr-old residence.

We installed permanent monitoring stations: 68 at IFG, 34 at Novato, and 12 at Larkspur (Lewis et al. 1998). At IFG, we found 3 distinct hydrocarbon phenotypes—53 of our monitoring stations were characterized as phenotype A; 10 as phenotype B; and 5 as phenotype C. Two additional hydrocarbon phenotypes were characterized from our monitoring stations in Marin County—16 monitoring stations sampled in Marin County were characterized as A′, 4 as A, and 14 as D (Haverty and Nelson 1997). Each month all foragers collected from each monitoring station were taken to the laboratory. Two hundred workers were used for determination of hydrocarbon phenotype and worker weight and voucher specimens (≈5 soldiers and 5 workers) were kept for each monitoring station each month. One voucher specimen for each monitoring station was deposited in the Essig Museum, University of California, Berkeley, and identified by cuticular hydrocarbon phenotype. Excess foragers were placed in culture.

Separate laboratory cultures were established from foraging termites collected from each monitoring station. Cultures were augmented each month if excess foragers were available. Cultures were maintained in the laboratory for up to 24 mo in containers provided with sand/vermiculite/water (1:1:0.8 vol.) (Haverty 1979). Cultures were supplied wood from old bait bundles (Lewis et al. 1998) and remoistened as needed.

Association of Foraging Groups. Once monitoring stations were visited regularly by termites, all termites collected from a single monitoring station inhabited by each cuticular hydrocarbon phenotype at each site were fed filter paper impregnated with 0.1% (wt:wt) Nile Blue A (Sigma, St. Louis, MO) for 14 d. After 14 d, the fat body of most workers was blue. Stained termites were returned to the single monitoring station from which they had been collected (Grace et al. 1989, Su et al. 1993, Haagsma and Rust 1995). All monitoring stations were checked 2 wk later. Unstained termites subsequently collected from monitoring stations containing marked termites were marked (as above) and released to their respective monitoring stations. This mark–release–recapture scheme was repeated 3 times. This was done with Sudan Red 7B (Sigma) (Su et al. 1983, Grace and Abdally 1989) for 1 colony at IFG and 1 at the Novato site.

Using the mark–release–recapture regime, we were able to associate foraging groups for 4 colonies at IFG, 4 colonies at Novato, and 2 colonies at Larkspur. When marked workers appeared in a monitoring station that had not previously contained marked foragers, we assumed that the foraging groups within these monitoring stations were members of the same colony. For the purposes of this article, we considered a colony to be foraging groups of the same phenotype sharing interconnected galleries (Su and Scheffrahn 1998). Our definition assumes that these foragers also are associated with other conspecifics involved in cooperative rearing of offspring (Wilson 1971).

Agonism Bioassay. We paired 2 groups of 10 workers from different monitoring stations (or the same monitoring station as a control) to determine whether or not they would show aggressive behavior. Workers were placed in plastic petri dishes (5 cm diameter) with tight-fitting lids, provisioned with a 47-mm absorbent pad (Gelman Sciences, Ann Arbor, MI), and moistened with 1 ml of distilled water. After all the groups of 10 workers from each monitoring station culture had been counted, the aspirator (metal tube and styrene tube) was disassembled and cleaned to remove possible contamination by semiochemicals before it was used to count workers from another culture.

Immediately upon combining the groups, behavior was observed for ≈2 min. When the lid was opened to place the 2nd group of workers in an arena, the workers already in the arena were usually disturbed by the air movement. Upon placing the 10 new workers in the arena, the workers investigated one another. The subsequent behaviors were similar to those expressed by pseudergates of *Zootermopsis* species (Haverty and

Table 1. Number and percentage of encounters with or without immediate aggression and number of encounters resulting in high, low, or equivocal levels of mortality among foraging groups of the same or different cuticular hydrocarbon phenotypes from the Institute of Forest Genetics near Placerville, CA

Pairing	N	Immediate aggression					No immediate aggression					No behavior observations			
		n	%	24-h mortality[a]			n	%	24-h mortality[a]			n	24-h mortality[a]		
				High	Low	Equiv.			High	Low	Equiv.		High	Low	Equiv.
Controls[b]	79	0	0	—	—	—	53	100	0	52	1	26	0	26	0
Intraphenotype[c]	470	25	8.0	25	0	0	289	92.0	151	127	11	156	49	88	19
Intracolonial	2	0	0	—	—	—	2	100	0	2	0	0	0	0	0
Intercolonial	468	25	8.0	25	0	0	287	92.0	151	125	11	156	49	88	19
Interphenotype[d]	261	72	61.5	71	1	0	45	38.5	45	0	0	144	139	2	3

Phenotypes A, B, and C as defined by Haverty and Nelson (1997).

[a] High mortality resulted in 0–10 workers alive, low mortality 17–20 workers alive, and equivocal mortality 11–16 workers alive after 24 h.

[b] Pairings of 2 groups of 10 workers from the same foraging group.

[c] Pairings of 2 groups of 10 workers from different foraging groups of the same cuticular hydrocarbon phenotype determined to be either from the same colony (intracolonial) or from different colonies (intercolonial) by mark–release–recapture studies.

[d] Pairings of 2 groups of 10 workers from foraging group of different cuticular hydrocarbon phenotypes. There were 179 pairings of A versus B, 42 of A versus C, and 40 of B versus C.

Thorne 1989) as follows: (1) no apparent reaction, (2) additional antennation and grooming each other, (3) clumping or thigmotaxis, (4) tapping of head or body on the substrate, (5) running in circles, (6) obvious avoidance of each other, (7) voiding gut contents, (8) or immediate aggression (chasing, lunging, or biting).

After 24 h, the surviving termites were counted. Mortality was considered high if 0–10 workers were alive after 24 h and low if 17–20 workers were alive after 24 h; the level of mortality was considered equivocal if 11–16 workers were alive after 24 h. High, low, or equivocal mortality counts were kept separately for pairings with immediate aggression or no immediate aggression.

Of the 549 intraphenotype and 261 interphenotype bioassays (a total of 810) staged for foraging groups from the monitoring stations at IFG, no behavioral observations were made for 326 (Table 1). Six hundred thirty intraphenotype and 461 interphenotype bioassays were arranged for foraging groups from Marin County (Table 2).

We did not set up an equal number of replicates for all possible combinations. The number of replicates per pairing varied in part by the number of workers available or because of the response of the initial pairing. For some tests we used only workers freshly collected from monitoring stations that contained >500 workers. For others we used laboratory cultures. In some cases we were interested in increasing the number of replicates for monitoring stations involved in the mark–release–recapture studies or those adjacent to the monitoring stations in the mark–release–recapture studies; thus the number of replications for each possible combination was not equal.

If the first pairings resulted in immediate aggression or high mortality, further pairings were deemed unnecessary because the workers were obviously not from the same colony. If the first pairings did not result in immediate aggression or high mortality, additional bioassays usually were conducted to confirm the lack of aggressive behavior. Thus, our intraphenotype results tended to be biased toward nonaggressive results because of the increased number of bioassays resulting when aggression was not evident.

Results

Association of Foraging Groups. We could establish connections among only a few of the monitoring stations at our research sites using the mark–release–

Table 2. Number and percentage of encounters with or without immediate aggression and number of encounters resulting in high, low, or equivocal levels of mortality among foraging groups or subcolonies of the same or different cuticular hydrocarbon phenotypes from 2 sites in Marin Co., CA

Pairing	N	Immediate aggression					No immediate aggression				
		n	%	24-h mortality[a]			n	%	24-h mortality[a]		
				High	Low	Equiv.			High	Low	Equiv.
Controls[b]	104	2	1.9	0	2	0	102	98.1	0	101	1
Intraphenotype[d]	526	64	12.2	58	6	0	462	87.8	266	175	21
Intracolonial	127	5	3.9	0	5	0	122	96.1	0	122	0
Intercolonial	399	59	15.0	58	1	0	340	85.0	266	53	21
Interphenotype[e]	461	225	48.8	225	0	0	236	51.2	234	2	0

Phenotypes A, A′, and D as defined by Haverty and Nelson (1997).

[a] High mortality resulted in 0–10 workers alive, low mortality 17–20 workers alive, and equivocal 11–16 workers alive after 24 h.

[b] Pairings of 2 groups of 10 workers from the same foraging group.

[c] Pairings of 2 groups of 10 workers from different foraging groups of the same cuticular hydrocarbon phenotype, determined to be either from the same colony (intracolonial) or from different colonies (intercolonial) by mark–release–recapture studies.

[d] There were 326 pairings of A′ versus D, 92 of A′ versus A, and 43 of D versus A.

recapture regime. We expected marked termites placed in one monitoring station to show up in numerous other stations and occupy an area extending ≥100 m or more in any direction, as has been demonstrated for *R. flavipes* in Florida (Su et al. 1993) and Canada (Grace et al. 1989, Grace 1990). What we observed was more similar to the small foraging territories for *Reticulitermes* spp. in Georgia (Forschler 1994, 1996; Forschler and Ryder 1996) and *R. hesperus* in California (Haagsma and Rust 1995).

Stained workers returned to a phenotype A monitoring station at IFG during the first mark–release–recapture attempt did not move to another station. In a different phenotype A monitoring station, marked individuals traveled to only 2 additional stations. In 2 phenotype B stations, marked termites never moved from their original monitoring station, even though another phenotype B station was 4 m away from one of them.

The mark–release–recapture studies of Marin County colonies also indicated limited foraging territories for California *Reticulitermes*. Dyed termites were placed in 1 phenotype A′ monitoring station and were recovered in only 1 additional monitoring station 6.4 m away. Stained workers placed in a phenotype D monitoring station did not move to any other monitoring station. Phenotype A′ workers, marked with Sudan Red 7B so that they would not be confused with foragers from any near-by phenotype A′ monitoring stations, eventually showed up in a monitoring station on the opposite side of the building (13.4 m away), but nowhere else. After 3 mark–release–recapture cycles, blue-stained workers released in yet another phenotype A′ monitoring station at Novato were collected in 7 other monitoring stations a maximal distance of 25 m apart. Stained workers placed in a phenotype A′ monitoring station at Larkspur never appeared in another monitoring station. Marked termites placed in a phenotype A monitoring station at Larkspur did move to a monitoring station ≈2 m away, but nowhere else.

At IFG and Novato, we observed one hydrocarbon phenotype displace another within a monitoring station. At IFG, this was corroborated by morphological measurements of soldiers in voucher specimens (Haverty and Nelson 1997), and confirmed by changes in aggressiveness in agonistic behavior bioassays. In addition, we never observed ≥2 hydrocarbon phenotypes occupying the same monitoring station at the same time.

In summary, of the 114 monitoring stations at our 3 sites, we identified only 5 (1 at IFG and 4 in Marin County) colonies occupying >1 monitoring station. Because stained workers moved among them, we are certain that these foragers belong to the same colony. Thus, we could use these connected monitoring stations to assess the validity of our agonistic behavior bioassays by pairing foraging groups determined to be from the same colony.

Evaluation of the Bioassay. The bioassay used in this study was a forced encounter. It forced the workers to confront the opposite set of 10 workers placed in, or already within, the arena. Only once did the 2 sets of 10 workers partition the space; 1 group managed to become tightly clumped below the absorbent pad for the duration of the 24-h exposure.

Bouts with immediate aggression almost always resulted in high mortality (0–10 workers alive after 24 h); few resulted in low mortality (17–20 workers alive after 24 h) or equivocal levels of mortality (11–16 workers alive after 24 h). In about half of the interphenotype bouts (38.5 and 51.2%) and in most of the intraphenotype, intercolonial encounters (92.0 and 85.0%), there was no obvious immediate aggression during the 1st 2 min (Tables 1 and 2). Immediate aggressive behavior is not the sole cause of high mortality in interphenotype pairings. Two-thirds (66.5%) of the intraphenotype, intercolonial bouts with no immediate aggression resulted in high mortality, whereas nearly all (98.8%) of these encounters with immediate aggression resulted in high mortality after 24 h (Tables 1 and 2).

To determine whether our bioassay itself elicited agonistic behavior, we paired 2 sets of 10 workers from the same monitoring station. Nearly all of 183 such pairings (98.9% combined for IFG and Marin County) resulted in low mortality at 24 h; in only 2 instances was mortality in the equivocal range at 24 h (Tables 1 and 2). Two of the 104 controls for Marin County displayed immediate aggressive behavior, whereas none of the controls for IFG displayed immediate aggression. Neither of these 2 pairings with immediate aggression resulted in high, or even equivocal, levels of mortality. We conclude that the artificial nature of the bioassay does not elicit aggressive behavior that results in even moderate mortality; nearly all such pairings resulted in no immediate aggression and all resulted in low mortality.

Interphenotype Agonism. When termites of different cuticular hydrocarbon phenotypes were placed together, results were usually unequivocal. Interphenotype encounters for IFG and Marin County resulted in immediate aggression 61.5 and 48.8% of the time, respectively. In contrast, only 8.0 and 15.0% of the intraphenotype, intercolonial bouts resulted in immediate aggression. Furthermore, 98.9% of the interphenotype bouts resulted in high mortality after 24 h, whereas only 63.3% intraphenotype, intercolonial pairings resulted in high mortality at both sites (Tables 1 and 2).

A high level of aggression was observed in pairings of groups of phenotype A′ and A. Only 1.5% of the pairings of these phenotypes resulted in low mortality; all the others resulted in high mortality. Consistent, aggressive responses strongly suggests that cuticular hydrocarbon phenotypes A′ and A from northern California represent separate, although closely related, taxa.

Intraphenotype Agonism. Forced encounters between workers of the same cuticular hydrocarbon phenotype from different foraging groups are more difficult to interpret than the interphenotype agonistic encounters. Pairings with high mortality probably are composed of termites from separate, conspecific colonies (Tables 1 and 2). Specific pairings with repli-

Table 3. Number of survivors from pairings of 2 sets of 10 workers from 2 different monitoring stations of the same hydrocarbon phenotype from either the Institute of Forest Genetics near Placerville, CA, or 2 sites in Marin Co., CA

Pairing[a]	No. survivors in each pairing[b]
Suspected intracolonial	
IFG: Wc7 vs Wd7	20, 20, 20, 20, 20
IFG: Yr19 vs Yt19	20, 20, 20, 20, 20, 20, 20, 20, 19
IFG: Yv32 vs Yv34	19, 19, 20, 20, 20, 19, 20, 20, 18, 20
StF: 12 (old) vs 21	20, 20, 20, 20, 20, 19, 19, 20, 20, 20, 20, 20, 20, 20, 20
StF: 314 vs 333	20, 20, 20, 20, 20, 20
Suspected intercolonial	
IFG: Wt51 vs Yr19	20, 20, 20, 20
IFG: Wt51 vs Yr34	20, 20, 20, 20
IFG: Xt10 vs Ze4	19, 19, 20, 20
IFG: Yr19 vs Ze4	20, 20, 19, 18
StF: 12 vs 35	6, 1, 1, 1
StF: 12 vs 57	4, 7, 20, 4
StF: 12 vs 63	1, 19
StF: 12 vs 66	4, 5, 0, 2
StF: 12 vs 69	3, 6, 0, 7
StF: 12 vs 71	10, 2, 8, 20
StF: 12 vs 78	20, 2, 1, 3
StF: 12 vs 87	20, 11, 20, 1
StF: 18 vs 35	3, 4, 0, 5
StF: 18 vs 57	3, 4, 20, 4, 20, 18, 14, 7, 1, 19, 20, 11, 19, 20
StF: 18 vs 63	17, 20
StF: 18 vs 66	19, 2, 0, 1, 5, 0, 1, 0, 0, 0, 2, 0, 1, 2
StF: 18 vs 69	3, 1, 18, 2
StF: 18 vs 71	1, 5, 20, 20
StF: 18 vs 78	18, 3, 13, 5, 10, 7, 6, 6, 2, 12, 6, 5, 0, 2
StF: 18 vs 87	20, 20, 20, 3, 20, 8, 20, 20, 18, 16, 13, 20, 19, 20
StF: 25 vs 35	0, 1, 4, 13
StF: 25 vs 57	2, 4, 1, 4
StF: 25 vs 63	2, 7
StF: 25 vs 66	3, 1, 1, 1
StF: 25 vs 69	2, 3, 16, 9
StF: 25 vs 71	3, 1, 3, 1
StF: 25 vs 78	1, 11, 2, 1
StF: 25 vs 87	5, 1, 0, 18
Unequivocal intercolonial	
L 4 vs StF 12 (new)	9, 20, 20, 1
L 4 vs StF 18	19, 1, 1, 20
L 4 vs StF 25	20, 1, 1, 6, 2, 5, 1, 1, 20, 1, 1, 5, 20, 20
L 4 vs StF 57	1, 4, 1, 1
L 4 vs StF 60	20, 20
L 4 vs StF 63	12, 1
L 4 vs StF 66	1, 1, 1, 5
L 4 vs StF 69	0, 1, 0
L 4 vs StF 71	3, 1, 1
L 4 vs StF 78	2, 2, 2, 1
L 4 vs StF 87	20, 8, 1, 5
L 6 vs StF 3	3, 16
L 6 vs StF 12 (old)	17, 0, 1, 1
L 6 vs StF 21	7, 5, 4, 4
L 6 vs StF 253	1, 1, 10, 11
L 6 vs StF 314	20, 12, 7, 6

[a] Monitoring stations from the Institute of Forest Genetics (IFG), St. Francis Church in Novato (StF), or Larkspur (L).

[b] After 24 h, the number of workers alive in the bioassay arena for each pairing. For example, in IFG: Wc7 versus Wd7 there were 4 pairings, each with 20 workers surviving.

cated low mortality, especially when they are closely situated, may indicate a high degree of relatedness between foraging groups collected from these monitoring stations (Table 3). They could represent workers from different foraging groups of the same colony (if mortality is always low) or satellite foraging units of the same colony with infrequent interaction (if an occasional pairing results in high mortality) (Thorne 1996).

We were able to connect multiple monitoring stations for only 1 colony at IFG; the other 3 colonies that we marked apparently restricted themselves to a single monitoring station. There were only 2 intracolonial pairings for this colony; they resulted in no immediate aggression and low mortality (Table 1). At our Marin County sites, we were able to connect multiple monitoring stations for 4 separate colonies. Of the 127 intracolonial bouts, 5 resulted in immediate aggression; all with resulting low mortality. The remaining 122 bouts all resulted in low mortality (Table 2).

The great majority of the monitoring stations at IFG was not included in our mark–release–recapture studies. In Marin County, 63% of the monitoring stations were not included. Agonistic pairings among stations that were never evaluated with mark–release–recapture studies are considered intercolonial (intraphenotype) encounters, even though some of the monitoring stations may be connected. As stated above (*Interphenotype Agonism*), a majority (63.3%) of the intraphenotype, intercolonial encounters resulted in high mortality (Tables 1 and 2).

At IFG, only 8% of the intraphenotype, intercolonial bouts resulted in immediate aggression, but all of these resulted in high mortality after 24 h. No immediate aggression was observed in 92% of these pairings; of these 52.6% (151 of 287 bouts) resulted in high mortality after 24 h (Table 1). Of particular interest are the 136 bouts that resulted in either low or equivocal levels of mortality. Some of these (suspected to be intracolonial) were from monitoring stations that were ≤4 m apart and probably were inhabited by foraging groups from the same colony, even though we never connected the monitoring stations by mark–release–recapture (Table 3). Conversely, at IFG there were a few monitoring station pairs of the same cuticular hydrocarbon phenotype that showed no immediate aggression and low mortality, but that we suspect are not connected (suspected intercolonial) because of the great distances (≥50 m) between them (Table 3).

At the Marin County sites, 15% of the intraphenotype, intercolonial pairs resulted in immediate aggression; all but 1 of these resulted in high mortality after 24 h (Table 2). No immediate aggression was observed in 85% of these pairings; of these 78.2% resulted in high mortality. As with the bioassays with workers from IFG, 21.8% of the intraphenotype, intercolonial bouts with no immediate aggression resulted in low or equivocal levels of mortality (Table 2). These also were probably from monitoring stations that were inhabited by foraging groups from the same colony that were never connected by mark–release–recapture.

For example, monitoring stations StF 12 and StF 21 were occupied by cuticular hydrocarbon phenotype D foraging groups but were never connected during mark–release–recapture studies. They are 9.8 m apart. However, all 16 agonistic encounters between work-

ers from these 2 monitoring stations resulted in low mortality levels (Table 3). Likewise, StF 314 and StF 333, both occupied by phenotype D, were never connected, but are 16.2 m apart. None of the 6 bouts between workers from these monitoring stations resulted in any mortality (Table 3). There were also monitoring station pairs of the same cuticular hydrocarbon phenotype from Marin County that resulted in high mortality responses mixed with patterns of low (occasionally equivocal) mortality (see Table 3, pairings with StF 18 or StF 25).

Intraphenotype pairings of termites from monitoring stations from different cities 18.5 km apart also resulted in responses of low (occasionally equivocal) levels of mortality. When termites from station 4 from Larkspur were paired with phenotype A′ workers from Novato, 22.9% (11 of 48) of the pairings ended with low levels of mortality after 24 h (Table 3). Likewise, when station 6 from Larkspur was paired with phenotype D monitoring stations from Novato, 27.8% of the bouts ended with low or equivocal levels of mortality after 24 h (Table 3).

These monitoring stations from Larkspur obviously are not connected to any colonies in Novato, yet a large proportion of the encounters resulted in low mortality. Therefore, it is apparent that workers from foraging groups that are not part of the same colony, especially within cuticular hydrocarbon phenotype A′ from Marin County in this study, occasionally do not recognize alien workers as such, or there were no individuals in the groups of 10 that might instigate an aggressive interaction.

Discussion

We are confident that the 4 hydrocarbon phenotypes, A, B, C, and D, represent separate taxa, probably subspecies or even different species, of *Reticulitermes*. Each phenotype has a discrete hydrocarbon mixture (Haverty and Nelson 1997) and soldier defense secretion mixtures of different components or in significantly different proportions (M.I.H. et al., unpublished observations). Phenotypes A and A′ may be variants of the same species or closely related species. Their hydrocarbon mixtures are similar, with the exception of the presence or absence of 2 isomers of pentacosatriene (Haverty and Nelson 1997); however, the components and relative abundance of the soldier defense secretions are nearly identical (M.I.H., unpublished observations). Because cuticular hydrocarbon mixtures are thought to be species specific in termites (Haverty and Nelson 1997), different cuticular hydrocarbon phenotypes will not be found in the same colony. Therefore, there is no doubt that foraging groups with different hydrocarbon phenotypes in different monitoring stations are not from the same colony.

By connecting the monitoring stations with mark–release–recapture studies, we feel certain that there is an exchange of individuals among these monitoring stations, and that each represents a subdivision or foraging group of the same colony. From our bioassay it is clear that workers recognize and accept members of their colony from other foraging groups. This was demonstrated by the lack of aggressive behavior in the controls and between foraging groups that were connected by mark–release–recapture studies.

In all the cuticular hydrocarbon phenotypes of *Reticulitermes* that we studied, workers could recognize differences at the colony level, as shown by both significant immediate aggression and 24-h mortality following pairings of workers from different colonies of the same phenotype. This is in contrast to reports of a lack of, or minimal, aggressive behavior between colonies of *R. flavipes* and/or *R. virginicus* (Grace 1996, Polizzi and Forschler 1998) and *Coptotermes formosanus* Shiraki (Su and Haverty 1991, Shelton and Grace 1997). This difference could be a result of the greater genetic variation in indigenous species, such as the *Reticulitermes* from northern California, than in the introduced *R. flavipes* in Ontario and *C. formosanus* in Hawaii and Florida. And clearly, all *Reticulitermes* phenotypes in northern California recognize different hydrocarbon phenotypes and react aggressively toward them.

The termites we studied from El Dorado and Marin counties are probably indigenous. Phenotypes A, A′, and D have wide distributions throughout northern California; phenotypes B and C have been collected thus far only in Placerville and do not match other cuticular hydrocarbon surveyed from the southeastern or southwestern United States (Haverty et al. 1996, 1999; Haverty and Nelson 1997). Within each site all cuticular hydrocarbon phenotypes are sympatric and are probably active competitors. There is a high cost of not recognizing kin being excluded from food or nesting resources. Therefore, agonistic behavior is a logical mechanism for protecting resources and, at the same time, partitioning these resources with more closely related colonies.

Our studies are artificial in that they involve forced encounters that may have left the workers only 2 choices, fighting or not fighting; they were not given the option to leave. In a natural setting, other behaviors (not yet studied), such as avoidance, may occur because they are less costly. This behavior prevents cross-breeding (mixing of allospecific colonies) and thereby supports our assumption that the hydrocarbon phenotypes correlate with different species. Correlation of negative agonism within our defined colonies and positive agonism among many of the intraphenotype pairings suggests that conspecifics can distinguish between related colonies and nonrelated colonies.

The equivocal results of intraphenotype pairings may support the idea of an open–closed colony (Clément 1986). Clément (1986) expounds on Wallace's (1963) idea that a social insect colony is open when members accept alien conspecifics and closed when rejecting conspecifics through aggressive displays. Clément (1986) showed that aggression varied seasonally in 2 European species, *R. (l.) grassei* and *R. (l.) lucifugus*—open in summer and closed in winter. In an open colony, enzymatic studies suggested that

those colonies were generally polygynous. Accordingly, in geographical areas where these colonies were generally closed, monogyny tended to be prevalent.

It has long been thought that *Reticulitermes* colonies do not have a centralized nest and use budding and fusion as predominant reproductive strategies rather than establishing incipient colonies by primary reproductives (Thorne 1996). Thorne et al. (1997) estimated that it takes an average of 2 yr for *R. flavipes* in the laboratory to develop significant numbers from primary reproductives under ideal conditions. More cost-effective strategies may be simply to build strength in numbers quickly by budding from a larger colony and differentiating replacement reproductives, or by fusing 2 smaller colonies rather than waiting years to build up a population of thousands of individuals from an incipient colony.

Monogynous colonies, such as *R. (l.) banyulensis*, are composed of true siblings and show distinct intraspecific, intercolonial agonism (Clément 1980). Population expansion and genetic exchange would be possible only during swarming. Each colony, containing a pair of founding reproductives, differs genetically from the others within the same population (Clément 1981). However, polygynous colonies that were formed from budding or fusion would share a common genetic background, and genetic uniformity among at least some colonies in a population would be likely. Genetic similarity would be advantageous for open colonies, because it would homogenize recognition systems, eliminating behavioral obstacles, such as aggression, that prevent mixing of colonies. Polygynous colonies that share a common genetic background might have a harder time distinguishing between kin and nonkin where kin recognition is genetically based. Errors of both acceptance and recognition could occur when both kin and nonkin are genetically alike.

This pattern that we found with our bioassays—separate foraging groups of the same cuticular hydrocarbon phenotype that sometimes fight and sometimes do not fight—could be explained by Clément's (1986) open–closed hypothesis. If there is some genetic uniformity among colonies, even if they are separate at the time, there could be kin recognition errors of both acceptance and rejection. The recognition characters may not be discrete between homogenous colonies.

Kin discrimination also may be based upon environmental cues (Shelton and Grace 1997), and because of the artificial environment to which we exposed our foraging groups, mistakes may have been made by the workers. Studies of kin recognition in termites suggest that errors of both acceptance and recognition occur, especially when recognition is based on a finite set of endogenous and exogenous cues (Thorne and Haverty 1991, Shelton and Grace 1996). It also is quite possible that alien workers were recognized as such, but that the releasers for aggressive behavior were absent (Su and Haverty 1991).

These results demand further research into agonistic behavior in *Reticulitermes*. Immediate and delayed aggression imply more than one mechanism. Cuticular hydrocarbons play a role in recognition by Hymenoptera (Clément et al. 1987, Morel and Van der Meer 1987, Van der Meer et al. 1989, Page et al. 1991.) and termites (Howard et al. 1982b, Takahashi and Gassa 1995). Characterization of the semiochemical(s) involved will be the subject of future research by members of this group.

In summary, we have shown that agonism between colonies of the same species or between species of *Reticulitermes* from northern California is common. It was not apparent that our laboratory cultures decreased in potential for aggression over time as has been suggested (Nel 1968, Clément 1986, Shelton and Grace 1997), although we did not conduct specific tests to demonstrate this. Our monitoring stations (Lewis et al. 1998) can be used by different species of *Reticulitermes* sequentially, but only 1 species is present at a given time. Results of our mark–release–recapture studies suggest that there is not much movement among monitoring stations by workers of the same colony over a 2-wk period, and foraging site fidelity is probably common.

Therefore, we caution that the lack of exchange of marked individuals among monitoring stations does not prove that the foraging groups within adjacent or nearby monitoring stations are not part of the same colony. Movement of marked workers among stations unequivocally demonstrates that workers in different monitoring stations are members of the same colony. Conversely, lack of agonistic behavior does not confirm that workers from different, nearby foraging groups are from the same colony, whereas immediate aggression or high mortality in one or more pairings strongly indicates that the foraging groups are not from the same colony. Agonistic bioassays, coupled with characterization of the cuticular hydrocarbons (to determine species) and mark–release–recapture studies, can greatly enhance our understanding of colony foraging territory and colony distributions (Fig. 1).

Our results suggest that agonistic behavior could be a useful bioassay for determining whether or not foraging groups of *Reticulitermes* belong to the same colony as has been shown with *Heterotermes aureus* (Snyder) (Jones 1990). In some cases it is possible to determine whether groups are different species solely on the size of the soldiers and workers; phenotypes A, A′, and D are significantly larger than phenotypes B and C (Haverty and Nelson 1997). However, when morphology cannot be used, forced encounters could be used to ascertain relationships. If the termites are different species, 1–2 pairings should provide unequivocal evidence; immediate aggression would be likely and high mortality after 24 h is assured. If the workers are the same cuticular hydrocarbon phenotype or species, then $\leq$10 bouts would be necessary to determine, with minimal doubt, the relationship between the groups. If none or only a few of the pairings result in immediate aggression, but many result in high mortality after 24 h, then it is highly likely that the foraging groups belong to different colonies. If there is no

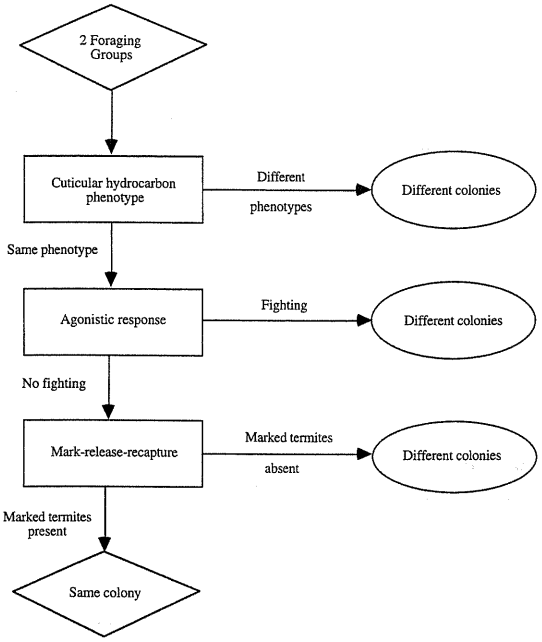

Fig. 1. Sequential use of cuticular hydrocarbon characterization, agonistic behavior, and mark–release–recapture technology to determine colony affiliation of foraging groups of *Reticulitermes*.

immediate aggression with little or no delayed mortality, then the foraging groups probably belong to the same colony, or are closely related, and use the same recognition cues for identifying kin. In the future it may be possible to use this bioassay, or semiochemical assays derived from this bioassay, to delimit colonies to reduce failures in bait treatments to suppress or eliminate *Reticulitermes* colonies.

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