Population Density, Species Abundance, and Breeding Structure of Subterranean Termite Colonies in and Around Infested Houses in Central North Carolina

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ABSTRACT Pressure from subterranean termites is known to vary geographically across the United States, but there are few quantitative studies concerning the threat of structural infestation for any geographic region. We assessed the number and locations of termite colonies present on 20 infested residential properties in central North Carolina, where subterranean termite pressure is considered to be heavy. This was achieved by using microsatellite markers to determine colony identity of termites collected over 6–14 mo from mud tubes in structures, below-ground monitors, and wood debris in the yard. In total, we identified 188 distinct colonies and determined their breeding structures. *Reticulitermes flavipes* (Kollar) was by far the most common species, accounting for nearly 90% of all colonies; the remaining colonies belonged to *Reticulitermes hageni* Banks and *Reticulitermes virginicus* (Banks). In four cases, there were two colonies infesting a structure simultaneously; in all other cases only a single colony was detected in the structure. Colony densities were high, averaging 62 colonies per ha (25 per acre) with a maximum of 185 colonies per ha (75 colonies per acre). Foraging ranges of *R. flavipes* and *R. hageni* colonies were generally small (<30 linear m), and most colonies were headed by a single pair of monogamous reproductives with nearly all the remaining colonies headed by relatively few inbreeding descendants of the original monogamous pair. These results provide the most detailed picture to date of the number, distribution, and colony characteristics of subterranean termite colonies located in and around residential structures.

KEY WORDS *Reticulitermes flavipes*, *Reticulitermes virginicus*, *R. hageni*, microsatellites, population genetics

Subterranean termites are major economic pests in the United States, where they account for >$1 billion per yr in damage and control costs (Su and Scheffrahn 1998). Despite their economic importance, many fundamental features of the ecology and population biology of subterranean termites remain poorly understood. The main obstacle to progress in these areas has been the difficulty of identifying and delineating individual colonies by using traditional field methods. Because populations of subterranean termites, like those of other social insects, are made up of collections of individual colonies (Thorne et al. 1999), acquiring such basic information as colony density, boundaries of colony foraging areas, and colony–colony dynamics, requires that individual colonies be located and their positions mapped. Several studies have used the technique of mark–release–recapture to investigate subterranean termite populations by marking and mapping individual colonies, especially around structures (Su and Scheffrahn 1988, Grace 1990, Su et al. 1993, Tsunoda et al. 1999, Haverty et al. 2000, Nobre et al. 2007). However, there are several limitations of this method that make it difficult to study large numbers of colonies. Among the major drawbacks are 1) the large number of termites needed to effectively mark a colony because of the low recapture rate; 2) the amount of time and effort required to install traps, collect, mark and release termites, and then monitor traps for the presence of marked termites; 3) the limited number of dyes available for marking; and 4) the short-lived nature of the dyes (Thorne et al. 1996).

Molecular markers have emerged as a powerful means to provide unequivocal identification of large numbers of colonies to investigate colony foraging areas and population dynamics, as well as for inferring colony breeding structure (reviewed in Husseneder et al. 2003). For example, in a recent study, Vargo et al. (2006a) identified 49 different colonies of four species of subterranean termites in a South Carolina state park from samples collected over a 2-d period. In a longer term study, DeHeer and Vargo (2004) mapped the locations of 32 colonies of three species of *Reticulitermes* at two undisturbed field sites and followed the colony dynamics over a 3-yr period, including the first documentation of colony fusion of subterranean ter-

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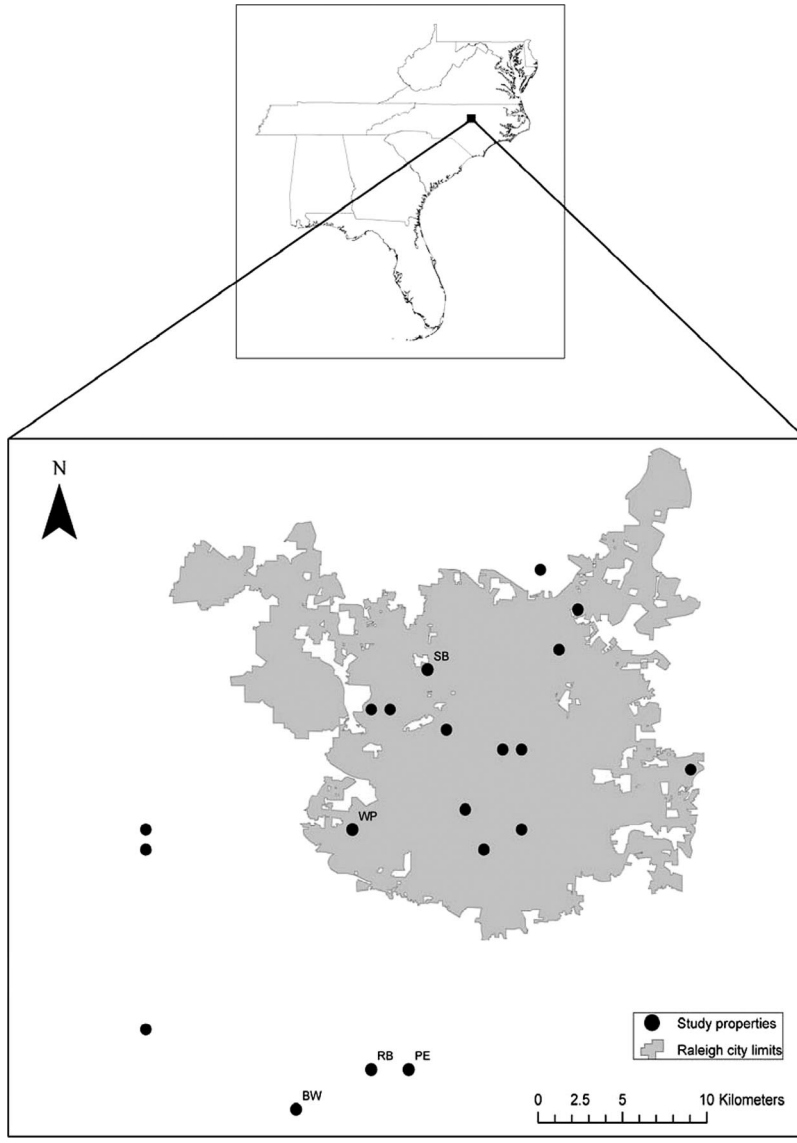

Fig. 1. Locations of study properties located in Wake County, NC. Details maps of properties BW and WP are provided in Figs. 3 and 4. The other locations indicated refer to properties mentioned in the text.

mites in the field. Finally, in a study of colonies located near structures, Vargo (2003a) monitored the fate of 35 colonies of *Reticulitermes flavipes* (Kollar) and one colony of *Reticulitermes virginicus* (Banks) for at least 1 yr after feeding on hexaflumuron bait.

To obtain a more detailed view of the number and distribution of *Reticulitermes* sp. colonies located in and around infested structures in central North Carolina, we used genetic markers to genotype samples collected from mud tubes, closely spaced in-ground monitors, and natural wood debris in the yards of 20 residential properties. From these data, we were able to determine the numbers of colonies infesting each structure, the locations and distributions of colonies on the properties, and the breeding structure of colonies in each yard. Our results on 188 colonies provide the most comprehensive view to date of colony density, size and shape of colony foraging areas, and colony breeding structure in residential areas.

Materials and Methods

Twenty houses with active termite infestations in and around Raleigh, Wake County, NC, were selected for this study (Fig. 1). Sixteen of these properties were located in the city of Raleigh, two were in the neighboring town of Cary, and one each was located in the nearby towns of Holly Springs and Fuquay Varina. The mean ± SD distance between sites was 13.9 ± 7.5 km, with a minimum of 0.8 km and a maximum of 32.1 km.

There was no treatment performed during the study, and the treatment history of the properties before the study started was unknown.

Monitoring stations were installed around the structures and in the yard areas between April 2001 and May 2004. For each property, the monitoring stations were installed either in a single day or over two consecutive days. Monitoring stations consisted of a 30-cm length of 6-cm-diameter polyvinyl chloride (PVC) pipe with 15-cm-long grooves cut down the sides containing two pieces of 20-cm-long pieces of pine (3 cm in width and 1.5 cm in thickness) attached with plastic ties. The stations were capped at ground level with a PVC cap. Holes for inserting the monitoring stations were bored with a hand auger or power auger. There was a mean ± SD of 69.5 ± 23.8 monitors installed per property (range, 37–121). Monitors were placed in a more or less concentric set of rings, with the inner ring located ≈0.5 m from the foundation wall and spaced approximately 3–6 m apart. Mean number (± SD) of monitors in the inner rings was 25.6 ± 11.3 (range, 13–53). The outer ring was located ≈6 m from the inner ring, and occasionally, there was a second ring of outer monitors with some stations located up to 23 m from the structure. In two cases, wooden survey stakes were placed in the ground as accessory monitoring stations, either under a deck or in the crawl spaces. Monitors were checked monthly a mean of 7.4 ± 2.8 times (range, 4–13) over an average period of 8.4 ± 2.9 mo (range, 4.9–14.4 mo). Mud tubes along the foundation wall also were checked for the presence of termites monthly. In addition, areas further out in the yard containing stumps, firewood, and wood debris were checked for the presence of termites at least twice during the sampling period. Workers and, when present, soldiers, were collected from monitoring stations, mud tubes, and wood debris, placed in vials containing 95% ethanol and stored at 4°C until DNA extraction. On average, 37.6 ± 25.5 samples were collected per property (range, 9–105).

Termites were extracted using either the DNeasy tissue kit (QIAGEN, Valencia, CA) or a modification of the Gentra PureGene kit (Gentra Systems, Inc., Minneapolis, MN). Species identification was based on the polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP) method of Szalanski et al. (2003). Termites were genotyped at 10 microsatellite loci: *Rf 24-2*, *Rf 21-1*, *Rf 15-2*, *Rf 6-1*, *Rf 5-10*, *Rf 1-3* (Vargo 2000), and *Rs 16*, *Rs 33*, *Rs 62*, *Rs 76* (Dronnet et al. 2004). PCR conditions were as described in Vargo (2000) and Dronnet et al. (2004). The resulting fluorescently labeled products were run on 6.5% polyacrylamide, and the bands were detected and recorded using a Li-Cor 4300 automated DNA sequencer (Li-Cor, Inc., Lincoln, NE). Size standards were run about every tenth lane. Allele sizes were determined using Gene Profiler version 3.56 (Scanalytics, Inc., Fairfax, VA).

Termites from each station containing foragers were analyzed. However, if the same station had foragers on multiple occasions during the sampling period (up to a maximum of seven times over a 13-mo period), we analyzed foragers collected during at least three time points, including the first and last samples taken and one point in the middle. In all cases, the same colonies were found to be present at these three time points and we therefore assumed that they were the sole occupants of the stations throughout the entire sampling period.

To determine colony identity of samples, we used exact tests of genotypic differentiation as implemented in the program GenePop on the Web (Raymond and Rousset 1995). Samples on the same property which shared the same genotypes and did not differ significantly ($P > 0.05$) in genotype frequencies according to the exact test were considered to belong to the same colony. At least 10 individuals per sample were used to determine colony identity. Colony breeding structure was determined by analysis of at least 20 worker genotypes per colony by using the classification of several recent studies (DeHeer and Vargo 2004, Vargo and Carlson 2006, Vargo et al. 2006a). Colonies were considered simple families if the workers had genotypes consistent with the progeny of a monogamous pair of reproductives and the genotypic frequencies did not deviate significantly from expected for full siblings ($P < 0.05$, *G*-test summed across loci). Colonies were classified as extended families if they had no more than four alleles per locus (the maximum number of alleles for colonies descended from a monogamous pair of founders) and the workers either had genotypes inconsistent with full siblings (e.g., five genotypic classes or three classes of homozygotes), or they had genotype frequencies that deviated significantly from expected for simple families. Finally, colonies were considered mixed families if they had more than four alleles at one or more loci. In all such colonies, the additional alleles present occurred in numerous individuals and at numerous loci.

The genetic structure of colonies was further assessed as described in several previous studies (Thorne et al. 1999, Bulmer et al. 2001, Vargo 2003b, DeHeer and Vargo 2004, Vargo et al. 2006a) through an analysis of inbreeding coefficients (*F*-statistics) and coefficient of relatedness (r) among colony members as implemented in the program FSTAT version 2.9.3.2 (Goudet 2001). In these analyses, colonies were treated as subpopulations, and genetic variation was partitioned among the total (T), colony (C) and individual (I) components. The resulting values of F_{IT}, F_{IC}, F_{CT}, and r were compared with values predicted for various breeding structures of subterranean termites based on computer simulations (Thorne et al. 1999, Bulmer et al. 2001).

The size of the house and the size of the lawn and natural areas of each property were determined using a combination of direct measurements on the ground and estimates from aerial photographs obtained through the Wake County, North Carolina Geographic Information Services website (http://www.wakegov.com/gis/default.htm).

We used the data on colony identities to estimate linear foraging distances and colony foraging areas.

For colonies collected at multiple sampling sites (monitoring stations or natural area locations), linear foraging distance was the maximum distance separating the sampling sites. For colonies collected at a single sampling site, the maximum foraging distance was set to 1 m, because we assumed that termites located at a site could forage at least 0.5 m out in all directions from the sampling point. Foraging area was estimated by calculating a simple polygon connecting the various sampling points from which a colony was collected. For colonies present at two sampling locations, foragers were assumed to be active up to 0.5 m on either side of the straightest line connecting the points. Thus, a colony occupying two stations 10 m apart was given a foraging area of 10 m^2. Colonies collected at only a single sampling site were assigned a foraging area of 1 m^2. The estimates of the linear foraging distance and foraging areas are admittedly imprecise and represent only the points where termites were detected and sampled. Thus, the results reported here should not be taken as accurate assessments of foraging ranges, but rather as the minimum sizes of foraging distance and area. For the purposes of this study, the values used were mainly for comparisons within and across the various properties investigated and were deemed valid for this objective.

Results

The mean ± SD size of the 20 properties was 1,854 ± 1,860 m^2 (0.5 ± 0.5 acres), with a range of 364–7,568 m^2. The houses averaged 293 ± 175 m^2 (3,154 ± 1,884 $feet^2$) and ranged in size from 364 to 789 m^2. In total, 751 samples were collected. The number of samples varied greatly across properties, ranging from nine over a period of 344 d to 105 over 415 d. Of these, the majority were *R. flavipes.* From these samples, we identified a total of 188 colonies, of which 169 (89.9%) were *R. flavipes*, 14 (7.4%) were *R. hageni*, and five (2.7%) were *R. virginicus.* In most cases, when *R. hageni* or *R. virginicus* were present on a property they were represented by a single colony, although one property (SB) had five colonies of *R. hageni* out of a total of 24 colonies. In only one case (property PE) were both *R. hageni* and *R. virginicus* present on the same property.

There was an average ± SD of 9.4 ± 9.2 colonies per property, with a range of 1–34 (Fig. 2A). Most houses had only a single colony infesting them, although four houses (20% of the total) were being attacked by two colonies simultaneously. In all but one case, colonies infesting structures were *R. flavipes;* the only exception was a single colony of *R. virginicus* infesting house RB. Two representative properties showing the detailed locations of colonies are given in Figs. 3 and 4. Figure 2B shows the distribution of colonies throughout the yard. There was an average of 2.2 ± 1.7 colonies (range, 1–8) either in the structure or in the inner ring of monitors within 0.5 m from the structure. In the outer ring of monitors located 6–25 m from the structure, there was an average of 3.7 ± 2.2 colonies (range, 1–10). And in the natural areas, we found an

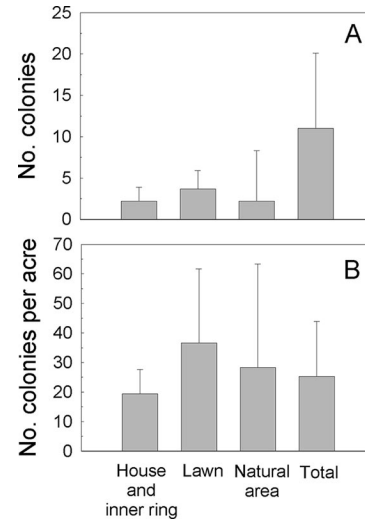

Fig. 2. Numbers, densities, and distributions of subterranean termite colonies (mean ± SD) located on 20 residential properties in central North Carolina.

average of 6.4 ± 7.1 colonies, with a range of 0–26. After adjusting for property size, colony density averaged 61.8 ± 50.7 ha (range, 11.1–185.3), the equivalent of 25.3 ± 20.5 colonies per acre (range, 4.5–75.0).

Table 1 shows the number of each family type present for colonies of all three species. Of the 188 unique colonies sampled, four (two *R. flavipes* and two *R. hageni*) had too few workers present (<10) to do a robust analysis of family structure and were not included in the subsequent analyses. Of the remaining 167 *R. flavipes* colonies, the majority (86%) were simple families, some 11% were extended families, and there were three colonies of mixed families. Interestingly, all three of the mixed family colonies were located on the same property (BW). The 12 *R. hageni* colonies for which we determined the family type were also predominately simple families (71%), with the remainder of colonies all extended families. Of the five *R. virginicus* colonies studied, they were about evenly split between simple families and extended families.

The family types for the colonies infesting structures were representative of the colonies present throughout the properties. Of the 23 *R. flavipes* colonies infesting structures, 20 of them (87% of the total) were simple families, a proportion nearly identical to the population at large (86%). The remaining three structure infesting colonies of *R. flavipes* were extended families. The one case of *R. virginicus* infesting a house involved an extended family colony (property RB).

The coefficients of inbreeding and of nestmate relatedness for colonies of all three species are given in Table 2, along with values predicted for different breeding structures based on computer simulations. Simple families of *R. flavipes* had values that did not differ significantly from those expected for populations consisting of colonies headed by monogamous

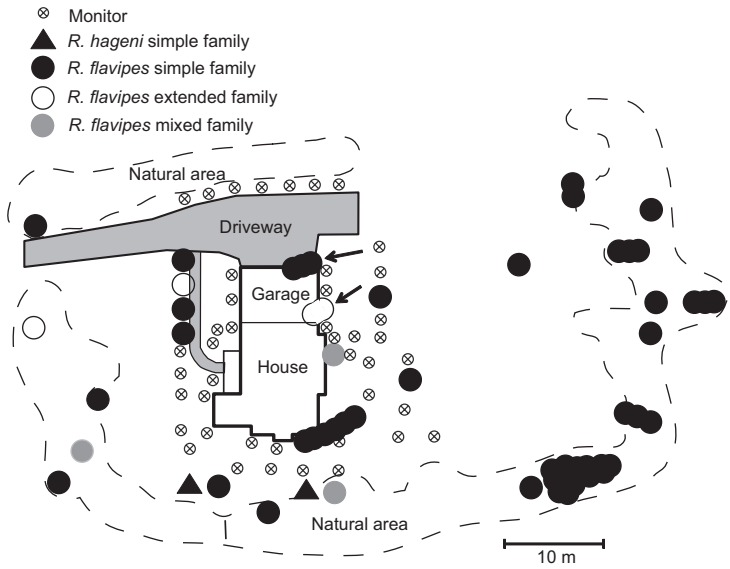

Fig. 3. Study property BW located in Wake County, NC. We identified a total of 30 colonies during the 208-d monitoring period. The arrows indicate the locations of two colonies found to be infesting the garage. This property had three mixed family colonies. Colonies found to be foraging at only a single point are indicated by a circle (*R. flavipes*) or triangle (*R. hageni*), and they were assumed to have foraging areas of 1 m^2. Colonies with more expansive foraging ranges are shown by overlapping symbols. The yard areas between the house and the natural areas was largely lawn. Natural areas were wooded areas with little or no landscaping and abundant wood debris. Colony density on this property was estimated at 100.2 colonies per ha (40.5 colonies per acre).

pairs of unrelated reproductives (Table 2, case A; all $P > 0.05$, one-sample *t*-test). The simple families of *R. hageni* had values significantly greater (all $P < 0.01$, one-sample *t*-test) than those expected for outbred simple families. The two *R. virginicus* simple families were significantly more differentiated from each other based on pairwise F_{ST} and had coefficients of relatedness higher than expected (both $P < 0.05$, one-sample *t*-test) for outbred colonies.

In all three species, extended family colonies were less inbred (higher F_{IT}) than expected for colonies containing three neotenics, the fewest that could be present in an extended family colony; this difference was significant in both *R. flavipes* and *R. virginicus* (both $P < 0.02$, one-sample *t*-test). For all three species, F_{IC}, the coefficient of inbreeding in individuals relative to others in their colony, was highly negative (all $P < 0.001$, one-sample *t*-test), suggesting relative few functional reproductives present on average, most likely fewer than 10 (Table 2, cases B1 and 2). However, due to the very small sample sizes for *R. hageni* and *R. virginicus* ($n = 2$ and 3, respectively) caution should be used in drawing conclusions regarding the breeding structure of these colonies.

The three mixed family colonies of *R. flavipes*, all of which were located on the same property, also had a significantly negative F_{IC} value ($P < 0.025$, one-sample *t*-test), although it was significantly greater that than the F_{IC} values for either the simple or extended families of this species (both $P < 0.03$, *t*-test). All three colonies were considered mixed families because they had five alleles at one or more loci, indicating the presence of two or more unrelated same sex reproductives. In one colony, workers could be grouped into one of two distinct families based on their genotypes, as has been done for other mixed family colonies of this species (DeHeer and Vargo 2004, Vargo and Carlson 2006, Vargo et al. 2006a), indicating the presence of separate non interbreeding sets of reproductives. However, in the other two colonies, we could not clearly differentiate workers belonging to different family groups, suggesting some degree of interbreeding among the cohabiting families. More complete analysis of these mixed families are reported separately (DeHeer and Vargo 2008).

Data on colony foraging ranges are presented in Table 3. Of the three species studied, *R. virginicus* had the highest proportion of colonies spanning two or more collection sites (3/5; 60%), followed by *R. flavipes* (80/167; 48%) and then *R. hageni* (2/12; 17%). Conducting pairwise comparisons of the proportions of multiple site colonies among the species using Fisher Exact Test, we found that only the difference between *R. flavipes* and *R. hageni* was significant ($P < 0.05$, two-tailed test). On average, colonies of *R. virginicus* had the longest foraging distances, with one colony spanning 48.8 m, compared with maximum distances of 26 m for *R. flavipes* and 10.7 m for *R. hageni*. However, foraging distances in *R. virginicus* did not differ significantly from either of the other two species (both $P > 0.20$, two-sample *t*-test not assuming equal variances), most likely due to the small number of *R. virginicus* colonies studied. The average foraging distances of *R. flavipes* colonies was greater than that

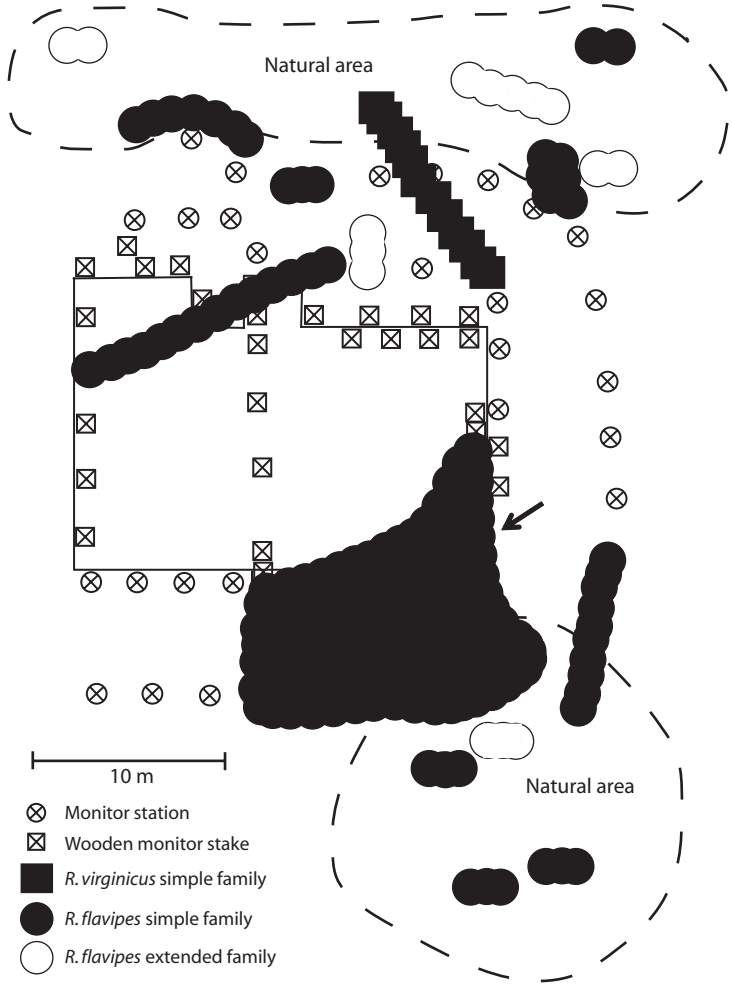

Fig. 4. Study property WP located in Wake County, NC. The structure was a quadriplex, but only two of the units are shown. Monitoring was conducted using in-ground monitoring stations, wooden stakes inserted in the crawl space, and sampling mud tubes and natural wood debris. The arrow indicates the only colony known to be infesting the structure; it was collected from numerous in-ground monitoring stations, multiple wooden stakes, mud tubes and wood debris located outside the structure. One other colony was found feeding on an in-ground monitor outside the structure and on wooden stakes in the crawl space but it was not considered to be infesting the structure because it was not found in mud tubes or in the structure itself. Nine colonies were detected during the 431 d monitoring period. Colonies found to be foraging at only a single point are indicated by a circle (*R. flavipes*) or a square (*R. virginicus*) and were assumed to have foraging areas of 1 m^2. Colonies with more expansive foraging ranges are shown by overlapping symbols. Colony density on the property was estimated to be 185.3 colonies per ha (75 colonies per acre), the highest of any of the study properties.

of *R. hageni* ($t_{17} = 2.28, P < 0.04$, two-sample *t*-test not assuming equal variances). Similarly, the mean foraging area was greatest in *R. virginicus*, in which one of the five study colonies occupied a foraging area of 318.2 m^2, but this value was not significantly greater than the foraging areas of the other two species (both $P > 0.25$, two-sample *t*-test not assuming equal variances). The mean foraging area of *R. flavipes* colonies, in which only four colonies (2.4% of all colonies) had foraging areas >100 m^2 and a maximum of 173.9 m^2, was significantly greater than that of *R. hageni* ($t_{39} = 2.23, P < 0.04$, two-sample *t*-test not assuming equal variances), which had a maximum foraging area of 21.1 m^2.

Table 1. Colony family types of three species of *Reticulitermes* spp. sampled on residential properties in the Raleigh, NC, area

Species	Total no. colonies	No. simple family colonies (%)	No. extended family colonies (%)	No. mixed family colonies (%)
R. flavipes	167	145 (86.8)	19 (11.4)	3 (1.8)
R. hageni	12	10 (83.3)	2 (16.7)	0
R. virginicus	5	2 (40)	3 (60)	0

Colony foraging range was not associated with family type in *R. flavipes*, because there was no significant difference between simple and extended families in either mean foraging area or maximum linear distance

Table 2. Inbreeding and nestmate relatedness coefficients for workers of subterranean termite colonies sampled in the Raleigh, NC, area and expected values for possible breeding systems based on computer simulations

Species/colony family type	F_{IT}	F_{CT}	F_{IC}	r
R. flavipes (10 loci)				
All colonies ($n = 167$)	0.029	0.262	−0.315	0.509
(SE)	(0.014)	(0.008)	(0.009)	(0.009)
Simples families ($n = 145$)	0.012	0.26	−0.335	0.514
(SE)	(0.013)	(0.007)	(0.01)	(0.009)
Extended families ($n = 19$)	0.134	0.278	−0.201	0.491
(SE)	(0.027)	(0.016)	(0.019)	(0.019)
Mixed families ($n = 3$)	0.153	0.227	−0.095	0.394
(SE)	(0.05)	(0.029)	(0.04)	(0.039)
R. hageni (8 loci)				
All colonies ($n = 12$)	0.39	0.514	−0.256	0.742
(SE)	(0.067)	(0.052)	(0.017)	(0.04)
Simples families ($n = 10$)	0.396	0.523	−0.268	0.752
(SE)	(0.076)	(0.059)	(0.019)	(0.045)
Extended families ($n = 2$)	0.16	0.295	−0.193	0.512
(SE)	(0.088)	(0.054)	(0.038)	(0.056)
R. virginicus (8 loci)				
All colonies ($n = 5$)	0.05	0.309	−0.376	0.589
(SE)	(0.066)	(0.031)	(0.041)	(0.028)
Simples families ($n = 2$)	0.195	0.442	−0.445	0.743
(SE)	(0.128)	(0.074)	(0.07)	(0.056)
Extended families ($n = 3$)	0.031	0.272	−0.332	0.529
(SE)	(0.051)	(0.029)	(0.046)	(0.042)
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) $N_f = 2$, $N_m = 1$, $X = 1$[a]	0.26	0.35	−0.14	0.55
(2) $N_f = N_m = 10$, $X = 1$[b]	0.33	0.34	−0.01	0.51

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

[a] From Vargo (2003b).

[b] From Thorne et al. (1999).

(both $t \leq 0.89$, $P > 0.3$). It is interesting to note that all three of the mixed family colonies of this species were found in individual monitoring stations, suggesting they had limited foraging ranges, but we consider a sample size of three to be too small to draw strong conclusions. There were too few samples in one or more of the family classes in both *R. hageni* and *R. virginicus* to make meaningful comparisons concerning foraging range and colony family structure. Finally, looking across properties, there was no significant correlation between colony density and either linear foraging distance ($r = -0.21$, $P > 0.2$) or foraging area ($r = 0.02$, $P > 0.7$), suggesting that colony density did not influence foraging ranges of colonies.

Table 3. Minimum linear foraging distances and estimated foraging areas of colonies of subterranean termites on residential properties in central North Carolina

Species	n	Mean ± SD linear foraging distance (m)	Mean ± SD foraging area (m^2)
R. flavipes	167	4.4 ± 5.5	8.4 ± 21.4
R. hageni	12	2.2 ± 3.0	3.1 ± 5.9
R. virginicus	5	16.3 ± 21.7	82.6 ± 137.3

Discussion

In a map of subterranean termite pressure often cited by the urban entomology community (e.g., Mallis 1997), central North Carolina sits on the border between "very heavy" and "heavy to moderate" pressure. However, to our knowledge this classification has not been based on any quantitative measure. Obviously, subterranean termite pressure is best measured as the relative incidence of infestation. This will largely be determined by a host of factors, including the species composition of the subterranean termite community located around structures, the density of colonies, and the foraging range of the resident colonies. Our results provide the most comprehensive picture to date of all three of these parameters for residential properties.

We found an average of 62 colonies per ha (25 colonies per acre), up to a maximum of 185 colonies per ha (75 colonies per acre), located on residential properties in the Raleigh, NC, area. This amounts to a mean of 9.4 colonies present on an average sized property measuring 1,854 m^2. Although these values should prove a useful guide for predicting the numbers of colonies located around residential structures in central North Carolina, we wish to acknowledge two caveats regarding our results. First, these values are almost certainly underestimates of the true colony densities present on the study properties because large portions of the yards, especially the open lawn areas where there was little or no wood debris and where few monitoring stations were installed, went unsampled. Second, because our study involved only residences with active infestations, it is possible that such properties have higher population densities than similar-sized properties without infestations. If so, our results may not be representative of all residences in this area. Similar studies of properties chosen at random with respect to the presence of termite infestation would be needed to determine whether colony densities differ between properties with or without structural infestations.

It is difficult to know how the values for colony densities obtained in the current study compare with those across geographic locations, because little equivalent data exist for other areas. There are, however, two previous studies involving careful censusing of the subterranean colonies present in forest habitats, to which these results can be compared. One of these studies was also conducted in Raleigh, NC, the location of the current study. Using molecular markers to identify colonies, DeHeer and Vargo (2004) conducted detailed studies over a 3-yr period of colonies located in undisturbed forested sites. Although these authors did not report colony densities, these values can be estimated from the number of colonies reported and the size of the study areas to yield values of 300 and 125 colonies per ha (121 and 51 colonies per acre, respectively). Thus, these forest sites had colony densities two- to five-fold higher than the average for the residential properties in the current study. This difference may indicate that colony densities on res-

idential properties are lower on average than in undisturbed forest areas possibly due to fewer suitable food sources, especially in the lawn areas of the yard. Alternatively, our monitoring efforts could have underestimated the actual number of colonies present on residential properties as described above. In any event, we found considerable variation among residential properties in the current study, and the colony density in some cases approached or exceeded those in the undisturbed areas. It is of interest to note that the average colony density reported here for residential properties was substantially greater than that reported for an undisturbed site in Massachusetts where the density of *R. flavipes* colonies (determined from the data given in the paper) was seven colonies per ha (three colonies per acre; Bulmer et al. 2001), suggesting much lower colony densities in the northern part of the range of subterranean termites than in North Carolina. In a population from southern Mississippi, Howard et al. (1982) reported a density of 6.8 colonies per ha of *R. flavipes and R. virginicus* combined, a value close to that reported for Massachusetts and nearly 10 times lower than we found in the current study. However, this was almost certainly an underestimate because the only criterion these authors used for separating colonies was a minimum distance of 15 m, most likely causing them to miss many of the colonies present. Detailed studies similar to the current study are needed from several locations spanning the range of *Reticulitermes* spp. within the eastern United States to accurately quantify subterranean termite abundance in different regions and to determine the degree to which it varies geographically.

There are few data on colony densities of other subterranean termite species. Haverty et al. (1975) reported a density of the desert subterranean termite *Heterotermes aureus* (Snyder) of 190 colonies per ha in a natural site near Tucson, AZ. However, the accuracy of this value is questionable because these authors used imprecise methods to distinguish among colonies; groups of foragers in below ground monitors were assigned to colony based on relative numbers of individuals and proximity to other foraging groups. And in another urban site, Messenger and Su (2005) studied a 12.75-ha park in New Orleans, LA, using mark–release–recapture over a 4-yr period and found a colony density of 1.5 colonies per ha, primarily of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki ($n = 13$ colonies), with a small number of *R. flavipes* colonies present ($n = 6$).

As expected, we found a preponderance of *R. flavipes* colonies among the 188 colonies studied (89.8% of total), with relatively few colonies of *R. hageni* (7.2% of total) and only five colonies of *R. virginicus* (3% of total). These values are similar to those found in Umstead State Park in Raleigh, where colonies of subterranean termites were studied in two 2-km perpendicular transects in undisturbed forest habitat (C. DeHeer, T. Juba and E.L.V., unpublished data). Of the 147 colonies sampled in that study, 134 (91.2%) were *R. flavipes*, seven (4.8%) were *R. hageni*, and six (4.1%) were *R. virginicus.* Thus, relative species abundance around residential properties is very similar to that in forested sites in this area. Further east in North and South Carolina, *R. hageni* becomes much more common. Vargo et al. (2006a) studied 49 colonies of subterranean termites from a state park in Charleston, SC, where they found *R. hageni* to be the most common species (43% of the total), followed by *R. flavipes* (37%), *C. formosanus* (12%), and *R. virginicus* (8%). And in a survey of 38 colonies of subterranean termites in the Coastal Plain of North Carolina, E.L.V. and Dalton (unpublished data) found 74% were *R. hageni*, 21% were *R. flavipes*, and 5% were *R. virginicus.*

Studies of *Reticulitermes* in other parts of the eastern and central United States based on samples collected in or around structures (Scheffrahn et al. 1988; Messenger et al. 2002; Austin et al. 2004a,b,c), show considerable variation in species composition from one region to another. These studies show that *R. flavipes* was the most common species in all areas studied, accounting for 58–78% of all subterranean samples from Florida, Louisiana, Texas, Arkansas, and Oklahoma. The relative abundance of the other two species was highly variable; *R. hageni* ranged from 1 to 19% and *R. virginicus* occurred at a frequency of 2–38%. Surprisingly, the relative species abundances found in the Raleigh, NC, area are more similar to Texas and Oklahoma, where *R. flavipes* comprised >70% of the samples and the other two species made up <10% each (Austin et al. 2004a,c), than to the species compositions in Florida, Arkansas, or Louisiana (Scheffrahn et al. 1988, Austin et al. 2004b).

One important component of relative pressure from subterranean termites is the spatial expansiveness of colonies; a single large colony could potentially pose a greater structural threat than two or more small colonies if it fed over a bigger area and consumed more wood. Our results indicated that colonies of *R. flavipes*, by far the most abundant species present, had relatively limited foraging ranges, with a maximum linear distance of 26 m and an estimated maximum foraging area of 174 m^2. These results are consistent with previous findings on this species in both undisturbed (Vargo 2003b, DeHeer and Vargo 2004, Vargo and Carlson 2006, Vargo et al. 2006a) and urban sites (Vargo 2003a) in North and South Carolina. The even more limited foraging ranges of *R. hageni* are also in agreement with results of previous studies in this region (DeHeer and Vargo 2004, Vargo and Carlson 2006, Vargo et al. 2006a). Our finding that some colonies of *R. virginicus* can have relatively extensive foraging ranges, corresponds with results of previous studies showing foraging activity over greater distances than sympatric colonies of *R. flavipes* or *R. hageni*, spanning distances up to 125 linear meters (Vargo and Carlson 2006, Vargo et al. 2006a).

Our results on the colony genetic structure of *Reticulitermes* spp. are similar to those of previous studies from central North Carolina. Combining the results of several earlier studies from both natural areas (Vargo 2003b, DeHeer and Vargo 2004, Vargo and Carlson 2006) and urban sites (Vargo 2003a), 69% of 152 *R. flavipes* colonies were simple families, 29% were ex-

tended families, and 2% were mixed families. Of 17 *R. hageni* colonies studied, 65% were simple families, 35% were extended families, and there were no mixed families. From six *R. virginicus* colonies previously studied, 83% were simple families and 17% were extended families. In addition, the genetic structure of the colonies of each species was similar to the present results. In this and previous studies, simple families of *R. flavipes* had inbreeding and relatedness coefficients consistent with colonies headed by monogamous outbred reproductives, whereas extended family colonies likely contained relatively few neotenics that were the F_1 offspring of the founding pair. Also, simple family colonies of *R. hageni*, unlike those of *R. flavipes*, were highly inbred ($F_{IT} = 0.40$), a result similar to previous studies on this species in North and South Carolina (Vargo and Carlson 2006, Vargo et al. 2006a), suggesting that simple family colonies of this species are often headed by related reproductives.

The mixed family colonies of *R. flavipes* found in the current study were of particular interest because they were all located on the same property. Although this property had 30 colonies, the second highest number of any of the properties, the probability that the only three mixed family colonies would all be located on this property by chance alone is extremely small ($P < 0.00001$). These results suggest that there may be local "hot spots" for genetically complex colonies, most likely arising through colony fusion. This conclusion is supported by the findings that all three of the other mixed family colonies reported from the Raleigh, NC, area, including a colony in which the fusion of two genetically distinct colonies was documented (DeHeer and Vargo 2004), were located in Schenck Forest, a small forest area belonging to North Carolina State University. Another reason these mixed family colonies were of interest is that only one of them had clearly identifiable family groups, a pattern seen in five other naturally occurring mixed family colonies studied in depth (DeHeer and Vargo 2004, 2008; Vargo and Carlson 2006; Vargo et al. 2006a), suggesting the presence of distinct groups of reproductives with little or no interbreeding between them. Two mixed family colonies found in the current study had apparently experienced interbreeding between groups of reproductives, a phenomenon not observed in the field studies mentioned above or in a laboratory study of colony fusion (Fisher et al. 2004). Thus, mixed family colonies arising through colony fusion can sometimes result in interbreeding between reproductives originating from more than one of the source colonies. Alternatively, the complex genetic patterns present among workers in these two colonies could possibly have arisen through some mechanism other than colony fusion, such as pleometrosis or adoption of unrelated reproductives by established colonies, but to date only colony fusion has been demonstrated as a mechanism producing mixed family colonies of subterranean termites (DeHeer and Vargo 2004).

Mixed family colonies have been reported in other populations of *R. flavipes* (Jenkins et al. 1999, Bulmer et al. 2001), where they also occur at relatively low frequencies. They also have been found in low frequencies in Spanish populations of *R. grassei* (E.L.V., C. DeHeer and A. G. Bagnères, unpublished data) but not in French populations (DeHeer et al. 2005), and at least one naturally occurring mixed family colony of *R. speratus* in Japan has been observed (Matsuura and Nishida 2001). However, there are no reports of genetically complex colonies occurring in other species that have been studied rather intensively, including *R. hageni* and *R. virginicus* in North and South Carolina (Vargo 2003a, DeHeer and Vargo 2004, Vargo and Carlson 2006, Vargo et al. 2006a) or in several introduced populations of *C. formosanus* (Vargo et al. 2003, 2006b; Husseneder et al. 2005). Thus, the evidence available so far indicates that genetically complex colonies in subterranean termites are uncommon and rather spotty in their occurrence, both within and among species.

In summary, we used a combination of intensive sampling and molecular markers to determine the species composition, numbers, locations and characteristics of 188 subterranean termite colonies in and around 20 residential structures in central North Carolina. These parameters are all important determinants of the likelihood of subterranean termite infestation. Our data therefore provide a useful baseline for establishing the level of pressure that subterranean termites exert on residential structures, at least in a single geographic area. No doubt there are several other factors that we did not measure that are likely to influence subterranean termite pressure, such as soil composition and abundance of food resources near structures. Future studies similar to the present one covering a broader geographic range and incorporating some of these additional factors together with data on the frequency of structural infestation would contribute greatly to our ability to predict the probability of attack by subterranean termites at both large (geographic region) and small (individual property) spatial scales.

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