

Relative Abundance and Comparative Breeding Structure of Subterranean Termite Colonies (*Reticulitermes flavipes*, *Reticulitermes hageni*, *Reticulitermes virginicus*, and *Coptotermes formosanus*) in a South Carolina Lowcountry Site as Revealed by Molecular Markers

EDWARD L. VARGO,¹ THOMAS R. JUBA, AND CHRISTOPHER J. DEHEER²

Department of Entomology, Box 7613, North Carolina State University, Raleigh, NC 27695

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ABSTRACT There are several species of subterranean termites in the United States, some of which occur sympatrically over broad geographic regions. However, there is little information on the relative abundance of the different species or the extent to which they differ with respect to colony social and spatial organization. We used microsatellite markers to investigate the relative numbers of colonies, to infer colony breeding structures, and to delineate colony foraging areas in four species of subterranean termites occurring in a state park in Charleston, SC. The two most abundant species, *Reticulitermes hageni* Banks and *Reticulitermes flavipes* (Kollar), which together accounted for 80% of the 49 colonies sampled, had fairly localized foraging ranges of <30 m across. In contrast, *Reticulitermes virginicus* (Banks) and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, had far fewer colonies, but these colonies were more expansive, spanning distances >100 linear m. Colonies of all species were primarily simple families each headed by a single monogamous pair of reproductives. Generally, the remaining colonies of each species were consistent with being extended families, i.e., headed by multiple neotenic reproductives descended from simple families. Only in *R. flavipes* was a mixed family colony detected, with workers from two distinct families occurring together. These results from molecular markers reveal how the various species in a relatively diverse subterranean termite community can vary in abundance, size of colony foraging area and breeding structure, thereby setting the stage for subsequent studies to identify the factors shaping these communities.

KEY WORDS population genetics, colony genetic structure, microsatellite, Formosan subterranean termite, eastern subterranean termite

Throughout many parts of the temperate and tropical regions of the world, including much of the United States, subterranean termites (Rhinotermitidae) play an important ecological role as decomposers of cellulose materials and are highly destructive pests of human structures. Species of the genus *Reticulitermes* are the most widespread subterranean termites in the United States, where there are six described species and probably several unrecognized species (Austin et al. 2002, Copren et al. 2005). There are large areas of overlap in the distribution of some of the species, especially in the southeastern United States, where three recognized species, *Reticulitermes flavipes* (Kollar), *Reticulitermes virginicus* (Banks), and *Reticulitermes hageni* Banks, are often sympatric.

Because of their cryptic foraging and nesting habits, many features of subterranean termite biology have

been difficult to study by using the traditional methods of field investigations. The main obstacle has been a lack of practical methods for delineating large numbers of colonies (Husseneder et al. 2003). Because the colony is the basic ecological unit in social insect populations (Wilson 1971, Thorne et al. 1999, Lepage and Darlington 2000), such fundamental information as relative species abundance, population density, and intra- and interspecific interactions requires that individual colonies be identified and distinguished from each other. The application of molecular genetic markers to field populations of subterranean termites provides a powerful way to discriminate among large numbers of conspecific colonies in a population as well as to determine colony breeding structure (Husseneder et al. 2003). There is an accelerating number of studies using molecular markers in subterranean termite populations to elucidate colony breeding structure (Kaib et al. 1996; Husseneder et al. 1999, 2005; Jenkins et al. 1999; Bulmer et al. 2001; Husseneder and Grace 2001b; Vargo 2003a, Vargo et al. 2003, 2006; DeHeer and Vargo 2004; Matsuura et al.

¹ Corresponding author, e-mail: ed_vargo@ncsu.edu.

² Current address: Department of Entomology, University of Nebraska, Lincoln, NE 68583.

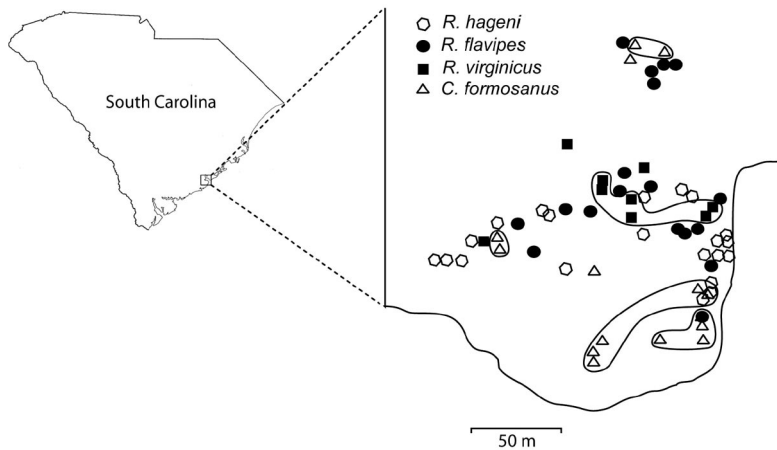


Fig. 1. Relative locations of collections points in Charles Towne Landing State Historic Site, Charleston, SC. Collection points determined to belong to the same colony are encircled.

2004; DeHeer et al. 2005; Vargo and Carlson 2006), colony spatial organization (Bulmer and Traniello 2002a, DeHeer and Vargo 2004, Messenger et al. 2005), colony-colony interactions (Bulmer and Traniello 2002b, DeHeer and Vargo 2004), and as a means to track colonies over time after insecticide treatment (Vargo 2003b, Messenger et al. 2005).

In the United States, most work involving the application of molecular markers in subterranean termites has concerned two species, the eastern subterranean termite, *R. flavipes* (Reilly 1987; Jenkins et al. 1999; Bulmer et al. 2001; Bulmer and Traniello 2002a,b; Vargo 2003a,b; DeHeer and Vargo 2004) and the invasive Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Husseneder et al. 2005; Messenger et al. 2005). There have been limited analyses of relatively few colonies of other species, including *R. virginicus* (Vargo 2003b, DeHeer and Vargo 2004), *R. hageni* (DeHeer and Vargo 2004, Vargo and Carlson 2006), and *R. hesperus* Banks (Copren 2004).

Because of its relatively high species richness, the southeastern United States is a particularly good place to examine subterranean termite community structure. In this study, we used microsatellite markers to investigate the relative abundance of colonies of four subterranean termite species—three native species (*R. flavipes*, *R. virginicus*, and *R. hageni*) and an introduced species (*C. formosanus*)—in a coastal South Carolina site where they occur sympatrically. In addition, we used the genetic data to compare colony breeding systems and size of foraging areas.

Materials and Methods

Sample Collection. Samples were collected from natural wood debris in Charles Towne Landing State Historic Site in Charleston, SC, on 13 and 19 May 2003. Areas with wood debris (heavily wooded locations and those with tree stumps) were searched in more or less transect manner as reported in other studies (e.g.,

Vargo 2003a, DeHeer et al. 2005). Samples consisting of groups of foragers and soldiers, and occasionally alates, were collected and placed directly into vials containing 95% ethanol. Usually, samples were located at least 15 m apart, but they sometimes occurred closer together. The distances and compass directions between neighboring collection points were measured. In total, 64 samples were collected; their locations are shown in Fig. 1. The *C. formosanus* colonies have been reported on elsewhere as part of a larger study of 21 colonies of this species from Charleston (Vargo et al. 2006), but the six colonies present at Charles Towne Landing State Historic Site are included here for comparative purposes.

Species Identification. Samples of *C. formosanus* were identified based on the rounded shape of the soldier head (Scheffrahn and Su 1994). *Reticulitermes* spp. specimens were identified by restriction fragment length polymorphism of a polymerase chain reaction (PCR)-amplified portion of the cytochrome oxidase II gene according to the methods of Szalanski et al. (2003).

Genetic Analyses. Ten to 30 workers per sample were genotyped at eight to 12 microsatellite loci according to the methods of Vargo (2000), Vargo and Henderson (2000), and Dronnet et al. (2004). Individuals were extracted using the Genra PureGene kit (Genra Systems, Inc., Minneapolis, MN). Workers of *R. flavipes* and *R. hageni* were genotyped at eight polymorphic loci (Table 1). Workers of *R. virginicus* also were genotyped at these same loci, but in this species one locus, *Rf 15-2*, was monomorphic. Therefore, another locus, *Rs 68*, from Dronnet et al. (2004), was used on this species. As reported by Vargo et al. (2006), 12 loci were run on *C. formosanus*, but only eight of these loci were polymorphic.

Colony Affiliations. To determine colony affiliation of the collection points, we followed the method of several other studies on subterranean termites (Vargo 2003a; Vargo et al. 2003, 2006; DeHeer and Vargo 2004; DeHeer et al. 2005; Husseneder et al. 2005) by testing

Table 1. Number of alleles at the assayed microsatellite loci in sympatric populations of three species of *Reticulitermes* and *C. formosanus* from Charleston, SC

Locus	<i>R. flavipes</i> (n = 18)	<i>R. hageni</i> (n = 21)	<i>R. virginicus</i> (n = 4)	<i>C. formosanus</i> (n = 6)
Rf 1-3	9	8	6	
Rf 5-10	5	8	3	
Rf 6-1	10	10	6	1
Rf 15-2	4	7	1	
Rf 21-1	19	12	7	
Rf 24-2	19	12	7	
Rs 10	8	10	2	
Rs 15	14	12	4	
Rs 68			3	
Cf 1-1				1
Cf 4:1 A2-4				3
Cf 4:1 A2-5				2
Cf 4-4				2
Cf 4-9A				2
Cf 4-10				2
Cf 8-4				2
Cf 10-4				1
Cf 10-5				2
Cf11-1				1
Cf 12-4				2
Mean ^a (± SD)	11.0 ± 5.8	9.9 ± 2.0	4.8 ± 2.0	2.1 ± 0.4

n refers to the number of colonies sampled.

^a Polymorphic loci only.

all pairs of collection points within each species for genotypic differentiation by means of a permutation test by using the program FSTAT (Goudet 2001). Pairs of collection points that were significantly differentiated from each other ($P < 0.05$) were considered to belong to different colonies; those that were not significantly differentiated were grouped into the same colony.

Colony Breeding Structure. We followed the classification system of other authors (Vargo 2003a,b; Vargo et al. 2003; DeHeer and Vargo 2004; DeHeer et al. 2005; Dronnet et al. 2005) in grouping colonies into three types based on worker genotypes: simple families, extended families, and mixed families. Simple family colonies had worker genotypes consistent with being the offspring of a monogamous pair of reproductives, and in which the frequencies of the genotypes did not differ significantly from expected. Deviation from expected frequencies was determined by means of a G -test by summing all the locus-specific G -values for an overall G -value for each colony with $\alpha = 0.05$. Colonies were considered extended families if they had no more than four alleles per locus, the most possible in colonies founded by a pair of primary reproductives, and the worker genotypes were incompatible with the presence of a single monogamous pair of reproductives (e.g., five or more genotypic classes), or if genotypes were present that were compatible with full siblings, the frequency of the genotypes differed significantly from expected ($P < 0.05$, G -test). Extended families were inbred colonies presumably headed by multiple neotenic reproductives, either by themselves or together with one or more of the original primary reproductives. Such colonies were likely inbred descendants of simple family colonies. Mixed

family colonies were those with more than four alleles at one or more loci, indicating the presence of two or more unrelated same-sex reproductives. Mixed family colonies could possibly arise through colony founding by three or more primary reproductives, by adoption of new primary reproductives by established colonies, or by colony fusion.

The genetic structure of colonies was further quantified by estimating F -statistics and the coefficient of relatedness from worker genotypes by using the program FSTAT (Goudet 2001). For each species, we conducted a two-tier analysis of genetic variation using the methods of Weir and Cockerham (1984), in which each colony is considered a subpopulation. We followed the notation of Thorne et al. (1999) and Bulmer et al. (2001) in identifying the different components of variation as the individual (I), colony (C), and total (T). According to this notation, F_{IT} in the current study is equivalent to the standard inbreeding coefficient, F_{IS} , and denotes the degree of inbreeding within individuals relative to others in the population. F_{CT} is similar to F_{ST} , representing the degree of genetic differentiation among colonies. The final term F_{IC} is the colony inbreeding coefficient and has no biologically equivalent term in solitary organisms. This term is especially sensitive to the numbers of reproductives within colonies and their mating patterns and is therefore particularly informative in inferring colony breeding structure (Thorne et al. 1999). Standard errors of the F values and of the relatedness coefficient were generated by jackknifing over loci. Significance of the values was determined by means of a one-sample t -test. A two sample approximate t -test assuming unequal variances (Sokal and Rohlf 1981) was used to determine significance in comparing the values between two groups.

Genotypes of reproductives in each simple family colony were inferred from the genotypes of the worker offspring and were used to investigate the breeding structure within simple families in more detail. We used these reconstructed genotypes to estimate F_{IS} by using the program Genetic Data Analysis (Lewis and Zaykin 2000). Ninety-five percent confidence intervals (CIs) were obtained by bootstrapping across loci (1,000 replicates). The average degree of relatedness between reproductives in these colonies was estimated using the program Relatedness version 5.0.8 (Queller and Goodnight 1989; available at <http://www.gsoftnet.us/GSoft.html>). Also, we identified pairs of reproductives within these colonies that were likely full siblings by using the program Kinship version 1.3.1 (Goodnight and Queller 1999; available at <http://www.gsoftnet.us/GSoft.html>). In this test, we used zero as the null hypothesis and the coefficient of relatedness among nestmate workers of each species as the hypothesis. To determine the statistical significance, we used 10,000 permutations.

Isolation by Distance. To determine whether there was significant isolation by distance among colonies of each species, the correlation between F_{CT} and geographic distance among all pairs of colonies was determined. Because of the nonindependence of data

Table 2. Species abundance of four sympatric species of subterranean termites from Charleston, SC

Species	No. samples (% of total)	No. colonies (% of total)
<i>R. flavipes</i>	18 (28.1)	18 (36.7)
<i>R. hageni</i>	21 (32.8)	21 (42.9)
<i>R. virginicus</i>	10 (15.6)	4 (8.2)
<i>C. formosanus</i>	15 (23.4)	6 (12.2)

points in the correlation, the significance of the coefficient was determined by means of a Mantel test as implemented in the program GenePop on the Web (Raymond and Rousset 1995; available at <http://wbiomed.curtin.edu.au/genepop/>).

Results

Species Identification and Colony Designations. *R. flavipes* and *R. hageni* were the most abundant species, together accounting for some 60% of the 64 samples collected and $\approx 80\%$ of the colonies (Table 2). Colony affiliations of the samples are shown in Fig. 1. Each of the *R. flavipes* and *R. hageni* samples were considered separate colonies, whereas at least some colonies of *R. virginicus* and *C. formosanus* spanned multiple collection points. Seven of the *R. virginicus* colonies shared all the same alleles and the same genotypes and were not significantly differentiated (all $P > 0.12$, exact test for genotypic differentiation). These were considered part of the same colony with a maximum linear distance between collection points of 125 m. The other three samples were significantly differentiated from the large colony and from each other (all $P < 0.0001$, exact test for genotypic differentiation) and were thus considered separate colonies, yielding a total of four colonies of *R. virginicus*. As described previously (Vargo et al. 2006), the 15 *C. formosanus* samples were grouped into six colonies; two of which were represented by a single collection point each, two by two collection points each, one by four, and one by five. The most expansive colony of *C. formosanus* spanned 133 linear m. Pairwise comparisons using the Wilcoxon rank sums test indicated that the frequency of such expansive colonies was significantly greater in *R. virginicus* and *C. formosanus* than in *R. flavipes* and *R. hageni* (all $P < 0.04$), but there was no significant difference between *R. virginicus* and *C. formosanus* ($P = 0.49$).

Colony Breeding Structure. The majority of colonies of all species were simple families (Table 3), ranging from all four of the *R. virginicus* colonies, including the very expansive colony comprising seven collection points, to some 72% of colonies in *R. flavipes*. All but one of the *C. formosanus* colonies were simple families, but in a more extensive sample of 25 colonies from the Charleston population, including the six from Charles Towne Landing State Historic Site in the current study, 48% were simple families. The genetic structure of colonies of each species is given in Table 4. Considering the overall level of inbreeding, F_{IT} , only workers of *C. formosanus* were

Table 3. Breeding structure of colonies of four sympatric species of subterranean termites from Charleston, SC

Species	No. simple family colonies (%)	No. extended family colonies (%)	No. mixed family colonies (%)
<i>R. flavipes</i>	13 (72.2)	4 (22.2)	1 (5.6)
<i>R. hageni</i>	20 (95.2)	1 (4.8)	0 (0)
<i>R. virginicus</i>	4 (100)	0 (0)	0 (0)
<i>C. formosanus</i>	5 (83.3)	1 (16.7)	0 (0)

significantly inbred ($F_{IT} > 0$, $P < 0.05$, two-tailed one-sample t -test), and only nestmate workers of *R. hageni* were significantly more related than expected for full siblings ($P < 0.02$, two-tailed one-sample t -test). Considering just simple family colonies, only workers of *R. hageni* were significantly inbred ($F_{IT} = 0.12$, $P < 0.03$, two-tailed one-sample t -test). In none of the other species did the F -statistics or the coefficients of relatedness differ significantly from values expected for colonies headed by monogamous pairs of unrelated reproductives (Table 4, case A; all $P \geq 0.06$ two-tailed one-sample t -test).

Extended family colonies were present in all species except *R. virginicus*, although both *R. hageni* and *C. formosanus* had only a single such colony. Further analysis of extended family colonies was limited to *R. flavipes*, because only in this species was there more than a single colony in this class ($n = 4$). Breeding structures with 20 or more reproductives and with three or more generations of inbreeding could be excluded for these colonies due to one or more of the statistical measures differing significantly from expected values (Table 4, case B). Thus, on average, the extended family colonies of *R. flavipes* most likely were headed by three to 10 neotenics who were the direct descendants of the founding pair of primaries.

Only a single mixed family colony was found. Analysis of the 30 workers genotyped in this colony showed the presence of two distinct family groups, consisting of 24 and six individuals, respectively. Out of a total of 37 alleles present in the two families, they shared only 11 (29.7%) in common; of a total of 35 genotypes present, they shared only four (11.4%) in common. The average \pm SE degree of relatedness among all nestmates in this colony ($r = 0.34 \pm 0.05$) was significantly lower than the population average ($r = 0.48 \pm 0.02$; $P < 0.03$, two-tailed approximate t -test). Between the two cohabiting families, workers were not related to each other ($r = -0.09 \pm 0.19$), but within each family, workers were as related as full siblings ($r = 0.50 \pm 0.08$ and 0.58 ± 0.065 , for family 1 and 2, respectively).

The degree of inbreeding and of nestmate relatedness among reproductives in simple family colonies is shown in Table 5. Similar to the results for the degree of inbreeding in workers overall (F_{IT}), reproductives in *C. formosanus* and *R. hageni* were the most inbred, but not significantly so (based on overlap of the 95% CIs with zero) and not significantly more so than those of *R. flavipes* or *R. virginicus* (based on overlapping 95% CIs). The coefficient of relatedness among nest-

Table 4. Colony genetic structure of four sympatric species of subterranean termites from Charleston, SC

	F_{IT}	F_{CT}	F_{IC}	r
Empirical values				
<i>R. flavipes</i>				
All colonies ($n = 18$)	0.03	0.25	-0.29	0.48
(SE)	(0.03)	(0.02)	(0.02)	(0.02)
Simple family colonies ($n = 13$)	0.01	0.25	-0.32	0.50
(SE)	(0.02)	(0.01)	(0.02)	(0.01)
Extended family colonies ($n = 4$)	0.17	0.27	-0.14	0.47
(SE)	(0.05)	(0.04)	(0.04)	(0.06)
<i>R. hageni</i>				
All colonies ($n = 21$)	0.14	0.34	-0.31	0.60
(SE)	(0.05)	(0.03)	(0.03)	(0.03)
Simple family colonies ($n = 20$)	0.12	0.32	-0.31	0.58
(SE)	(0.06)	(0.04)	(0.03)	(0.04)
<i>R. virginicus</i>				
Simple family colonies ($n = 4$)	-0.04	0.21	-0.32	0.44
(SE)	(0.07)	(0.03)	(0.05)	(0.03)
<i>C. formosanus</i>				
All colonies ($n = 6$)	0.17	0.38	-0.34	0.66
(SE)	(0.07)	(0.06)	(0.08)	(0.07)
Simple family colonies ($n = 5$)	0.11	0.35	-0.38	0.64
(SE)	(0.06)	(0.05)	(0.09)	(0.07)
Simulated values				
A. Simple families headed by outbred parents	0.00	0.25	-0.34	0.50
B. Extended families with inbreeding among neotenes				
(i) $N_f = 2, N_m = 1, X = 1$	0.26	0.35	-0.14	0.55
(ii) $N_f = 5, N_m = 1, X = 1$	0.27	0.34	-0.11	0.53
(iii) $N_f = N_m = 5, X = 1$	0.27	0.29	-0.03	0.46
(iv) $N_f = N_m = 10, X = 1$	0.33	0.34	-0.01	0.51

Simulated values are from Thorne et al. (1999) and Vargo (2003a). N_f , number of females; N_m , number of males; X , number of generations of inbreeding.

mate reproductives was also higher in *C. formosanus* and *R. hageni*, but only in the latter species was it significantly greater than zero ($P < 0.04$, two-tailed approximate t -test), and in neither species was it significantly greater than the other two species (all $P > 0.08$, two-tailed approximate t -test).

Isolation by Distance Analysis. There was no significant correlation between colony pairwise F_{CT} values and geographic distance for any of the four species (all $r \leq -0.06, P \geq 0.49$). Similar results were obtained using $F_{CT}/(1 - F_{CT})$ and natural log of distance (all $r \leq 0.01, P \geq 0.49$).

Discussion

These results give a detailed view of the structure of a relatively diverse community of subterranean termites consisting of all four species known to occur in the eastern United States, including three native and one introduced species. In addition, we conducted a

comparative study of colony spatial organization and breeding systems at a single site. There were marked differences among the species in relative abundance and spatial expanse of colonies, whereas the breeding systems showed both similarities and differences. *R. flavipes* and *R. hageni* were by far the most common species, together accounting for some 80% of all colonies. These two species formed localized colonies with foraging distances in most cases probably not exceeding 30 linear meters. Although *R. virginicus* and the invasive *C. formosanus* were less common, at least some colonies of these latter two species were more expansive, covering distances up to 125 and 133 linear meters, respectively. In terms of their breeding structures, colonies of all species consisted mainly or exclusively of simple families with a much smaller fraction of extended family colonies, but there were differences among species in the degree of inbreeding within colonies.

Our results on the relative abundance of species is admittedly based on a small area in only one habitat and may not be typical of the subterranean termite community structure across the entire eastern United States. Moreover, our results differ from what has been reported for several other areas in the southeastern and south central United States, although it should be mentioned that the current study was the only one in which individual colonies were identified. Our results show a higher relative abundance of *R. hageni* than has been reported in other geographic locations in the eastern and central United States. In a census of 24 1-ha plots in southern Mississippi, in which colony

Table 5. Comparative levels of inbreeding (F_{IS}) and nestmate relatedness (r) among reproductives in simple family colonies based on analysis of reconstructed genotypes

	<i>R. flavipes</i>	<i>R. hageni</i>	<i>R. virginicus</i>	<i>C. formosanus</i>
n	13	20	4	4
F_{IS}	0.00	0.07	-0.06	0.11
(95% CIs)	(-0.02-0.03)	(-0.01-0.15)	(-0.14-0.03)	(-0.17-0.39)
r	0.03	0.20	0.03	0.21
(SE)	(0.02)	(0.08)	(0.12)	(0.21)

n , number of colonies.

identities were assumed based on a minimum distance of 20 m between collection points, Howard et al. (1982) reported finding only *R. flavipes* and *R. virginicus*, with the latter species being nearly twice as abundant as the former (4.4 colonies per ha versus 2.4 colonies per ha). Surveys of termites in the southern United States involving pest management professionals (Scheffrahn et al. 1988; Messenger et al. 2002; Austin et al. 2004a,b,c) have found *R. flavipes* to be the most common species accounting for 58–78% of subterranean termite samples from Florida, Louisiana, Texas, Arkansas, and Oklahoma; *R. virginicus* was the second most common species in Florida and Louisiana, comprising 38 and 18%, respectively (Scheffrahn et al. 1988, Messenger et al. 2002), whereas it comprised only 9% of the samples from Arkansas and Texas (Austin et al. 2004a,c) and 2% of the samples from Oklahoma (Austin et al. 2004b). Of the previous studies, *R. hageni* was most common in Louisiana and Arkansas, where it comprised 19 and 16% of the subterranean termite samples, respectively (Messenger et al. 2002, Austin et al. 2004c), whereas this species accounted for only 1–8% of the subterranean termite samples from Florida, Oklahoma, and Texas (Scheffrahn et al. 1988; Austin et al. 2004a,b). Because the above-mentioned studies relied heavily on samples collected by pest control operators, they most likely involved termites mainly associated with structural infestations that may not be representative of the species composition as it occurs in natural areas.

In a study of a natural area of central Texas by using evenly spaced subterranean monitoring stations, Houseman et al. (2001) reported slightly more stations attacked by *R. hageni* (36) than by *R. flavipes* (25), possibly indicating that the former species was more abundant, although colony identity of the foraging groups was not known. The relatively high abundance of *R. hageni* in the present coastal site is similar to the results of a recent study in a Coastal Plain site in North Carolina where H. A. Dalton and E.L.V. (unpublished data) found 28 (74%) of 38 subterranean termite colonies were *R. hageni*, eight (21%) were *R. flavipes*, and two (5%) were *R. virginicus*. In two 22- by 22-m forest plots in central North Carolina studied intensively over 3 yr, DeHeer and Vargo (2004) found that *R. flavipes* colonies accounted for 91% of the 33 colonies sampled, whereas *R. virginicus* and *R. hageni* comprised 6 and 3%, respectively. Thus, it seems that the relative abundance of subterranean termites across the eastern United States can vary considerably from one area to another, although detailed studies of species abundance based on colony identity are needed to quantify this variation more precisely.

The present results concerning colony foraging areas are consistent with previous findings on each of the species studied. A number of studies from central North Carolina indicate that the foraging ranges of *R. flavipes* colonies in this area are generally <30 m in diameter and often much smaller (Vargo 2003a,b; DeHeer and Vargo 2004; Vargo and Carlson 2006). A recent study also from central North Carolina indicates that colonies of *R. hageni* are generally localized

as well (Vargo and Carlson 2006). In contrast, one of the four *R. virginicus* in the current study had a linear foraging distance of 125 m, which seems to be the maximum distance recorded for the genus *Reticulitermes* in the United States; colonies of *R. flavipes* (= *R. santonensis*; Austin et al. 2005) in France, where it is an introduced species, can span some 300 m (Dronnet et al. 2005). Although colony foraging areas of *R. virginicus* have not received much study, large, expansive colonies have also been reported from central North Carolina covering up to 122 linear meters (Vargo 2003b, DeHeer and Vargo 2004, Vargo and Carlson 2006), suggesting that colonies of this species tend to forage over much larger distances than sympatric colonies of *R. flavipes* or *R. hageni*. The large colonies of *C. formosanus* were consistent with previous findings of this species with linear foraging distances of well over 100 m (Su and Scheffrahn 1988a, Su 1994, Messenger and Su 2005, Vargo et al. 2006) and estimated worker populations sizes up to nearly 7 million (Su et al. 1984, Su and Scheffrahn 1988b).

There were important similarities and differences in colony breeding structure across the four species studied here. Simple families were the predominant colony type, ranging from all simple families in the four sampled *R. virginicus* colonies to 72% of the colonies in *R. flavipes*. Although all but one of the six *C. formosanus* colonies were simple families, these were part of a larger population of 25 colonies from Charleston reported on separately (Vargo et al. 2006) in which 12 (48%) were simple families. Based on the *F*-statistics and relatedness coefficients of nestmates, simple family colonies of *R. flavipes*, *R. virginicus*, and *C. formosanus* seemed to be largely headed by outbred reproductives. Only in *R. hageni* were reproductives within simple family colonies significantly related to each other. This could have been due to a portion of the colonies of this species each headed by either related primary reproductives and/or single monogamous pairs of neotenic reproductives. Our data do not allow us to distinguish between these two possibilities. *R. flavipes* was the only species in which there was a sufficient number of extended family colonies to perform statistical analysis, and the highly negative F_{TC} value in these colonies is indicative of low numbers of neotenic reproductives, on the order of three to 10. Also, *R. flavipes* was the only species in which a mixed family colony was found, suggesting that such genetically complex groups are not widespread within the study species, confirming the results of many other studies (Vargo 2003a,b; Vargo et al. 2003, 2006; DeHeer and Vargo 2004; Husseneder et al. 2005; Vargo and Carlson 2006).

The results concerning the breeding system of *R. flavipes* in this study population show strong similarities to what was previously reported for central North Carolina in four recent studies (Vargo 2003a,b; DeHeer and Vargo 2004; Vargo and Carlson 2006): some 70% of the 151 colonies studied previously were simple families compared with 72% here, 28% were neotenic-headed extended families descended from simple families compared with 22% here, and there were three

mixed family colonies (2% of the total) compared with the one mixed family colony found here. In addition, as found previously in central North Carolina, the reproductives in simple family colonies were mostly outbred, and the numbers of neotenes were relatively low (three to nine) with only a generation or two of inbreeding. Finally the mixed family colony documented in the current study shared characteristics with three other colonies found in previous studies (DeHeer and Vargo 2004, Vargo and Carlson 2006), in which there also were distinct family groups, with no evidence of interbreeding among the reproductives in the different groups.

We found a lack of significant isolation by distance for all four of the species studied here, although sample sizes for *R. virginicus* and *C. formosanus* were too small for robust analyses in these species. An absence of isolation by distance at small spatial scales suggests that successful colony foundation follows relatively long-range mating flights rather than through budding or short range (<30 m) flights. These results on *R. flavipes* support previous findings on this species in central North Carolina (Vargo 2003a; Vargo and Carlson 2006) and Massachusetts (Bulmer et al. 2001). Together, these results from populations spanning a large portion of the range of *R. flavipes* (South Carolina, North Carolina, and Massachusetts) suggest that colony reproduction primarily occurs through long-range mating flights rather than through short-range mating flights or budding. The lack of isolation by distance in the present *R. hageni* population, however, is at odds with previous results for this species. Vargo and Carlson (2006) found significant isolation by distance in a population of *R. hageni* in central North Carolina, which the authors attributed to short range mating flights and frequent pairing of related reproductives during colony founding.

Studies of other subterranean termites, also suggest that colony reproduction by budding may not be all that common in this group. In a study of the European species *Reticulitermes grassei* Clément, DeHeer et al. (2005) found no significant isolation by distance in three populations in southwestern France. In several studies of the Formosan subterranean termite, no significant isolation by distance has been detected in two populations in Japan (Vargo et al. 2003); two populations in New Orleans, Louisiana (Husseneder et al. 2005, Vargo et al. 2006); and populations in Rutherford County, NC, and Charleston, SC (Vargo et al. 2006).

R. flavipes, *R. hageni*, and *R. virginicus* occur sympatrically over much of their ranges in the southeastern United States (Snyder 1954), where they sometimes co-occur with the introduced *C. formosanus*. Because all four species play similar ecological roles, it is reasonable to assume that interspecific competition contributes to resource partitioning among them, especially in the three native species, which have presumably contended with each other for millennia. No doubt a combination of biotic and abiotic factors determines the relative species abundance within a given habitat. Although little effort has been made to identify specific factors affecting the distribution and

abundance of individual *Reticulitermes* spp., a few studies point toward local variation in temperature and soil moisture (Howard et al. 1982, Houseman et al. 2001) and in susceptibility to flooding (Forschler and Henderson 1995) as being among the contributing abiotic factors. In this study, we used molecular genetic markers to provide a detailed view of the relative abundance of colonies of all members of a subterranean termite community and to glean insight regarding variation in the breeding structure and spatial expansiveness of colonies. This and other similar studies will help lay the groundwork for more detailed ecological studies aimed at explaining the observed patterns.

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