Genetic Structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) Colonies in an Urban Habitat and Tracking of Colonies Following Treatment with Hexaflumuron Bait

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ABSTRACT Colony and population genetic structure was determined for *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) collected from Sentricon Termite Colony Elimination System monitoring stations at an apartment complex in Raleigh, NC. Once in each of 2000, 2001, and 2002, samples of termites were collected from monitoring stations just before the installation of bait tubes containing 0.5% hexaflumuron. Twenty workers from each sample were genotyped at five microsatellite loci. Comparison of worker genotypes among samples provided unambiguous colony associations. Analysis of worker genotypes within colonies coupled with estimates of *F*-statistics and nestmate relatedness showed that three fourths (30) of the 41 *R. flavipes* colonies and all three of the *R. virginicus* colonies were simple families headed by pairs of outbred monogamous reproductives. The remaining *R. flavipes* colonies were extended families, apparently headed by a few neotenic reproductives. Most colonies appeared to be localized, occupying only a single monitoring station. Termite pressure was initially heavy, with up to five colonies present around a single building simultaneously, but it progressively decreased over time. Of 35 *R. flavipes* colonies and 1 *R. virginicus* colony baited in 2000 or 2001, only a single *R. flavipes* colony was found again 1 yr later, but this colony was not detected the following year. These results suggest that although treatment with hexaflumuron bait successfully suppresses or eliminates *Reticulitermes* spp. colonies, new colonies can quickly move into areas vacated by treated colonies, but over time continuous baiting can reduce termite pressure and effectively protect structures.

KEY WORDS Eastern subterranean termite, microsatellite markers, colony fate, social organization, breeding system

TERMITES COMPRISE AN ECOLOGICALLY and economically important group of social insects. Ecologically, termites are major decomposers in many terrestrial ecosystems (Pearce 1997, Bignell and Eggleton 2000). As consumers of cellulose materials, many termite species are important economic pests of wooden structures. In the United States, subterranean termites (Rhinotermitidae), especially species of *Reticulitermes*, are the most widespread group, inhabiting all but the northern most regions of the mainland. The cost as a result of damage and control of *Reticulitermes* spp. is estimated at approximately $1 billion annually in the United States (Su and Scheffrahn 1998).

The cryptic nesting and foraging habits of subterranean termites have hindered our understanding of many important features of their population biology, especially the distinctness of colonies and the breeding system within colonies. Colony distinctness can potentially range from "unicolonial" populations with no clear colony boundaries, to distinct colonies that are spatially separated. The breeding structure of subterranean termites can assume a variety of forms, from simple families headed by a single pair of unrelated reproductives, to genetically complex groups with multiple reproductives. Multiple same-sex reproductives can be present in colonies, and these can differ in number, the degree of relatedness, and the extent to which they inbreed. There are several possible origins of multiple reproductives. They can arise through associations of multiple same-sex founders during colony initiation (pleometrosis), the production of neotenic (nonalate-derived) reproductives within the colony, adoption of new reproductives into established colonies, or colony fusion. Investigations of colony distinctness have been conducted using one or more of a variety of methods, including the mark-release-recapture technique (reviewed in Su and Scheffrahn 1996a), levels of agonism among workers (Jones 1990, Haverty et al. 1999), chemical phenotype, based primarily on cuticular hydrocarbons (Haverty et al. 1999), morphometric analysis (Husseneder et al. 1998, Husseneder and Grace 2001a), and genetic analysis (Husseneder et al. 1998; Bulmer et al. 2001; Husseneder and Grace 2001a, 2001b; Vargo 2003). Of these different methods, genetic markers provide the most powerful means for delineating the boundaries of col-

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onies and for determining colony affiliation for groups of foraging workers (Thorne et al. 1999, Bulmer et al. 2001, Vargo 2003). Microsatellite markers, with their codominant nature and high variability, are especially useful for investigations of colony distinctness (Vargo 2003). Microsatellite markers have recently been developed for a number of termite species, including the termitids *Macrotermes michaelseni* (Sjöstedt) (Kaib et al. 2000) and *Cubitermes subarquatus* Sjöstedt (Harry et al. 2001), the mastotermitid *Mastotermes darwiniensis* Froggatt (Goodisman et al. 2001), and the subterranean species *Coptotermes lacteus* (Froggatt) (Thompson et al. 2000), *Coptotermes formosanus* Shiraki (Vargo and Henderson 2000), *Reticulitermes flavipes* (Kollar) (Vargo 2000), and *Reticulitermes speratus* (Kolbe) (Hayashi et al. 2002).

Genetic markers are also the most practical way to determine colony breeding structure (Thorne et al. 1999, Ross 2001), and there have been a growing number of genetic studies of breeding systems in termites (Luykx 1993; Atkinson and Adams 1997; Husseneder et al. 1997, 1999; Thompson and Hebert 1998a, 1998b; Husseneder and Grace 2001a, 2001b; Goodisman and Crozier 2002; Vargo et al. 2003), including some on *Reticulitermes* spp. (Clément 1981, 1984; Reilly 1987; Jenkins et al. 1999; Bulmer et al. 2001; Clément et al. 2001; Vargo 2003). The most detailed studies to date of the eastern subterranean termite, *R. flavipes*, are those of Reilly (1987) using allozymes, Bulmer et al. (2001) using allozymes and mitochondrial DNA haplotype data, and Vargo (2003) using microsatellites and mitochondrial DNA sequence data. These studies have revealed variation in colony social organization in *R. flavipes* from very highly inbred colonies in Tennessee (Reilly 1987), to a mixture of approximately one third simple families and two thirds inbred colonies headed by many neotenic reproductives in Massachusetts (Bulmer et al. 2001), to three fourths simple families and one fourth inbred families with only a few neotenics in North Carolina (Vargo 2003). The above studies were conducted in natural areas, and there are no comparable studies performed to date in urban areas around buildings. Colony social organization in *R. flavipes* may vary in response to local ecological conditions (Bulmer et al. 2001), raising the possibility that for a given geographic area, colonies in urban habitats may differ in their social organization from those in natural habitats. If so, then results of studies conducted in natural habitats may not completely apply to urban habitats, where most structural infestations occur. Indeed, in a study of *Reticulitermes hesperus* Banks in southern California, Haagsma and Rust (1995) found differences in colony size, foraging activity, and body weight between colonies in natural and urban habitats.

In addition to providing a powerful way to determine colony distinctness and to infer colony breeding structure in subterranean termites, molecular genetic markers are useful for applied studies, such as tracking colonies over time after exposure to an insecticide treatment and determining whether termites that reappear after treatment are part of the originally treated colony or are from a neighboring untreated colony that has moved into the area (Husseneder et al. 2003). Such studies are critical in the evaluation of management practices that target specific colonies and aim to eliminate them or greatly suppress their populations.

Baiting, in which a slow-acting active ingredient is placed in a suitable food source, is used extensively to target specific subterranean termite colonies for elimination or suppression (reviewed in Su and Scheffrahn 1998, Su 2003). Bating technology relies on the transfer of the active ingredient from foragers feeding directly on the bait to other colony members through trophallactic exchange. The Sentricon Colony Elimination System (Dow AgroSciences LLC, Indianapolis, IN), which uses the chitin synthesis inhibitor hexaflumuron as the active ingredient (but this is currently being replaced by novoflumuron), was the first termite baiting system registered for use in the United States. There have been a number of studies reporting colony elimination or suppression using the Sentricon baiting system (reviewed in Su 2003), but genetic markers were not used in any of these studies to track the fate of treated colonies, which in many cases resulted in ambiguity about the identity of colonies that reappeared in treated areas.

The objectives of the current study were twofold. First, to infer the colony social organization of *R. flavipes* in an urban habitat based on colony and population genetic structure and to compare this social organization to that in nearby natural habitats (Vargo 2003). The second objective was to track the fate of individual colonies after treatment with hexaflumuron bait in a 10-building apartment complex over a 2-yr period.

Materials and Methods

The Sentricon Termite Colony Elimination System (Dow AgroSciences LLC) was installed 22 June 2000 around 10 buildings in the Apartments of Westgrove, located at 4929 Faber Drive in Raleigh, NC. A total of 234 in-ground monitoring stations were installed according to the manufacturer's specification, with 5–38 stations per structure spaced 3–6 m apart. Only 1 of the 10 buildings had an active subterranean termite infestation, and two above-ground stations (Recruit AG, Dow AgroSciences LLC) were installed over active mud tubes on this structure. Unlike in-ground stations, above-ground stations are generally used for treatment only and are typically removed after termite activity in them ceases. The installation and monitoring was carried out by a licensed pest control technician, previously authorized by Dow AgroSciences LLC to install and service the Sentricon Termite Colony Elimination System. Data on the presence of termites in monitoring stations and bait consumption were recorded using the Dolphin data scanner (Dow AgroSciences LLC), and these data were stored in the Prolinx database (Dow AgroSciences LLC).

Samples of termites (workers and soldiers) from active stations were collected only once per year during regularly scheduled monthly or quarterly service visits. In accordance with the manufacturer's protocol, all monitoring stations were checked each visit, and the monitoring devices from all active stations were removed and a Baitube (Dow AgroSciences LLC) was placed inside the station. On 17 August 2000, 29 samples were collected from in-ground stations and two from above-ground stations. Samples were again collected 17 October 2001 from 17 in-ground stations. Finally, in 2002, samples from a total of 10 stations were collected: one sample on 15 August, six on 18 October, and three samples on 22 November. In nearly all cases, 20–100 individuals were collected per sample, although in a few cases samples were limited to ≈10 individuals. Live termites were placed directly into vials containing 95% ethanol and were stored at 4°C until extracted. Soldiers were examined for species identification using the key of Scheffrahn and Su (1994). Voucher specimens have been deposited in the North Carolina State University Insect Collection.

Genomic DNA was extracted from individual termites using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA). A total of 899 workers were genotyped at each of five microsatellite loci: *Rf 1–3*, *Rf 5–10*, *Rf 6–1*, *Rf 15–2*, and *Rf 24–2*. Details of the polymerase chain reaction conditions and genotype scoring procedures are given in Vargo (2000). Mendelian inheritance and conformance to Hardy-Weinberg equilibrium of all five loci have been previously confirmed in nearby populations (Vargo 2003). To determine whether workers in different samples belonged to the same or different colonies, comparisons were made of the allelic composition and genotypes present in all pairs of samples to ascertain whether there were private alleles (those present in only one sample in each pair of samples) and/or unique genotypes. In addition, F_{ST}-values were generated for all pairs of samples. Workers from different samples were considered to belong to the same colony if the F_{ST} between collection points was not significantly different from zero (based on 95% confidence intervals overlapping zero), and if the workers had all the same alleles present. As it turned out, pairs of samples that did not have significant F_{ST}-values shared all the same alleles and had identical genotypes, whereas sample pairs with significant F_{ST}s differed in the presence of many alleles. Because of the high variability of the markers and the close genetic affinities of colony mates (see "Results"), colony identifications were straightforward and unambiguous.

Colonies were classified as simple families or extended families based on worker genotypes. A colony was considered a simple family if workers had genotypes consistent with being the progeny of a single monogamous pair of reproductives, and if the frequencies of the genotypes did not differ significantly from Mendelian ratios, i.e., those expected for the offspring of a monogamous pair of reproductives. Significant deviation from Mendelian ratios was determined by performing a *G*-test for goodness-of-fit between observed and expected genotypes for each locus and then producing an overall *G*-value for each colony by summing the locus specific *G*-values across the five loci. A colony was considered an extended family if there were more worker genotypes than is possible with a single pair of reproductives, or if Mendelian genotypes were present but their frequencies differed significantly from expected based on the *G*-test ($P < 0.05$). The term "extended family" seemed appropriate for these colonies, because they all had genotypes consistent with being headed by neotenic reproductives descended from the founding primary king and queen, or possibly a combination of one or more primaries together with neotenics (see "Results").

To examine colony and population genetic structure, *F*-statistics were estimated for each species using the program Genetic Data Analysis (Lewis and Zaykin 2000). I followed the notation of Thorne et al. (1999), in which genetic variation is partitioned among the individual (I), the colony (C), and total (T) components. Using this notation, F_{IT} is equivalent to the standard inbreeding coefficient F_{IS}. F_{CT} is similar to F_{ST} and represents genetic differentiation among colonies. F_{IC} represents the colony inbreeding coefficient, which is expected to be strongly negative for simple families, will increase toward zero with greater numbers of reproductives, and will become positive if there is assortative mating among multiple groups of reproductives within colonies or there is mixing of individuals from different colonies. Ninety five percent confidence intervals (CIs) were generated by bootstrapping over loci 1,000 times. Relatedness among nestmate workers was estimated using the program Relatedness 5.08 (Queller and Goodnight 1989) with colonies weighted equally, and 95% CIs were generated by jackknifing over loci. To infer features of the breeding system in each species, the *F*-statistics and relatedness coefficients were compared with previously published values generated by computer simulations for possible breeding systems of subterranean termites (Thorne et al. 1999, Bulmer et al. 2001). In addition, simulations were performed using the methods of Thorne et al. (1999) to generate values for a population consisting of three fourths simple families and one fourth extended families with few neotenics, corresponding to the composition of the *R. flavipes* colonies (see "Results").

The quantity of bait consumed by all colonies sampled in 2000 and 2001 was used to investigate possible factors that might help explain the persistence of colony BR00-25, the colony that was present in both 2000 and 2001 (see "Results"). I retrieved information on the amount of bait consumed 1 mo after samples were collected (at which time the Baitubes were installed) from the Prolinx data base. The following categories of bait consumption were recorded: bait tube full (no consumption), 75–99% remaining, 51–74% remaining, 26–50% remaining, 1–24% remaining, and empty (100% consumed). Consideration was given to tracking bait consumption over a longer period; however, because samples were collected only once per year, there was no way to verify whether the same colonies

Table 1. Allelic diversity of microsatellite markers in 41 *R. flavipes* colonies and three *R. virginicus* colonies in an urban North Carolina study area

Locus	*R. flavipes*		*R. virginicus*	
	No. alleles	Freq. of most common allele	No. alleles	Freq. of most common allele
Rf 1–3	11	0.28	5	0.53
Rf 5–10	7	0.57	2	0.71
Rf 6–1	11	0.53	4	0.42
Rf 15–2	4	0.91	1	1.0
Rf 24–2	26	0.10	4	0.53
Mean ± SD	11.8 ± 7.6		3.1 ± 1.5	

that were genotyped were those that continued feeding on the bait months after the samples were collected. Thus, to avoid possible confusion over the identity of colonies feeding on baits, analysis was limited to bait consumption data for the first month after installation.

Results

Colony Designations. Of the 58 samples, 53 (91%) were *R. flavipes*, whereas the remaining samples were *Reticulitermes virginicus* (Banks), with four of the five samples of the latter species turning up during the last sampling period. The high level of variability of the microsatellite loci (Table 1), together with the close family structure of colonies (see below), made for straightforward differentiation among colonies. Examples of the clear genetic distinction between colonies of *R. flavipes* are shown in Table 2. Colony designations unambiguously showed a total of 45 colonies when summing the number of colonies present each year (Table 3); however, as discussed below, there was one *R. flavipes* colony present in both 2000 and 2001 (Table 2). There was strong and significant differentiation among the *R. flavipes* samples belonging to different colonies [mean (±SD) $F_{ST} = 0.26 \pm 0.07$; range, 0.12–0.45]. Moreover, despite the fact that no sample had more than four alleles at a locus, all pairs of samples considered to belong to different colonies had an average of 13.7 ± 2.7 private alleles (range, 5–23) for an average of 2.7 private alleles per locus. In fact, the number of private alleles in these pair-wise comparisons accounted for approximately two thirds (68.2%) of the total number of alleles (mean = 20.1 ± 2.0) present in each of the colony pairs being compared, indicating that the samples considered as different colonies were genetically distinct groups. In contrast, pairs of *R. flavipes* samples considered to belong to the same colony had F_{ST}-values not significantly different from zero (mean = 0.01 ± 0.02, range, 0.0–0.05). In addition, pairs of samples from the same colony had identical alleles across all loci, as well as the same genotypes present among the workers (see example in Table 2). Finally, relatedness between workers in different samples belonging to the same colony were high ($r = 0.471 \pm 0.064$) and nearly identical to the relatedness values among workers in the same sample ($r = 0.464 \pm 0.078$) and among all members of the presumed colony ($r = 0.449 \pm 0.071$).

Table 2. Genotypes at two representative loci of *R. flavipes* worker groups collected from the same monitoring station in 2000 and 2001; samples BR00-25 and BR01-16 were from the same colony, whereas BR00-4 and BR01-3 belonged to different colonies

Locus/ genotype	Same station/ same colony		Same station/ different colonies	
	BR00-25 ($n = 20$)	BR01-16 ($n = 10$)	BR00-4 ($n = 20$)[a]	BR01-3 ($n = 20$)[a]
Rf 5–10				
147/153				4
147/162				2
150/153			9	
150/162			9	
153/153	9	5		4
153/162	11	5		10
Rf 24–2				
128/155	3	2		
128/176	4	3		
155/194	8	4		
161/188			9	
161/191			7	
164/179				7
164/200				5
170/179				3
170/200				3
176/194	5	1		
179/188			3	
179/191			1	

[a] Only 18 genotypes were available for *Rf 24–2*.

Only three *R. virginicus* colonies were found at monitoring stations, one during 2001 and two during 2002. As with the *R. flavipes* samples, colony designation was unambiguous. Pairwise F_{ST}-values between samples belonging to different colonies were significantly greater than zero (mean $F_{ST} = 0.24 \pm 0.03$), whereas those belonging to the same colony were much lower and not significant (mean $F_{ST} = 0.00 \pm 0.02$). In addition, samples belonging to different colonies had an average of 3.2 ± 1.5 private alleles, whereas those from the same colony shared all the same alleles and had identical genotypes. Finally, all three *R. virginicus* colonies were simple families, making the colony designations for these samples especially clear.

Most of the 45 colonies found were present during the first sampling period, followed by successive declines in both the number of monitoring stations being

Table 3. Numbers of colonies of *R. flavipes* and *R. virginicus* present in samples collected from monitoring stations around buildings

Year	Total no. samples	No. *R. flavipes* colonies	No. *R. virginicus* colonies	Total no. colonies
2000	31	24	0	24
2001	17	12[a]	1	13[a]
2002	10	6	2	8
Total	58	41	3	44

[a] Includes a colony sampled in both 2000 and 2001; this colony is counted only once in the total.

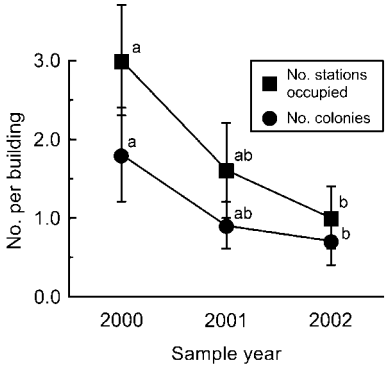

Fig. 1. Decrease in the mean (±SE) number of active monitoring stations and number of subterranean termite colonies detected per building at a 10-building apartment complex during three years of sampling. There was a significant difference among samples in each comparison (one-way analysis of variance; both $F_{2,27} \geq 3.31, P \leq 0.05$). Samples with different lower case letters in each comparison differed significantly ($P < 0.05$, Tukey test).

"hit" and by the number of colonies detected (Table 3; Fig. 1). The decrease in both the number of active monitors and number of colonies present around buildings over time were significant, with the 2002 sample containing significantly fewer than the 2000 samples in both cases (Fig. 1). This reduction was caused by fewer buildings being hit and fewer hits in the buildings with active monitors. In 2000, 8 of the 10 buildings had termites present in monitors with a maximum of eight active stations in a building. In 2001, there were seven buildings with occupied monitors and a maximum of six active monitors at a building. By 2002, there were five buildings with active monitors and the maximum number of monitors hit at a building was three.

Table 4. Numbers of simple families and extended (inbred) families of *R. flavipes* present in each of the samples collected in an urban habitat in North Carolina

Year	Total no. colonies	No. simple families (%)	No. extended families (%)
2000	24	18 (75.0%)	6 (25.0%)
2001	12[a]	7[a] (58.3%)	5 (41.7%)
2002	6	6 (100.0%)	0 (0.0%)
Total	41	30 (73.2%)	11 (26.8%)

[a] Includes a colony sampled in both 2000 and 2001; this colony is counted only once in the total.

Figure 2 shows the locations of the different colonies sampled in 2000, at which time only *R. flavipes* was found. There were 31 samples representing 24 colonies, with five colonies occupying two monitoring stations and one colony occupying three stations. Interestingly, the two above-ground stations placed in the front and back of the same apartment unit were occupied by workers from different colonies. These samples were the only ones collected from colonies known to be infesting any of the structures. Activity in these stations ceased in October 2000 in one case and April 2001 in the other, and the stations were removed on these dates. As seen in Table 4, 75% of the colonies sampled in 2000 were simple families.

In 2001, all but one of the stations contained *R. flavipes*, representing 12 colonies (Fig. 2), 7 of which were simple families. Two colonies occupied three stations each, and both were simple families. Five colonies occupied monitoring stations that had been active during the previous year's sample. In four cases, the possibility that the new occupants belonged to the previous colony could be excluded based on the large number of private alleles occurring between pairs of colonies collected from the same monitoring station during different years (mean = 13.3 ± 2.2, range,

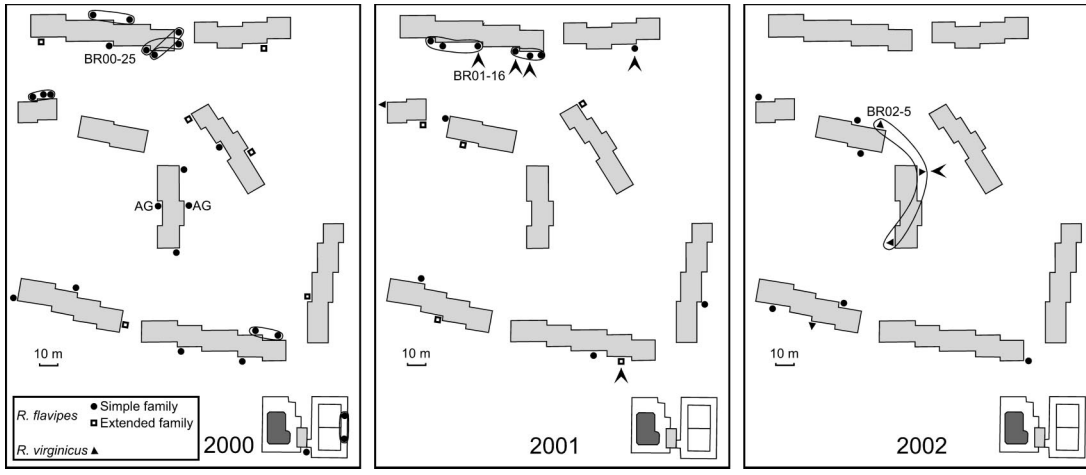

Fig. 2. Location of occupied monitoring stations during the three sampling periods. Except for the two above-ground (AG) stations, all stations were in-ground. Different stations containing termites from the same colony are encircled. Arrows mark stations in which termites had been collected during a previous sampling period.

11–16) and the high F_{ST}s between the colony pairs (mean $F_{ST} = 0.248 \pm 0.077$, range, 0.20–0.36). Moreover, the average degree of relatedness between nonnestmate workers in each pair was not significantly different from zero ($r = -0.033 \pm 0.086$), whereas relatedness among nestmates was equivalent to that between siblings ($r = 0.477 \pm 0.099$). Thus, it is clear from these analyses that in four cases the colonies present in 2001 were different from those that occupied the same monitoring stations in 2000. The one exception was workers from sample BR01-16, which were collected in 2001 from the same monitoring station (designated BR00-25) in 2000; these samples had the same alleles and same genotypes at every locus indicating they were part of the same colony (Table 2). Furthermore, the coefficient of relatedness between workers in the two samples ($r = 0.539$) was indistinguishable from the within-sample relatedness values ($r = 0.536$ and 0.534, for samples BR00-25 and BR01-16, respectively). Therefore, this colony, which was a simple family, went from occupying a single monitoring station in 2000 to occupying three adjacent monitoring stations in 2001. The four samples, one from 2000 and three from 2001, were considered a single colony and treated as such in subsequent analyses. The one *R. virginicus* colony found in 2001 was a simple family, and it occupied a monitoring station in which an *R. flavipes* colony had been present in 2000.

The 10 samples collected in 2002 represented eight colonies, of which six were *R. flavipes* occupying a single monitoring station each, and two were *R. virginicus*, one of which occupied a single station, whereas the other was found in three stations separated by up to 54.3 m. All of the colonies of both species collected in 2002 were simple families.

Colony Genetic Structure. All colonies of both species formed close family units. Despite the highly variable nature of the microsatellite markers in the *R. flavipes* samples (Table 1), there was a maximum of four alleles found at a locus in every colony. These results are consistent with each colony being a simple family or an extended family descended from a simple family. Because only three colonies of *R. virginicus* were sampled, far fewer alleles were found in this species. However, larger sample sizes of this species collected from the same area around Raleigh, NC, show similar levels of variability to those found in *R. flavipes* (C. DeHeer and E. Vargo, unpublished data). The three *R. virginicus* colonies investigated in the current study were simple families and were genetically distinct.

The *F*-statistics and relatedness coefficients are shown in Table 5, along with values generated by previous studies (Thorne et al. 1999, Bulmer et al. 2001) in which different possible breeding systems of *Reticulitermes* spp. were simulated. The *R. flavipes* colonies did not show signs of significant levels of inbreeding based on the observation that the 95% CIs for the F_{IT}-values overlapped zero. Values for all the *F*-statistics and for the relatedness coefficient among the simple family colonies were nearly identical to the values expected for simple families headed by outbred reproductives (Table 5, case A). The extended families had low levels of inbreeding compared with those expected for neotenic-headed colonies (Table 5, cases B1–6), and in particular, had strongly negative F_{IC}-values, suggestive of colonies with low numbers of reproductives. The values for the extended family colonies are a reasonably good match to the values expected for colonies headed by two female and one male neotenic that are the direct offspring of the original primary reproductives (Table 5, case C2i), although we cannot rule out other possible colony types with low numbers of reproductives, such as one or more primaries together with neotenics. The results of the computer simulations for a population consisting of 75% simple family colonies headed by monogamous reproductives and 25% extended family colonies with female neotenics and one male neotenic who are the direct offspring of the founding pair (Table 5, case C3) provide a reasonable fit to the data for all the *R. flavipes* colonies combined.

All of the *R. virginicus* colonies were simple families with *F*-statistics and relatedness coefficients very similar to those for the *R. flavipes* simple families as well as to the values expected for simple families headed by a pair of outbred monogamous reproductives (Table 5, case A).

Colony Spatial Organization. By far, most colonies were detected at only a single monitoring station. Fewer than 20% of the *R. flavipes* colonies simultaneously occupied two or more monitoring stations (Table 6). Five colonies, all sampled in 2000, were found at two stations, and three colonies, one sampled in 2000 and two sampled in 2001, were found at three monitoring stations. Each of these colonies occupied stations around a single building. All of these colonies were simple family colonies, although simple family colonies were not significantly more likely to occupy multiple stations than were extended family colonies ($X^2_1 = 0.209$, $P = 0.15$). In *R. flavipes*, the maximum linear distance between monitoring stations occupied by the same colony was 23.5 m (colony BR01-16 found at three stations, Fig. 2). Only one of the three *R. virginicus* colonies, sampled in 2002 (BR02-5, Fig. 2), was found at multiple stations. These were located around two different buildings, and the maximum linear distance between stations occupied by this colony was nearly twice that found in *R. flavipes*.

Bait Consumption by Colonies. There was considerable variation in the quantity of bait consumed (Fig. 3). Of the 24 colonies baited in 2000, nearly one half (11) consumed all of the bait within 1 mo of installation. The other colonies consumed various amounts, including six colonies (25%) that did not consume any bait. Colony BR00-25, the only colony found in more than 1 yr, was present in one station in 2000 and had consumed only 1–25% of the bait placed in that station. However, in 2001, this colony occupied three stations, all of which were subsequently baited. No detectable bait had been consumed in any of these stations 1 mo after baiting in 2001. However, two of these stations were baited again in spring 2002, and in both of these

Table 5. *F*-statistics and relatedness coefficients for worker nestmates of *R. flavipes* and *R. virginicus* from the urban North Carolina study site and expected values for possible breeding systems as derived from computer simulations

Species/Colony type	F_{IT}	F_{CT}	F_{IC}	r
R. flavipes				
All colonies (n = 41) (95% CI)	0.048 (−0.048 to 0.123)	0.262 (0.199 to 0.313)	−0.291 (−0.311 to −0.272)	0.496 (0.389 to 0.603)
Simple families (n = 30) (95% CI)	0.010 (−0.062 to 0.062)	0.251 (0.200 to 0.292)	−0.322 (−0.343 to −0.294)	0.478 (0.358 to 0.599)
Extended families (n = 11) (95% CI)	0.167 (−0.016 to 0.359)	0.296 (0.176 to 0.395)	−0.183 (−0.245 to −0.068)	0.538 (0.434 to 0.641)
R. virgincius				
All colonies (simple families, n = 3) (95% CI)	0.015 (−0.067 to 0.079)	0.235 (0.128 to 0.334)	−0.288 (−0.434 to −0.160)	0.473 (0.095 to 0.851)
Simulated breeding system				
(A) Simple families headed by outbred reproductive pairs[a]	0.00	0.25	−0.33	0.50
(B) Extended families with inbreeding among neotenics				
(1) $N_f = N_m = 1$, $X = 1$[a]	0.33	0.42	−0.14	0.62
(2) $N_f = N_m = 1$, $X = 3$[a]	0.57	0.65	−0.22	0.82
(3) $N_f = 2$, $N_m = 1$, $X = 3$[a]	0.52	0.59	−0.17	0.78
(4) $N_f = N_m = 10$, $X = 1$[b]	0.33	0.34	−0.01	0.51
(5) $N_f = N_m = 10$, $X = 3$[a]	0.37	0.38	−0.02	0.56
(6) $N_f = 200$, $N_m = 100$, $X = 3$[a]	0.33	0.34	−0.00	0.50
(C) Population of 75% simple families and 25% extended families[c]				
(1) Simple families headed by outbred reproductive pairs	0.00	0.27	−0.36	0.53
(2) Extended families with inbreeding among neotenics				
(i) $N_f = 2$, $N_m = 1$, $X = 1$	0.26	0.35	−0.14	0.55
(ii) $N_f = N_m = 2$, $X = 1$	0.26	0.32	−0.09	0.51
(iii) $N_f = 5$, $N_m = 1$, $X = 1$	0.27	0.34	−0.11	0.53
(iv) $N_f = N_m = 5$, $X = 1$	0.27	0.29	−0.03	0.46
(3) Simple families and extended families ($N_f = 2$, $N_m = 1$, $X = 1$) combined	0.07	0.29	−0.31	0.54

For the simulated breeding systems, X represents the number of generations of production of replacement reproductives within a colony; N_f and N_m represent the number of replacement females and males, respectively, produced per generation.

[a] From Thorne et al. (1999)

[b] From Bulmer et al. (2001)

[c] Results of simulations performed using the methods of Thorne et al. (1999).

Table 6. Spatial organization of *R. flavipes* and *R. virginicus* colonies in a North Carolina urban habitat

Species	No. colonies	No. colonies occupying multiple stations (%)	Intercolony distance between stations (m)	
			Mean (±SD)	Maximum
R. flavipes	41	8 (19.5%)	14.3 ± 4.8	23.5
R. virginicus	3	1 (33.3%)	54.3	54.3

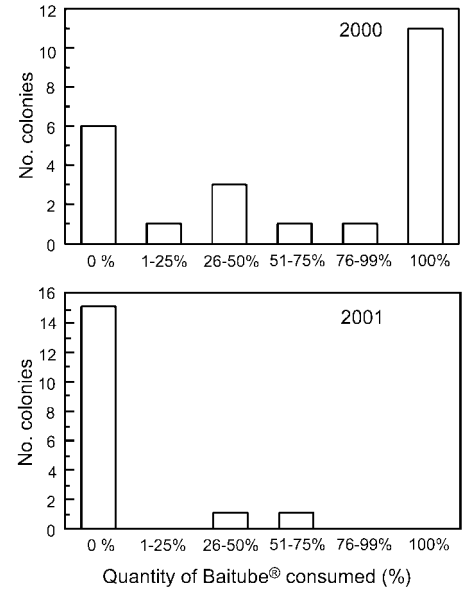

Fig. 3. Quantity of 0.5% hexaflumuron bait consumed by colonies within 1 mo of bait installation.

stations the entire Baitube was consumed; this colony was not detected again in the 2002 sample collected in November. In fact, of the 17 stations receiving Baitubes in October 2001, noticeable quantities of bait were consumed in only two cases (Fig. 3). It should be noted that 11 of the 15 stations in which no perceptible quantity of bait was consumed in 2001 contained termites during at least one of the subsequent monthly service visits, and these were baited again before sampling in 2002. In five cases, they were baited a third time before sampling in 2002. In these subsequent baitings, the entire Baitube was consumed in three cases, 26–75% was consumed in another two cases, and 1–25% was consumed in two cases, for a total of seven cases in which some perceptible quantity of bait was consumed upon rebaiting.

Discussion

The present results provide further evidence for the power of moderately to highly variable microsatellite markers to elucidate the breeding structure of subterranean termite colonies and to determine colony affiliations of worker groups, including colony identity of foragers that reappear in monitoring stations months or years after baiting. The results clearly show that all of the *R. flavipes* investigated were either simple families or extended families descended from simple families. Nearly three fourths of the colonies were simple families headed by outbred reproductives. The remaining colonies appeared to have low numbers of neotenics that were not highly inbred. Indeed, estimates of *F*-statistics and relatedness among colony mates suggest that, on average, the extended family colonies were headed by only two female neotenic and one male neotenic that were the direct offspring of the original founding pair. These results are very similar to those in a recent study of *R. flavipes* colonies in three natural forest habitats located 3.5–27 km away from the current study site (Vargo 2003). In the previous study, a total of 56 colonies were investigated, and, as in the current study, all of those colonies were either simple families or extended families derived from simple families. Moreover, 76.8% (43) of the colonies in the forest habitat were simple families, a percentage nearly identical to that in the current study (73.2%). The *F*-statistics and relatedness values for the simple-family colonies in natural habitats also strongly indicated that they were headed by outbred primary reproductives. The extended family colonies had *F*-statistics and relatedness values very similar to those in the current study ($F_{IT} = 0.21$, $F_{CT} = 0.34$, $F_{IC} = -0.20$, $r = 0.51$), suggesting that these colonies also had low numbers of neotenics that were the direct progeny of the original founding pair. Thus, the social organization of colonies in urban habitats in central North Carolina appears to be essentially the same as those found in natural habitats with regard to the types of colonies present (simple and extended families), their relative proportions, and the degree of inbreeding.

All three *R. virginicus* colonies investigated were simple families, apparently headed by outbred reproductives. Although the present results provide the first genetic analysis of social organization of *R. virginicus* colonies, the sample size is too small to draw any strong conclusions about this species, and it is likely that extended family colonies occur with some frequency (Snyder 1915).

Social organization of *R. flavipes* colonies in central North Carolina appears to differ somewhat from two other populations of this species that have been studied using allozyme markers. In a middle Tennessee forest, Reilly (1987) found workers were highly inbred ($F_{IT} = 0.62$) and that the coefficient of inbreeding in individuals relative to the colony was highly positive ($F_{IC} = 0.26$), suggesting extensive inbreeding within colonies with mixing of workers from either different reproductive centers within the colony or from different colonies altogether (Thorne et al. 1999). Reilly (1987) did not provide information on the family structure of individual colonies. In Massachusetts, Bulmer et al. (2001) found differences between two forest sites separated by 0.5 km. At one site, simple families headed by outbred reproductives com-

prised 37.5% (6 of 16) of the colonies, whereas the other colonies at this site were inbred with values of the relatedness coefficient and *F*-statistics indicative of tens or hundreds of neotenics inbred for several generations. At the other site, these authors found six colonies that showed signs of high levels of inbreeding and some commingling of workers from different reproductive centers or from different colonies. Bulmer et al. (2001) attributed the differences in colony social organization between sites to variation in age structure and/or soil conditions. Although there may be variation in *R. flavipes* social organization between different geographic regions and even between nearby sites within a region, the present results show that in central North Carolina social organization can be fairly uniform in habitats as different as undisturbed forests and an urban apartment complex.

The present results suggest that colonies of *R. flavipes* in the study population are fairly localized with a limited foraging range. More than 80% of the colonies detected were found only at a single monitoring station, and the maximum distance spanned by a single colony was 23.5 m. Again, these results are consistent with those from nearby forested areas, where termites were collected from natural wood debris at 15-m intervals, and only a single colony out of 56 sampled was found in two adjacent sampling points (Vargo in press). Combining the present findings with the previous results from natural areas ($n = 97$), it appears that *R. flavipes* colonies in central North Carolina in both natural and urban areas are localized, with foraging ranges generally not extending much >15–20 linear m. Results from detailed analysis of foraging areas of 31 *R. flavipes* colonies over a 2-yr time span in forested habitats near Raleigh also show limited ranges (C. DeHeer and E. Vargo, unpublished data). Thus, the spatial organization of *R. flavipes* colonies in urban habitats does not appear to be different from that in natural areas. Although there were only three *R. virginicus* colonies found, it is interesting that one colony had a foraging range nearly twice that of the most expansive *R. flavipes* colony. These results are consistent with findings that *R. virginicus* colonies are frequently much more expansive than sympatric colonies of *R. flavipes*, including one *R. virginicus* colony from a natural habitat near Raleigh that spanned 120 linear m (E. Vargo and J. Carlson, unpublished data).

The rather limited foraging ranges of *R. flavipes* colonies in the study population are similar to those reported for this species in urban habitats in Georgia (Forschler and Ryder 1996), but smaller than those found in other areas. In Toronto, Grace et al. (1989) studied two colonies and found linear foraging distances of 48 and 79 m. In south Florida, Su et al. (1993) reported foraging distances in residential sites of up to 71 m. Studies of other *Reticulitermes* spp. also reported limited foraging ranges in urban habitats, e.g., *R. hesperus* in California (Haagsma and Rust 1995; Haverty et al. 1999, 2000) and *R. speratus* in Japan (Tsunoda et al. 1999). Thus, although *R. flavipes* colonies can sometimes forage relatively long distances, the localized foraging activity found in the current study may be more typical of this species in many parts of its range and appears to be similar to that found in other *Reticulitermes* species.

The combination of highly variable markers and close family structure of colonies allow for a high degree of certainty in determining colony affinities, even in samples collected months or years apart. Using this method, I was able to collect information on 44 colonies, including 36 colonies that had been baited. This is a far greater number of colonies than has been previously reported in any single study. Su (2003) recently reviewed the literature involving field trials of hexaflumuron bait to control subterranean termites. Out of a total of 41 studies, the mean ($\pm$ SD) number of colonies tracked over time was 3.9 $\pm$ 4.9, with a maximum of 22 reported in the study of Kistner and Sbragia (2001) on *R. hesperus* in California. Clearly, the use of genetic markers, such as microsatellites, to "fingerprint" and track the fate of colonies allows for a substantially greater number of colonies to be studied than is practical using other methods.

In the current study, there was a surprisingly large number of colonies around buildings, averaging more than two per building at the start of the study with a maximum of five colonies around a single building. There have been a few other studies of *R. flavipes* reporting up to two colonies present simultaneously around structures as determined by mark-release-recapture (Forschler and Ryder 1996, Su and Scheffrahn 1996b, Prabhakaran 2001), but generally only a single colony has been found at one time (e.g., Su et al. 1993, Su 1994, Potter et al. 2001). Using cuticular hydrocarbon and mark-release-recapture data in California, Haverty et al. (2000) found two cases in which there were two colonies of *R. hesperus* present simultaneously at the same structure. Thus, the large numbers of colonies found in the current study are among the highest reported to occur simultaneously next to structures for any *Reticulitermes* spp., and results on residential properties in the Raleigh, NC, area indicate that it is not uncommon to find five or more colonies of *R. flavipes* present at one time around a structure (V. Parman and E. Vargo, unpublished data).

That only one colony out of 36 colonies that were baited in 2000 and 2001 was found again, and that this colony was not detected again in 2002, is consistent with elimination or substantial suppression of the baited colonies. Furthermore, this elimination/suppression generally occurred within 1 yr after baiting. If the colonies were indeed eliminated or significantly suppressed, then these results support the findings of several other studies documenting elimination or suppression of subterranean termite colonies by hexaflumuron baits (reviewed in Su 2003). However, as with any study attempting to track the fate of colonies in the field, the lack of detection of a colony does not necessarily mean that it was eliminated, only that no foragers of that colony were present at any of the monitoring stations at the time samples were collected. An alternative explanation is that almost all of the baited colonies moved out of the monitored area

after a few months, resulting in a nearly complete turnover each year. This seems unlikely, because it would mean that during both years of the study all colonies moved away from structures into the surrounding unmonitored area before the next year's sample, and that none relocated near structures where they could be found in monitoring stations. Moreover, in a detailed longitudinal study of *R. flavipes* colonies in natural habitats, we have detected very little movement of colonies over a 2-yr period (C. DeHeer and E. L. Vargo, unpublished data). Thus, the elimination or suppression of colonies is the most reasonable explanation for the failure to find most colonies again 1 yr after treatment.

There was a steady decline in both the average number of colonies detected around structures over time, from nearly two per structure at the beginning of the study to fewer than one by the end of the study 2 yr later. These results suggest that termite pressure declines over time with an on-going baiting program. Presumably, the elimination or suppression of colonies after baiting leads to unoccupied foraging areas that can be exploited by either established neighboring colonies or by newly founded colonies. These colonies may in turn discover and begin feeding on the monitoring stations, themselves becoming targets for baiting. It is possible that these colonies reuse foraging tunnels excavated by the original colony, thus facilitating the finding of previously visited monitoring stations (Grace and Su 2001). One would expect that, over time, the number of colonies found around structures would stabilize, reaching an equilibrium between neighboring established colonies relocating or expanding into vacated areas around structures and the foundation of new colonies in the area. Despite the continued but declining presence of termite colonies around the structures, none of the buildings had active termite infestations after the activity ceased in the above-ground stations in April 2001, indicating that the Sentricon Colony Elimination System effectively protected the structures.

In conclusion, microsatellite markers are powerful tools for inferring the breeding structure of subterranean termite species, for delineating the foraging ranges of colonies, and for differentiating between colonies. The results obtained here show that *R. flavipes* colonies in a central North Carolina urban site had localized foraging areas, and were comprised of a majority of simple families with a minority of extended families headed by inbred descendants of simple families. The results were very similar to previous findings on this species from nearby natural areas, indicating no major differences in the spatial or social organization of this species across two very different habitats. Finally, the markers allowed the tracking of large numbers of colonies after bait treatment, and analysis of these data suggest that hexaflumuron bait resulted in elimination of colonies or at least significant suppression of the colony population. Additional studies using these markers, or other genetic markers with similar properties, promise to greatly expand our understanding of subterranean termite social organization and foraging ranges. Moreover, future studies with these markers should improve our ability to evaluate termite management technologies through increased capacity to accurately detect colony elimination and/or suppression.

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