# Genetic Analysis of Colony and Population Structure of Three Introduced Populations of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) in the Continental United States

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**ABSTRACT** The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a major invasive pest species in many parts of the world. We compared the colony breeding system and population genetic structure in three introduced populations in the continental United States: Charleston, SC; City Park, New Orleans, LA; and Rutherford County, NC. Based on worker genotypes at 12 microsatellite loci, we found that colonies were mainly genetically distinct entities consisting of either simple families headed by monogamous pairs of reproductives or extended families containing multiple neotenic (replacement) reproductives descended from simple families. Populations varied from 48% simple families in Charleston to 82% simple families in City Park. Extended family colonies in all three populations were likely headed by <10 neotenic reproductives. There was no significant isolation by distance in any of the populations, suggesting that colonies reproduce by relatively long-range mating flights and/or human-mediated dispersal within each population. The Charleston population showed evidence of a recent genetic bottleneck and most likely was founded by very few colonies. Cluster analysis indicated that the Charleston and City Park populations are quite genetically distant from each other and most likely originated from different source populations. The more recently introduced Rutherford County population was genetically most similar to City Park. These findings, together with results from other infested sites, indicate considerable variation in the genetic structure and breeding system of introduced populations of this species, making it unlikely that there is a simple genetic or behavioral explanation for the success of *C. formosanus* as an invasive species.

**KEY WORDS** microsatellite markers, breeding system, social organization, Formosan subterranean termite, *Coptotermes formosanus*

INVASION OF BIOLOGICAL COMMUNITIES by exotic species is a serious and growing problem that is having enormous ecological and economic impacts around the world (Pimentel et al. 2000, Mooney and Cleland 2001). Invasive social insect species are especially damaging; several species of ants, wasps, and termites have severely disrupted ecological communities and/or caused significant economic damage in many of their introduced ranges (Vinson 1986, Williams 1994, Moller 1996). The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a highly invasive species that has become established in numerous parts of the world (Su and Tamashiro 1987, Su 2003). A native of East Asia, most likely mainland China (Kistner 1985), *C. formosanus* has invaded several other oriental locations (Taiwan, Japan, and Sri Lanka), the Pacific (Hawaii, Guam, Midway, and Marshall Islands), South Africa, and many areas of the continental United States. Its initial introduction into the U.S. mainland is closely associated with military ports receiving equipment and supplies from the Pacific theater after World War II (La Fage 1987). A specimen collected from Charleston, SC, in 1957 is the first record of *C. formosanus* on the U.S. mainland (Chambers et al. 1988). Other early reports of this species on the U.S. mainland include Houston, TX, in 1965 (Anonymous 1965) and New Orleans in 1966 (Spink 1967). *C. formosanus* is now widespread but patchily distributed on the U.S. mainland, occurring in 10 states, including virtually all the southeastern and southcentral states and California (Woodson et al. 2001).

In this study, we used microsatellite markers to investigate colony and population genetic structure in three populations of *C. formosanus* from the southeastern Unites States, including two populations that were among the first sites known to be invaded—

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Charleston, SC, and New Orleans, LA—and a recently discovered population from western North Carolina. The populations of Charleston and Rutherford County are relatively isolated (Chambers et al. 1988; unpublished data), whereas the City Park population is embedded in a much more extensive population in and around New Orleans (Woodson et al. 2001). Our first objective was to infer colony breeding structure in these three populations to determine whether there were common features that might help account for the exceptional invasion success of this species. In other highly invasive social insects, primarily ants, reduced genetic variability after introduction events can favor invasiveness by lowering intercolonial aggression, leading to large, unicolonial societies that are ecologically dominant (Holway et al. 2002, Tsutsui and Suarez 2003, Payne et al. 2004). Thus, it is conceivable that founder effects in *C. formosanus* could promote a particular type of colony social organization underlying its invasion success.

Genetic markers are a powerful tool for inferring colony breeding structure in social insects (Thorne et al. 1999, Ross 2001), and there has been an increasing number of genetic studies on colony social organization of termites (Atkinson and Adams 1997, Thompson and Hebert 1998a, b, Goodisman and Crozier 2002), especially subterranean termites (Clément 1981, 1984, Reilly 1987, Kaib et al. 1996, Husseneder et al. 1997, 1998, 1999, 2005, Jenkins et al. 1999; Bulmer et al. 2001, Clément et al. 2001, Husseneder and Grace 2001, Vargo 2003a, b, Vargo et al. 2003a, b, DeHeer and Vargo 2004, Dronnet et al. 2005, DeHeer et al. 2005). These studies indicate that colonies of subterranean termites are generally founded by pairs of unrelated primary (alate-derived) reproductives. As colonies age, the primary reproductives are replaced or supplemented by neotenics (precocious nonwinged reproductive forms that develop within the colony) who inbreed within the colony. In at least one species, *Reticulitermes flavipes* Kollar, there is evidence that a small percentage of colonies can contain three or more unrelated reproductives (Jenkins et al. 1999, Bulmer et al. 2001, DeHeer and Vargo 2004), and both laboratory (Fisher et al. 2004) and field (DeHeer and Vargo 2004) studies have shown that such genetically mixed groups can arise through colony fusion. It has been thought that, as colonies of subterranean termites grow and expand their foraging ranges, they frequently fragment into independent daughter colonies (Shellman-Reeve 1997, Myles 1999, Thorne et al. 1999). Although there is some genetic evidence suggesting that colony reproduction by budding may occur often in some species, e.g., the African subterranean termite *Schedorhinotermes lamanianus* (Sjöstedt) (Husseneder et al. 1998), it is not universal because intensive studies of a number of populations of *R. flavipes* have failed to find evidence for it (Bulmer et al. 2001, Vargo 2003a, DeHeer and Vargo 2004).

To date, we have studied the breeding structure of two introduced populations of *C. formosanus*, and these show considerable variation. In two populations from southern Japan, we found that 90% of the colonies were simple families headed by closely related reproductives, and the remaining 10% consisted of highly inbred extended family colonies (Vargo et al. 2003a). In contrast, we found that a little more than one-half the colonies (57%) in a New Orleans, LA, population were comprised of simple families headed by outbred reproductives, and the remaining colonies were only slightly inbred extended families (Husseneder et al. 2005). Comparative studies of additional introduced populations should help reveal whether there are features common to colony breeding structure across introduced populations, and, if so, what role these may play in the invasion success of this species. In addition, understanding the breeding structure of colonies may be important in management, especially in the case of *C. formosanus*, where there are efforts in place to eliminate all colonies within large areas. For example, if colonies frequently form spatially separated reproductive centers with limited movement of workers among them, this could restrict the distribution of bait toxicants, interfering with the ability of baits to eliminate entire colonies (Husseneder et al. 2003).

Our second objective was to study the genetic relationships among these three populations as well as two other introduced populations that were previously studied in Japan (Vargo et al. 2003a) and another population in New Orleans, LA (Husseneder et al. 2005). Analyses of the genetic relationships among introduced populations of invasive species can help determine whether they arose from a single introduction or multiple introductions, whether some introduced populations likely originated from the same source population, and to infer patterns of dispersal within introduced ranges (Goodisman et al. 2001, Buczkowski et al. 2004, Johnson and Starks 2004, Kolbe et al. 2004). For example, it is of interest to know whether there was a single introduction of *C. formosanus* to the U.S. mainland that spread to various locations through human-aided dispersal or whether there were multiple introductions from different source populations.

## Materials and Methods

**Sample Collection.** Samples were collected from natural wood or artificial feeding stations in three populations: City Park, New Orleans, LA; Charleston, SC; and Rutherford County, NC. Figures 1–3 show the relative locations of the collection points in each population. With few exceptions, samples from each collection point consisted of at least 20 individuals –workers, soldiers and sometimes alates. Termites were placed directly into 95% ethanol and stored at −20°C until DNA extraction.

**City Park, New Orleans, LA.** A total of 19 samples was collected between 19 March and 16 May 2001 (Fig. 1). As part of a larger study of termites infesting trees in City Park, bucket traps consisting of 15.25-cm circular valve box covers filled with rolled corrugated cardboard were installed in the ground within 1 m of a tree in spring 2001. Only trees and shrubs >10 cm in diameter at ground level were used. *C. formosanus* was

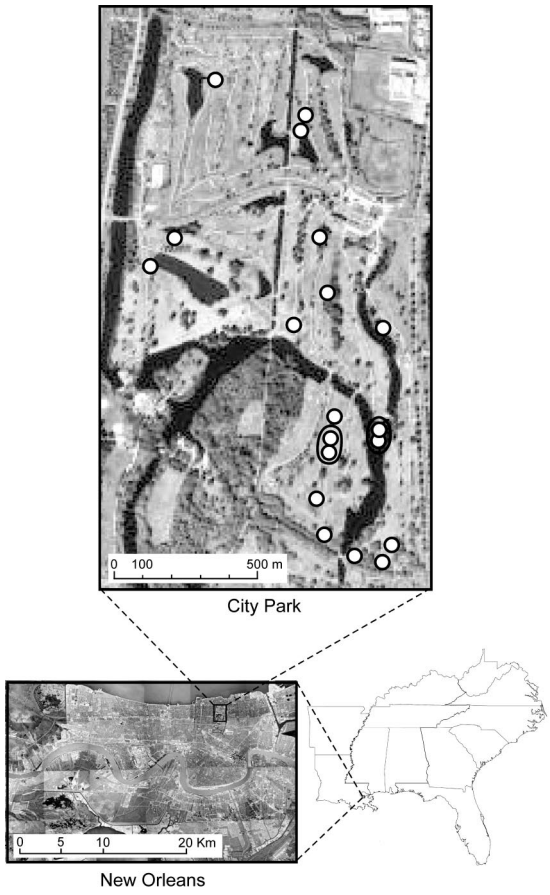


**Fig. 1.** Locations of *C. formosanus* samples from the City Park, New Orleans, LA, population. Dark shapes represent bodies of water. Encircled collection points were part of the same colony.

most commonly found with southern live oak (*Quercus virginiana* Miller), followed by southern yellow pine (*Pinus* sp.), Chinese tallow (*Sapium sebiferum* L. Roxburgh), and bald cypress (*Taxodium distichum* L.). The location of each sample was determined using a Trimble Pro XLR GPS (Trimble, Sunnyvale, CA) device.

**Charleston, SC.** Thirty-six samples were collected from Charleston, SC (Fig. 2). Samples of workers and soldiers, and in some cases, alates, were collected from the trunks or the bases of 21 trees in Charleston, SC, on 3 June 2002. Most of the samples were collected in or near Hampton Park located adjacent to the Citadel northwest of downtown Charleston. The location of each sample was determined using a Trimble GeoExplorer 3 handheld GPS device. An additional 15 samples were collected from trees and wood debris (tree stumps and down limbs) in Charles Towne Landing State Historic Site on 13 and 19 May 2003. This park is located ≈2 km from Hampton Park across the Ashley River. The location of samples was manually marked on a map, and the distance between collection points was measured on the ground using a tape measure.

**Rutherford County, NC.** Sixteen samples were collected from infested trees, buildings, and railroad ties along an ≈8-km stretch of U.S. Highway 221A running through the towns of Rutherfordton, Spindale, and Forest City in Rutherford County, NC, on 12 and 13 August 2003 (Fig. 3). This was part of a cooperative effort of the Structural Pest Control Division, North Carolina Department of Agriculture and Consumer Services, North Carolina Cooperative Extension Service, North Carolina State University, and local pest control companies to delineate the boundaries of a population first discovered in 1992 (M.G.W., unpublished data).

**Microsatellite Analysis.** Genomic DNA was extracted from individual termite whole bodies using the DNeasy Tissue Kit (Qiagen, Valencia, CA). Twenty workers from each collection point were genotyped at 12 microsatellite loci (Table 1), 11 of which were identified from *C. formosanus* (Vargo and Henderson 2000) and 1 of which, *Rf 6–1*, was identified from *Reticulitermes flavipes* (Vargo 2000). We followed the protocols of Vargo (2000) and Vargo and Henderson (2000) for polymerase chain reaction (PCR) amplification and genotype scoring.

**Colony Affiliations.** To determine if samples from nearby collection points represented the same or different colonies we used three criteria. First, all pairs of collection points within each population were tested for genotypic differentiation by means of a permutation test using the program FSTAT (Goudet 2001). Second, we compared the number of private alleles (the number of alleles present in one collection point but not the other) in each pair of collection points. Groups of workers with different alleles present were considered to belong to different colonies. Third, we took into account the distance between collection points. Because the maximum linear distance reported for foragers of a single *C. formosanus* colony is 115 m (Su and Scheffrahn 1988), collection points separated by much further (>500 m) were considered to belong to different colonies. In practice, collection points belonging to different colonies were significantly differentiated and had many private alleles. More emphasis was placed on analysis of private alleles in the Charleston population because of the low genetic variability present at this location.

**Classification of Colonies.** Following the classification of Vargo (2003a, b), Vargo et al. (2003a), and DeHeer and Vargo (2004), colonies were placed in one of the following two groups: simple families or extended families. Simple families were those presumably headed by a monogamous pair of reproductives. Colonies were considered simple families if the genotypes of the workers were consistent with those expected for a single pair of parents and if the ratios of the observed genotypes did not differ significantly from the expected Mendelian ratios as determined by a *G*-test. For each colony, an overall *G*-value was obtained by summing all the locus-specific *G*-values. In contrast to simple families, extended families are headed by multiple kings and/or queens. Extended family colonies were those having genotypes that

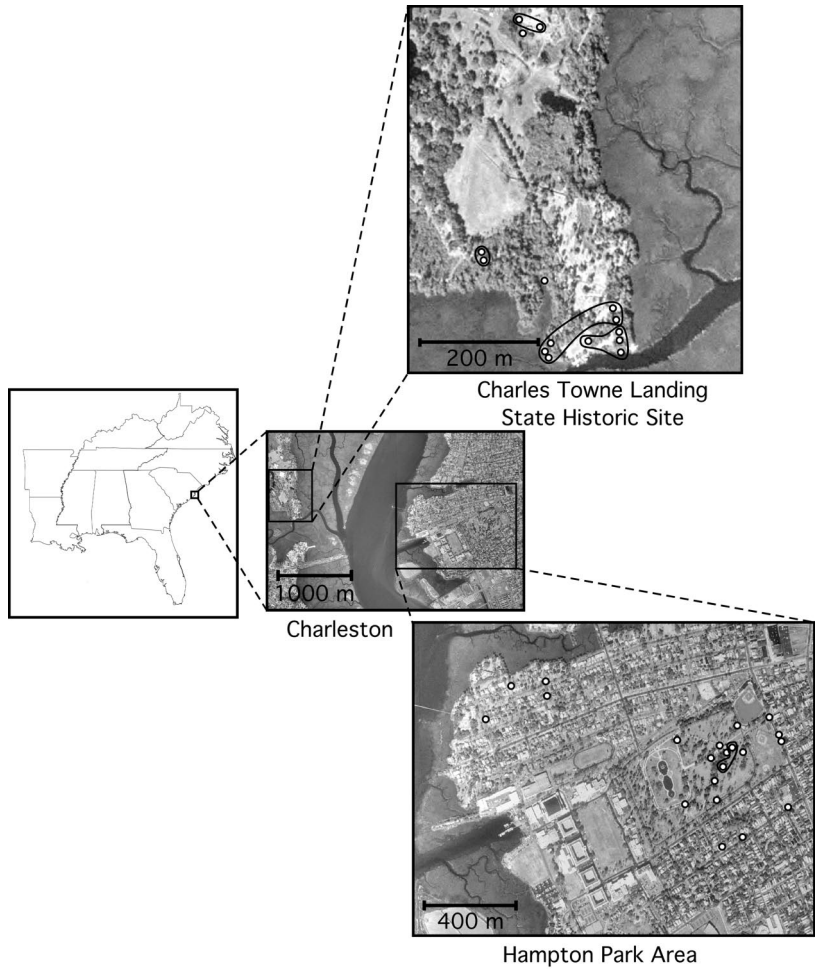


**Fig. 2.** Locations of *C. formosanus* samples from the Charleston, SC, population. Collection points that are encircled were part of the same colony.

were inconsistent with a single pair of reproductives (e.g., one or more loci with five or more genotypic classes or three classes of homozygotes) or those in which the genotypes were consistent with the presence of a single pair of reproductives but the observed frequencies of the genotypes deviated significantly from the expected values in simple families ($P < 0.05$, *G*-test).

**Colony and population genetic structure.** Colony and population genetic structure were assessed by estimating the coefficient of relatedness among nestmate workers and by estimating *F*-statistics. The average relatedness among colony mates was estimated using the program Relatedness v. 5.0.8 (Queller and Goodnight 1989) with colonies weighted equally. The SEs were obtained by jackknifing over loci. *F*-statistics were estimated using FSTAT (Goudet 2001). We followed the notation of Thorne et al. (1999) and Bulmer et al. (2001) in which each colony is treated as a subpopulation, and genetic variation is partitioned among the following components: the individual (**I**), the colony (**C**), and total (**T**). According to this notation, $F_{IT}$ is analogous to the standard inbreeding coefficient, $F_{IS}$, and is a measure of the level of inbreeding in individuals relative to the population. $F_{CT}$ is analogous to $F_{ST}$ and represents the genetic differentiation among colonies. $F_{IC}$ is the coefficient of inbreeding in individuals relative to their colony and is particularly sensitive to the numbers of reproductives present and their mating patterns within colonies. As shown by Thorne et al. (1999) and Bulmer et al. (2001), $F_{IC}$ is expected to be strongly negative in simple family colonies, to approach zero with increasing numbers of reproductives within colonies and to become positive with assortative mating among multiple groups of reproductives within colonies or with mixing of individuals from different colonies. For each of the *F*-components, SEs were estimated by jackknifing over loci. Significance of the *F*-values and the relatedness coefficients was determined by comparing them to predicted single point values by means of one-sample *t*-tests ($P < 0.05$). Pairs of empirical values were tested for significance using two-sample *t*-tests and not assuming equal variances. Pairwise genetic

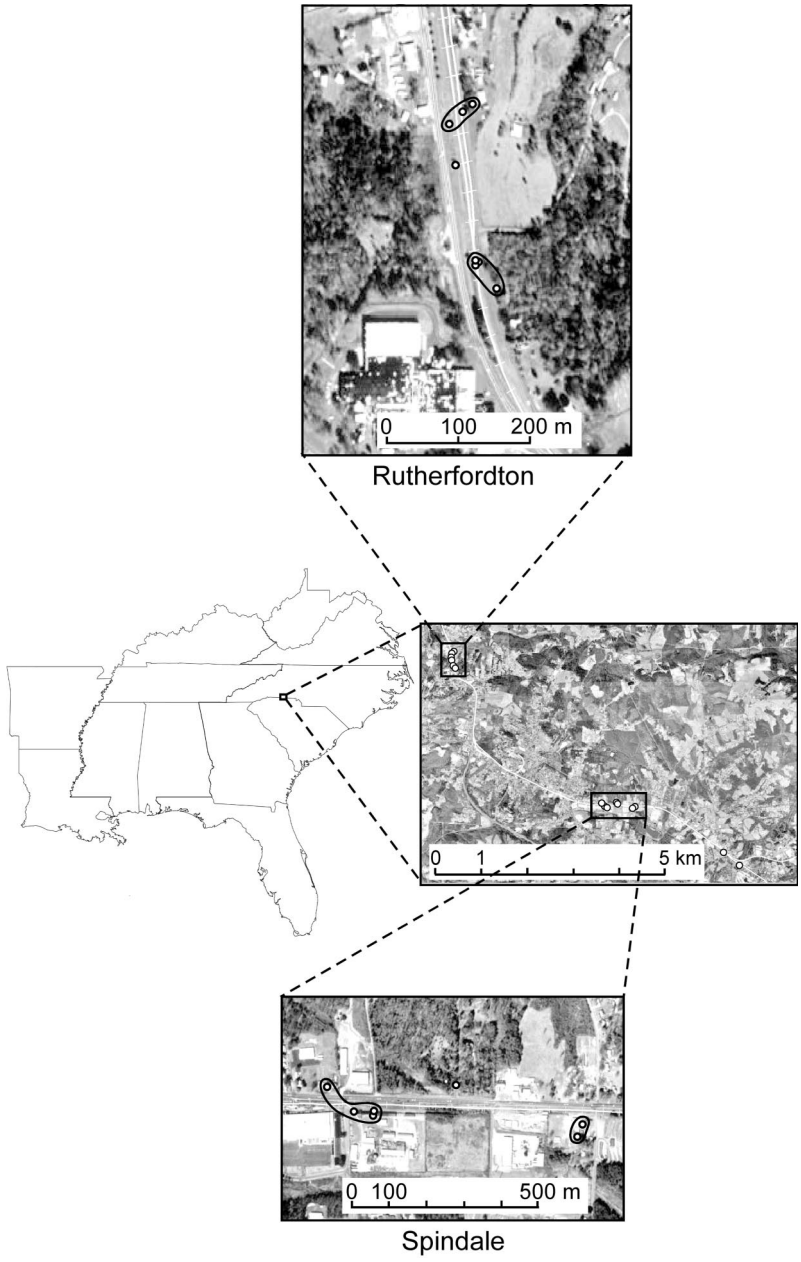


**Fig. 3.** Locations of *C. formosanus* samples from the Rutherford County, NC, population. Encircled collection points were part of the same colony.

differentiation ($F_{ST}$) among populations was determined using the program GDA (Lewis and Zaykin 2000) by performing a hierarchical analysis incorporating colony level structure simultaneously.

From the worker genotypes present in each of the simple family colonies, the genotypes of the parents were reconstructed, and *F*-statistics and the coefficient of relatedness among nest mate reproductives were estimated from the inferred genotypes. The likelihood that the two reproductives in simple family colonies were full siblings (nest mates) was tested using the program Kinship (Goodnight and Queller 1999). For the primary hypothesis, we used the average degree of nestmate relatedness for each population, and this was tested against a null hypothesis of zero. Log likelihood ratios were calculated based on 1,000 iterations.

**Isolation by Distance Analysis.** To assess isolation by distance, we first obtained pairwise $F_{CT}$-values for all pairs of colonies within each population. These values were converted to $F_{CT}/(1 - F_{CT})$, and the Pearson product correlation coefficient between these values

**Table 1. Variability of microsatellite loci in the Charleston, SC; Rutherford County, NC; and CityPark, New Orleans, LA, populations**

| Locus | City Park, New Orleans, LA | | | Rutherford Co. NC | | | Charleston, SC | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. alleles | Freq. most common allele | He | No. alleles | Freq. most common allele | He | No. alleles | Freq. most common allele | He |
| *Cf 1–1* | 1 | — | | 1 | — | | 2 | 0.82 | 0.29 |
| *Cf 4:1 A2–4* | 3 | 0.36 | 0.66 | 3 | 0.81 | 0.32 | 4 | 0.38 | 0.71 |
| *Cf 4:1 A2–5* | 2 | 0.71 | 0.41 | 2 | 0.95 | 0.10 | 2 | 0.94 | 0.11 |
| *Cf 4–4* | 2 | 0.57 | 0.49 | 5 | 0.58 | 0.57 | 7 | 0.33 | 0.76 |
| *Cf 4–9A* | 2 | 0.54 | 0.50 | 4 | 0.37 | 0.69 | 3 | 0.41 | 0.66 |
| *Cf 4–10* | 2 | 0.55 | 0.49 | 3 | 0.48 | 0.64 | 3 | 0.41 | 0.66 |
| *Cf 8–4* | 2 | 0.77 | 0.35 | 6 | 0.33 | 0.74 | 4 | 0.37 | 0.71 |
| *Cf 10–4* | 1 | — | | 3 | 0.72 | 0.42 | 3 | 0.76 | 0.39 |
| *Cf 10–5* | 2 | 0.75 | 0.37 | 3 | 0.63 | 0.49 | 3 | 0.60 | 0.53 |
| *Cf 11–1* | 1 | — | | 1 | — | | 1 | — | |
| *Cf 12–4* | 2 | 0.87 | 0.23 | 4 | 0.50 | 0.59 | 3 | 0.39 | 0.66 |
| *Rf 6–1* | 1 | — | | 2 | 0.76 | 0.36 | 2 | 0.80 | 0.40 |
| Mean ± SD[a] | 1.9 ± 0.6 | | 0.44 ± 0.13 | 3.5 ± 1.3 | | 0.49 ± 0.20 | 3.1 ± 1.5 | | 0.53 ± 0.21 |

Allele frequencies were calculated by the program RELATEDNESS (Queller and Goodnight 1989) using all worker genotypes and colonies weighted equally.

[a] Polymorphic loci only.

and the ln of geographic distance for all pairs of colonies was calculated. The significance of the correlation coefficient was assessed by means of a Mantel test with 1,000 iterations as implemented in Genepop on the Web (Raymond and Rousset 1995).

**Tests for Genetic Bottleneck.** Because *C. formosanus* is an introduced species with a relatively short history in the U.S. mainland, we expect that the study populations experienced a recent genetic bottleneck. To determine if there was evidence for a bottleneck in each population, worker genotypes were tested for heterozygosity excess using two tests implemented in the program Bottleneck v. 1.2.02 (Piry et al. 1999). One test, developed by Cornuet and Luikart (1996), determines whether there is a significantly greater proportion of loci with heterozygosity excess than expected for a population at mutation-drift equilibrium using a sign test, whereas the other test detects significant heterozygosity excess on average across loci using a Wilcoxon sign-rank test (Piry et al. 1999). The latter test is considered to be the more appropriate and powerful of the two tests for <20 loci (Piry et al. 1999). The tests were conducted on each of the 20 resampled data sets for each population using the two-phased model of mutation, which includes a mixture of single-step and multi-step mutations, as recommended by the authors for microsatellite loci (Piry et al. 1999). We also followed the authors' suggestion in setting the single-step mutations to 95% and the variance among multiple steps to 12.

In addition, for each locus, we calculated $M = k/r$, the ratio of the number of alleles to the range of allele sizes, where k = the number of alleles and r = the number of possible alleles sizes between the smallest and the largest observed alleles (Garza and Williamson 2001). We calculated mean M across all loci for each population. This mean value was compared with $M = 0.68$, the threshold value below which a population can reasonably be assumed to have undergone a recent reduction in population size (Garza and Williamson 2001).

**Unweighted Pair-Group Method with Arithmetic Average Clustering.** To study the relationships of the three populations to each other and to two other introduced populations previously studied in Japan (Vargo et al. 2003a), as well as a previously studied population from Louis Armstrong Park, New Orleans (Husseneder et al. 2005), we used the program Mega2 (Kumar et al. 2001) to construct a tree using the unweighted pair group method with arithmetic mean from pairwise $F_{ST}$ values in GDA. Twelve loci were used in this analysis for each population, except the Louis Armstrong Park population, for which eight loci were used.

## Results

**Genetic Variability.** The three populations differed considerably in genetic variability. As shown in Table 1, 11 of the 12 loci examined were variable in the City Park population, with two to seven alleles per locus. Ten loci were variable in the Rutherford County population, with two to six alleles per locus. Despite having the most samples and the most colonies present, the Charleston population was the least variable, with no more than three alleles per locus. Of the total of 19 alleles present in the Charleston population, only 2 were unique, both of which occurred at locus *Cf 4:1A2–4*.

**Colony Affiliations.** Based on permutation tests of genetic differentiation, 13 of the 19 collection points in City Park were genetically distinct and were considered separate colonies. Two pairs of collection points located 13 and 55 m apart, respectively, were not significantly differentiated and had identical alleles and genotypes. Each of these pairs was grouped together into a colony (Fig. 1). There were two other collection points that also were not significantly differentiated from each other, but there were two private allele and four genotypes unique to one of these collection points. Moreover, these were separated by 900 m. Because of the presence of private alleles and

**Table 2. Examples of genotypes of *C. formosanus* workers from pairs of collection points in which the genotypes within each pair were not significantly differentiated**

| Locus | Pair 1[a] | | Pair 2[a] | |
|---|---|---|---|---|
| | CT1–17 | CT1–8 | CT1–13 | CT1–16 |
| *Cf 4:1A2–4* | | | | |
| *197/197* | 1 | 1 | | |
| *197/188* | 11 | 10 | 7 | 9 |
| *197/185* | | | 13 | 11 |
| *188/188* | 7 | 3 | | |
| *188/185* | | 4 | | |
| *185/185* | | 2 | | |
| *Cf 4:1A2–5* | | | | |
| *163/163* | | | | |
| *163/160* | 10 | 13 | | |
| *160/160* | 10 | 6 | 20 | 19 |
| *Cf 4–4* | | | | |
| *248/248* | 11 | 13 | | |
| *248/230* | 9 | 7 | 11 | 10 |
| *230/230* | | | 9 | 10 |
| *Cf 4–9A* | | | | |
| *302/302* | 1 | 2 | 6 | 5 |
| *302/287* | 14 | 11 | 8 | 10 |
| *287/287* | 5 | 7 | 6 | 5 |
| *Cf 4–10* | | | | |
| *245/245* | 1 | 3 | 7 | 6 |
| *245/236* | 12 | 10 | 7 | 9 |
| *236/236* | 6 | 7 | 6 | 5 |
| *Cf 8–4* | | | | |
| *243/243* | 20 | 20 | 9 | 12 |
| *243/234* | | | 11 | 8 |
| *Cf 10–5* | | | | |
| *296/296* | 10 | 12 | 10 | 9 |
| *296/281* | 10 | 7 | 10 | 11 |
| *281/281* | | 1 | | |
| *Cf 12–4* | | | | |
| *191/191* | 17 | 12 | 20 | 20 |
| *191/173* | 3 | 8 | | |

[a] Pair 1 was considered to belong to different colonies because of the presence of one allele and three genotypes present in CT1–8 but not CT1–17 and the long distance between them (874 m); whereas pair 2 was considered part of the same colony because they shared all the same alleles and were located relatively close to each other (101 m).

**Table 3. Comparison of the breeding structure of three introduced populations of the Formosan subterranean termite**

| Population | No. colonies | Simple families | | No. reproductives in extended families |
|---|---|---|---|---|
| | | No. (%) | Inbred | |
| City Park, New Orleans, LA | 17 | 14 (82%) | − | 3–9 |
| Charleston, SC | 25 | 12 (48%) | − | 4–9 |
| Rutherford Co., NC | 8 | 6 (67%) | + | 3–9 |

the large distance between them, these two collection points were considered different colonies. In Rutherford County, we grouped the 16 collection points into eight genetically differentiated colonies, three of which were present at multiple sites spanning from 46 to 144 linear m (Fig. 3).

Because of the low variability of the microsatellite loci in the Charleston population, assigning colony affiliations was not as straightforward as in the other two populations. Of the 315 pairwise tests of genotypic differentiation, 49 (16%) were not significant ($P > 0.05$). However, we excluded 29 of these pairs as belonging to the same colony because there were one or more private alleles present. The other pairs were considered part of the same colonies. Thus, one pair of nearby collection points located 101 m apart in Hampton Park was grouped together into the same colony (Fig. 2). Table 2 gives examples of two pairs of samples that were not significantly differentiated. CT1–17 and CT1–8 were not significantly differentiated ($P > 0.8$, exact test of genotypic differentiation) but were assigned to different colonies because of the presence of one private allele (*185* at locus *Cf1:4A2–4*) and three genotypes unique to CT1–8 (*Cf 1:4A2–5:188/185* and *185/185; Cf 10–5:281/281*), as well as the long distance (874 m) between them. Samples CT1–13 and CT1–16 were grouped into the same colony because they were not significantly differentiated ($P > 0.9$, exact test of genotypic differentiation), they shared all the same alleles and genotypes, and were located relatively close to each other (101 m). The differences between CT1–13/1–16 and CT1–17 were more typical of different colonies; they were significantly differentiated ($P < 0.00001$, exact test of genotypic differentiation), and they differed in having three private alleles and seven unique genotypes. The Charles Towne Landing State Historic Site had four multiple-site colonies, consisting of two to five collection points, the most expansive of which spanned 133 linear m (Fig. 2). The five collection points that comprised this expansive colony all had identical genotypes, and this colony was a simple family.

**Classification of Colonies.** There was variation in the proportion of simple and extended family colonies present in the three populations, ranging from about one-half simple families in Charleston to >80% simple families in City Park (Table 3). Of the 13 extended family colonies present in Charleston, 7 had genotypes inconsistent with the presence of a single pair of reproductives (e.g., too many homozygous classes), whereas 6 had Mendelian genotypes but the frequencies differed significantly from those expected ($P < 0.05$, *G*-test summed across loci). Because of the low genetic variability in this population, we could not exclude the possibility that some of these "extended family colonies" were actually "mixed family colonies," i.e., headed by two or more unrelated same sex reproductives (DeHeer and Vargo 2004). However, given the high degree of relatedness among worker nestmates ($r = 0.49$) in this population, we can conclude that if such mixed family colonies exist, they are not common. In the Rutherford County and City Park populations, the extended family colonies all had genotypes incompatible with a single pair of reproductives. Despite greater genetic diversity in these two populations (seven alleles at one locus in City Park and two loci with five or more alleles in Rutherford County), no more than four alleles per locus were present in any of the colonies, suggesting that reproductives within extended family colonies in these pop-

**Table 4. *F*-statistics and relatedness coefficients for worker nestmates of *C. formosanus* from Charleston, SC; Rutherford County, NC; and City Park, New Orleans, LA, and values expected for some possible breeding systems of subterranean termites as derived from computer simulations**

| | $F_{IT}$ | $F_{CT}$ | $F_{IC}$ | $r$ |
|---|---|---|---|---|
| Empirical values | | | | |
| Charleston, SC | | | | |
| All colonies ($n$ = 25) | 0.139 | 0.280 | −0.196 | 0.492 |
| (SE) | (0.046) | (0.021) | (0.036) | (0.050) |
| Simple family colonies ($n$ = 12) | 0.036 | 0.285 | −0.349 | 0.584 |
| (SE) | (0.070) | (0.035) | (0.041) | (0.076) |
| Extended family colonies ($n$ = 13) | 0.194 | 0.238 | −0.058 | 0.420 |
| (SE) | (0.055) | (0.036) | (0.057) | (0.092) |
| Rutherford Co., NC | | | | |
| All colonies ($n$ = 8) | 0.239 | 0.379 | −0.225 | 0.637 |
| (SE) | (0.065) | (0.047) | (0.038) | (0.111) |
| Simple family colonies ($n$ = 6) | 0.132 | 0.323 | −0.282 | 0.616 |
| (SE) | (0.065) | (0.046) | (0.030) | (0.107) |
| Extended family colonies ($n$ = 2) | 0.434 | 0.499 | −0.127 | 0.684 |
| (SE) | (0.137) | (0.123) | (0.062) | (0.229) |
| City Park, New Orleans, LA | | | | |
| All colonies ($n$ = 17) | 0.085 | 0.339 | −0.384 | 0.609 |
| (SE) | (0.022) | (0.011) | (0.029) | (0.014) |
| Simple family colonies ($n$ = 14) | 0.008 | 0.306 | −0.428 | 0.596 |
| (SE) | (0.039) | (0.023) | (0.025) | (0.021) |
| Extended family colonies ($n$ = 3) | 0.380 | 0.475 | −0.176 | 0.649 |
| (SE) | (0.085) | (0.077) | (0.083) | (0.186) |
| Simulated breeding system | | | | |
| (A) Simple family colonies headed by outbred reproductive pairs[a] | 0.00 | 0.25 | −0.33 | 0.50 |
| (B) Extended family colonies with inbreeding among neotenics | | | | |
| (1) $N_f$ = $N_m$ = 1, X = 1[a] | 0.33 | 0.42 | −0.14 | 0.62 |
| (2) $N_f$ = 2, $N_m$ = 1, X = 1[b] | 0.26 | 0.35 | −0.14 | 0.55 |
| (3) $N_f$ = 2, $N_m$ = 1, X = 3[a] | 0.52 | 0.59 | −0.17 | 0.78 |
| (4) $N_f$ = 5, $N_m$ = 1, X = 1[b] | 0.27 | 0.34 | −0.11 | 0.53 |
| (5) $N_f$ = $N_m$ = 10, X = 1[c] | 0.33 | 0.34 | −0.01 | 0.51 |
| (6) $N_f$ = $N_m$ = 10, X = 3[a] | 0.37 | 0.38 | −0.02 | 0.56 |
| (7) $N_f$ = 200, $N_m$ = 100, X = 3[a] | 0.34 | 0.34 | 0.00 | 0.71 |

Empirical values were based on eight microsatellite loci in the Charleston, SC, population, 10 loci in the Rutherford County, NC, population, and 11 loci in the City Park, New Orleans, LA, population. For the simulated breeding systems, X represents the no. of generations of production of replacement reproductives within a colony; $N_f$ and $N_m$ represent the no. of replacement females and males, respectively, produced per generation.

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

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

[c] From Bulmer et al. (2001).

ulations were descended from monogamous pairs of reproductives.

**Colony and Population Genetic Structure.** The *F*-statistics and relatedness values estimated from the worker genotypes are shown in Table 4, along with values derived from computer simulations based on Thorne et al. (1999) and Bulmer et al. (2001). Overall, workers in all three populations were significantly inbred ($F_{IT}$ = 0.085–0.239; all $P < 0.05$, one-sample approximate *t*-test) and they were closely related to each other ($r$ = 0.49–0.64). The Rutherford County population was the most inbred overall ($F_{IT}$ = 0.239), but it differed significantly only from the City Park population ($P < 0.03$, two-sample approximate *t*-test). In all three populations, the extended family colonies were significantly more inbred and had significantly higher $F_{IC}$ values than their corresponding simple family colonies (all $P < 0.05$, approximate *t*-test). In City Park, $F_{CT}$ for extended families was significantly greater than that for simple families ($P < 0.03$, two-sample approximate *t*-test). The *F*-statistics and relatedness coefficient for workers in simple family colonies in the Charleston population were close to and not significantly different from those predicted for colonies headed by outbred pairs of monogamous reproductives, suggesting these colonies were headed by unrelated primary reproductives. However, workers in simple family colonies in Rutherford County were significantly more inbred than expected ($F_{IT}$ = 0.132, $P < 0.04$, one-sample approximate *t*-test), a result consistent with the elevated degree of relatedness between reproductives heading these colonies. Workers in the City Park simple families had significantly higher values of $F_{CT}$, $F_{IC}$, and $r$ than predicted (all $P < 0.03$, one-sample approximate *t*-test), most likely because the reproductives in these colonies originated from inbred colonies. As shown in Table 3, the main conclusions regarding the simple families in the three populations are the following: those in Charleston and City Park tended to be headed by unrelated reproductives resulting in outbred workers, whereas workers in the Rutherford County colonies are slightly inbred because nestmate reproductives are more closely related in this population.

Considering the extended family colonies, the presence of 10 or more neotenics of each sex (Table 4,

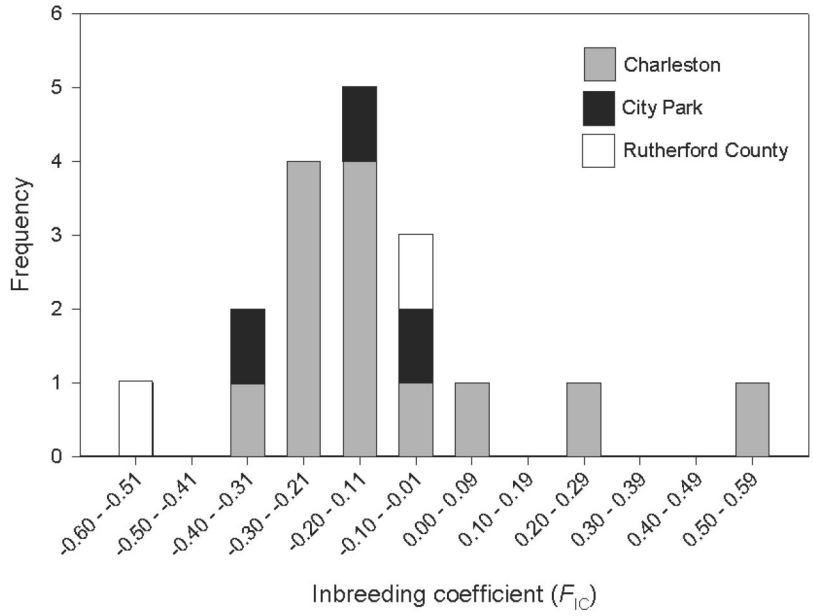


**Fig. 4.** Frequency distribution of point estimates for individual colony inbreeding coefficients ($F_{IC}$) of extended family colonies for three introduced populations of *C. formosanus.*

cases B5 and 6) could be excluded for all three populations, because they had either $F_{IC}$ values that were significantly lower than expected (Rutherford County and City Park, both $P < 0.05$, one-sample approximate *t*-test) or had significantly lower $F_{IT}$ and $F_{CT}$ values (Charleston, $P < 0.03$, one-sample approximate *t*-test). As discussed below, the relatively high value of $F_{IC}$ in the Charleston extended family colonies was because of two colonies with highly positive values. When these two colonies were excluded from the analysis, $F_{IC}$ for this class of colonies became more strongly negative (−0.178), a result consistent with relatively few neotenics present. Thus, extended families in all three populations appeared to have on average few neotenics, from three to nine (Table 3).

A detailed view of the individual colony inbreeding coefficients ($F_{IC}$) shows a more complex picture for the Charleston population (Fig. 4). The only colonies with positive $F_{IC}$-values were the two colonies from Charleston at the far right side of the graph in Fig. 4, with values of 0.28 and 0.59. The elevated values of these two colonies account for the relatively high mean $F_{IC}$-value for the extended family colonies in this population. In these colonies, all individuals at either one locus (in the colony with $F_{IC} = 0.28$) or two (in the colony with $F_{IC} = 0.59$) were one of two homozygous genotypes with no heterozygotes present. In the latter colony, there were only two homozygous classes at loci *Cf 4–4* and *Cf 4–10,* and these were associated such that individuals who were *230/230* at *Cf 4–4* were *245/245* at *Cf 4–10,* whereas *248/248* homozygotes at *Cf 4–4* were *236/236* at *Cf 4–10.* These results suggest some degree of assortative mating among the reproductives within these colonies.

Table 5 shows the values for the inbreeding coefficient ($F_{IS}$) for reproductives in simple family colonies and the relatedness coefficient between nestmate reproductives in simple family colonies based on their genotypes as inferred from their worker offspring. The reproductives had high coefficients of relatedness in Charleston and Rutherford County ($r = 0.209$ and 0.312, respectively), but in neither case was this significantly greater than zero (both $P > 0.1$, *t*-test). Compared with worker $F_{IT}$-values, the equivalent of $F_{IS}$ in the reproductives, functional reproductives within simple family colonies were slightly less inbred than workers in Charleston and Rutherford County and slightly more inbred than workers in City Park, but none of these differences were significant based on overlapping 95% confidence intervals. Results of the analysis with the program Kinship showed similar proportions of colonies headed by putative sib–sib pairs in all three populations: 21% (3 of 14) in City Park, 33% (4 of 12) in Charleston, and 33% (2 of 6) in Rutherford County.

**Table 5. Coefficients of relatedness (*r*) between nestmate reproductives and of inbreeding ($F_{IS}$) of reproductives in simple family colonies of *C. formosanus***

| Population | *r* (SE) | $F_{IS}$ (95% CI) |
|---|---|---|
| Charleston, SC | 0.209 (0.213) | 0.060 (−0.015–0.150) |
| Rutherford County, NC | 0.312 (0.240) | 0.104 (0.026–0.228) |
| City Park, New Orleans, LA | 0.015 (0.058) | 0.184 (0.119–0.254) |

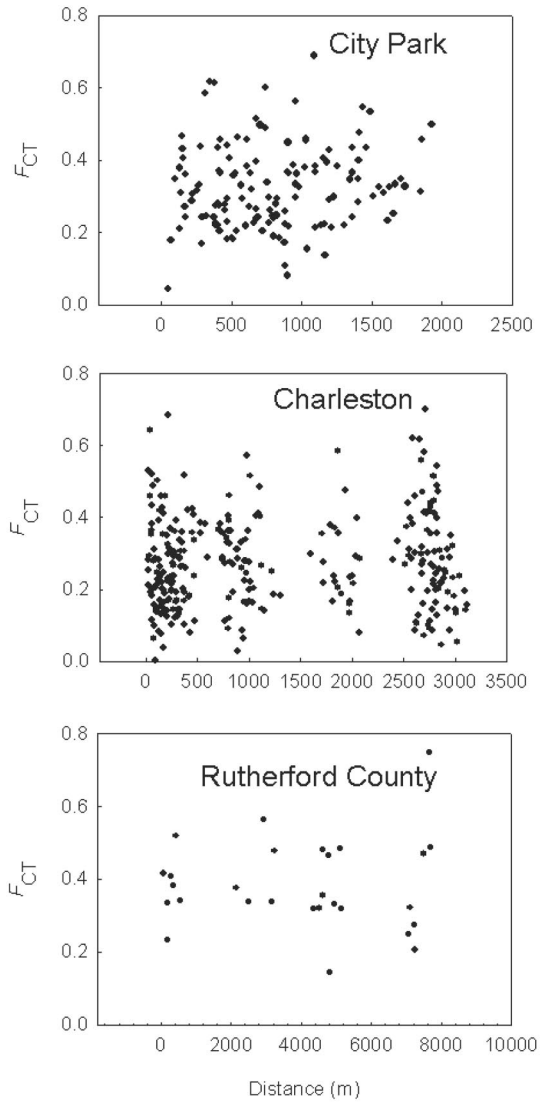


**Fig. 5.** Relationship between genetic differentiation ($F_{CT}$) estimated from microsatellite genotypes and physical distance among all pairs of *C. formosanus* colonies in the study populations.

**Isolation by Distance Analysis.** There was no strong isolation by distance in any of the populations (Fig. 5). Although there was a positive correlation between colony pairwise $F_{CT}$ values and geographic distance in all three populations, the correlations were not significant in either City Park ($r^2 = 0.096$, $P = 0.119$, Mantel test) or Rutherford County ($r^2 = 0.044$, $P = 0.618$, Mantel test). There was a weak but significant correlation in the Charleston population ($r^2 = 0.087$, $P < 0.02$, Mantel test), but this was most likely caused by the slight differentiation between the Hampton Park colonies and the Charles Towne Landing State Historic Site colonies, which were separated by >1 km of water, because this correlation disappeared when considering each population separately ($r^2 = 0.050$, $P > 0.30$ and $r^2 = -0.339$, $P > 0.75$ for the Hampton Park and Charles Towne Landing colonies, respectively).

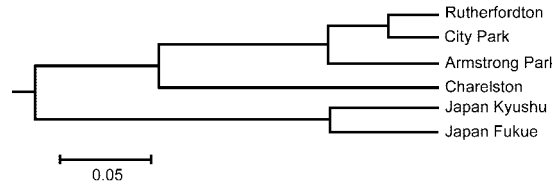


**Fig. 6.** Unweighted pair group method with arithmetic mean (UPGMA) tree showing the genetic relationships among the three studied populations of *C. formosanus* and two previously studied populations in Japan (Vargo et al. 2003a) and Louis Armstrong Park, New Orleans, LA (Husseneder et al. 2005). The tree was constructed from pairwise $F_{ST}$ values calculated from 12 loci in all populations except for the Louis Armstrong Park population, in which 8 loci were used.

**Tests for Genetic Bottleneck.** For the Charleston population, there was strong evidence of a genetic bottleneck. All of the 20 resampled data sets showed significant heterozygosity excess averaged across loci ($P \leq 0.05$, Wilcoxon sign-rank test). Similarly, a sign test for a greater proportion of loci with heterozygosity excess than expected was significant ($P \leq 0.05$, sign test) in all cases. The mean ± SD ratio of the number of alleles to the range in allele size (mean = 0.426 ± 0.276) was significantly lower than the threshold value of 0.68 ($P = 0.017$, one-sample *t*-test), providing further support of a strong, recent genetic bottleneck. For the City Park population, there was significant heterozygosity excess ($P < 0.05$) in only 3 of the 20 resampled data sets according to the Wilcoxon sign-rank test, and in no cases using the less powerful sign test. M = 0.631 ± 0.303 was lower than the threshold of 0.68 but not significantly different from it ($P = 0.33$, one-sample *t*-test). Also, there was no strong evidence of a recent bottleneck in the Rutherford County population; there was significant heterozygosity excess in only 2 of the 20 resampled data sets according to the Wilcoxon test and in only 1 of the data sets according to the sign test. The average ratio of the number of alleles to the range of allele sizes was again below the threshold value (mean = 0.631 ± 0.305) but not significantly so ($P = 0.31$, one-sample *t*-test).

**Unweighted Pair-Group Method with Arithmetic Average Analysis.** Results of the unweighted pair-group method with arithmetic average clustering (Fig. 6) suggest that the Rutherford County, NC; City Park, New Orleans; and Louis Armstrong Park, New Orleans populations are the most closely related, whereas the Charleston population is rather distant to these. The Japanese populations cluster together into a branch that is quite removed from the other four populations.

## Discussion

A principle finding of this study was that the three populations of *C. formosanus* investigated here, each

with its own distinct introduction history, exhibited considerable variation in colony breeding structures. Although all three populations were primarily comprised of colonies consisting of genetically distinct family groups, they varied in the relative proportions of simple and extended families and in the overall degree of inbreeding. The two populations differing the most in the proportions of the two family types were from the areas with the longest history of Formosan termites on the U.S. mainland: Charleston, with ≈50% extended families, and City Park, New Orleans, with <20%. Rutherford County, a relatively recently introduced population, was intermediate, with one-third extended families. Variation among the populations in the overall degree of inbreeding among workers was complex and did not merely reflect the proportions of extended family colonies in each population, as might be expected. For example, Rutherford County had the highest level of inbreeding ($F_{IT}$ = 0.24) even though this population had a lower percentage of extended families than did Charleston.

Based on *F*-statistics and the coefficient of relatedness, simple families in Charleston provided a reasonable match to the values expected for colonies headed by outbred monogamous pairs. The simple family colonies in Rutherford County and City Park varied somewhat from expected but in different ways. In the former population, workers were significantly more inbred than expected, presumably because nestmate reproductives were more closely related in this population than in the other two populations. In City Park, nestmate workers were more closely related to each other and less inbred relative to their own colonies (more negative $F_{IC}$) than expected, because functional reproductives in simple family colonies were significantly more inbred than the population average. One possible explanation for this finding is that alates in City Park issued predominately from extended family colonies, which produce individuals who are more inbred than do simple family colonies. Investigating whether simple and extended family colonies differ in their relative reproductive output would require quantifying alate production by colonies of known breeding structure.

The average inbreeding coefficients for extended family colonies in all three populations, especially the highly negative $F_{IC}$-values, were consistent with colonies headed by relatively few closely related neotenic reproductives, on the order of three to nine, all descended from monogamous pairs of founders. Higher numbers of reproductives can be excluded, because according to the predictions from computer simulations, this would have resulted in $F_{IC}$-values closer to zero (Thorne et al. 1999, Bulmer et al. 2001). Moreover, the presence of multiple unrelated reproductives in most colonies, which may arise through colony fusion, can also be ruled out because such colonies are expected to have positive $F_{IC}$-values. The fact that no more than four alleles per locus were found in extended family colonies in the City Park and Rutherford County populations, despite having up to seven and six alleles per locus, respectively, also supports this conclusion. The possibility of multiple unrelated reproductives in the Charleston extended families is more difficult to exclude. First, the low genetic variability in this population, with a maximum of three alleles per locus, prevented us from detecting the presence of any multiple unrelated reproductives within colonies. Second, the relatively small average $F_{CT}$-value obtained, indicating low genetic contrasts among colonies, is consistent with some fusion among colonies. However 10 of the 13 colonies had negative $F_{IC}$-values, making it unlikely that colony fusion was common, if it occurred at all, in this population. Nonetheless, two of these colonies had strongly positive $F_{IC}$-values suggesting some degree of assortative mating, one possible cause of which is fusion between two or more colonies. Other mechanisms that could also underlie assortative mating are the presence of physically separated reproductive centers within colonies with no breeding between the different groups of reproductives and selective mating within a single group of reproductives, such as two cohabiting monogamous pairs. Similarly high $F_{IC}$-values were obtained by Vargo et al. (2003a) in an introduced population of *C. formosanus* in Japan, suggesting some level of assortative mating within extended family colonies in this population as well. In an expansive extended family colony of *C. formosanus* in Louis Armstrong Park, New Orleans, Husseneder et al. (2005) found slight but significant genetic differentiation among spatially separated foraging sites, suggesting the presence of two or more reproductive centers with unequal mixing of workers produced in each. Similar spatial and genetic substructure has been found in the African subterranean termite *Schedorhinotermes lamanianus* (Husseneder et al. 1998). More detailed studies are needed of individual colonies to determine whether such genetic substructuring in *C. formosanus* colonies is widespread.

The presence of multiple unrelated reproductives in termite colonies has been shown using molecular markers in a few termites, such as the mastotermitid *Mastotermes darwiniensis* Froggatt (Goodisman and Crozier 2002) and the termitid *Nasutitermes corniger* (Motschulsky) (Atkinson and Adams 1997). In subterranean termites, the co-existence of multiple unrelated reproductives occurs at low frequency in *R. flavipes* (Jenkins et al. 1999, Bulmer et al. 2001, DeHeer and Vargo 2004). It was reported to be common in *R. grassei* Clément (Clément 1981), but new data for this species (DeHeer et al. 2005) suggest that it may be rare at best. Colony fusion has been observed in the field and laboratory as a mechanism leading to the co-existence of offspring produced by multiple unrelated reproductives (DeHeer and Vargo 2004, Fisher et al. 2004). Evidence for colony fusion in *C. formosanus* is sparse and indirect. Based on the location of dyed termites and changes in worker size over time, Su and Scheffrahn (1988) reported the possible fusion of two *C. formosanus* colonies in Florida. However, the lack of clear genetic evidence of fused colonies in a number of populations with sufficient allelic variability to detect it, including Oahu, HI (C.H., E.L.V., and

J.K.G., unpublished data), and Kyushu, Japan (Vargo et al. 2003a), from previous studies, and City Park, New Orleans, and Rutherford County in this study, suggests that, if colony fusion occurs in introduced populations of *C. formosanus*, it is rare.

The inferred number of neotenic reproductives in extended family colonies in the study populations was relatively low, more so than what might be expected based on field collections. Although collections of functional reproductives in colonies of *C. formosanus* are few, the numbers of neotenics observed within colonies ranges from 6 to >100 (King and Spink 1969, Myles 1999). We inferred an average of six or fewer functional neotenics per colony for the study populations, which is low compared with what has been found in the field. Possible explanations for this discrepancy are that only exceptionally large and old colonies with abnormally high numbers of reproductives were excavated, field collections came from populations different from the study populations (China, Hawaii, South Africa, and Lake Charles, LA) where neotenic numbers were higher, or there is significant disparity among the reproductive output of cohabiting neotenics resulting in a lower effective number of reproductives, as occurs in many social insects (Ross 2001).

The variation in the family types observed here among the different populations is well within the wide range reported for other introduced populations of *C. formosanus.* At one extreme, Vargo et al. (2003a) found that simple families made up 90% of 30 colonies in two Japanese populations. At the other extreme, 30% of 20 colonies were simple families in Oahu, HI (Vargo et al. 2003b). And between these extremes, Husseneder et al. (2005) found, in a detailed study of colonies in Louis Armstrong Park, another New Orleans population, 57% of 14 colonies were simple families. In contrast, a native population of 14 colonies from Guangdong Province, China, consisted of all extended families (C.H., E.L.V., and J.K.G., unpublished data). The reasons for this large variation are not clear, but could include age structure of the populations, local ecological conditions, or genetic make-up.

In this regard, it is of interest to compare these findings for City Park to previous findings on Louis Armstrong Park (Husseneder et al. 2005), a population located only 3 km away that was intensively studied using the same microsatellite loci employed in this study. The results from these two populations, which share not only common ecological conditions but a similar genetic composition, are alike. Both populations were comprised of a majority of simple family colonies. Although a higher proportion of simple family colonies was present in City Park (82%) than in Louis Armstrong Park (57%), this difference was not significant ($P = 0.23$, Fisher exact test). The coefficients of inbreeding and relatedness in both populations suggested that simple family colonies were headed by outbred pairs of primary reproductives and that extended families were headed by relatively few neotenics descended from the original founding pair. The degree of relatedness between nestmate reproductives in simple families was low in the two populations ($r = 0.02$ and 0.11 in City Park and Louis Armstrong Park, respectively) and not significantly different from zero. Also, there was a similar proportion of cohabiting reproductives that were putative siblings: 21% (3 of 14) in City Park and 17% (1 of 6) in Louis Armstrong Park. Moreover, there was no significant isolation by distance in either population, consistent with a lack of strong population viscosity within either population. These two populations are certainly more similar to each other than they are to other introduced populations that have been studied to date. Because City Park and Louis Armstrong Park share largely the same ecological conditions and are genetically similar, it is not possible to tease apart which of these two factors may be more important in shaping colony breeding structures. Future studies examining other urban populations along the Gulf Coast with distinct introduction histories or comparing nearby urban and natural populations may help in this regard.

Our results showing variation in the breeding system in different introduced populations of *C. formosanus* are consistent with the results of studies on native populations of subterranean termites. Studies of the eastern subterranean termite, *R. flavipes*, have revealed variation in colony breeding structure among geographically distant populations. Studies in central North Carolina (Vargo 2003a, b, DeHeer and Vargo 2004) show that about three-quarters of the colonies are simple families headed by outbred reproductives, about one-quarter are extended families descended from simple families and headed by relatively few reproductives, and a very small number of colonies (2%) are mixed families formed by the fusion of two or more colonies. In contrast, Bulmer and Traniello (2001) found that 27% of 22 colonies in Massachusetts were simple families headed by monogamous pairs of outbred reproductives, 59% were extended families headed by numerous neotenics, and 14% were comprised of mixed families. In a study of *R. grassei* in southwestern France, DeHeer et al. (2005) found variation among populations, ranging from all extended families with numerous neotenics in an area north of Bordeaux to nearly one-half simple families and one-half extended families in an area further south. Thus intraspecific variation in colony breeding structure may be common in subterranean termites, and a major question to address in future studies concerns the causes of this variation.

Our results are consistent with those of previous studies showing expansive colonies of *C. formosanus.* Although our sampling scheme was not designed to explicitly study the foraging areas of colonies, we found collection sites from the same colony separated by 144 m in Rutherford County, and one colony in Charleston spanned 133 linear m. These distances are close to the reported record for this species of 185 linear m observed by Su (1994) in Florida, where this author also reported a colony with a foraging distance of 100 m. Distances of ≈100 m also have been reported in Florida by Su and Scheffrahn (1988), in Louisiana

by King and Spink (1969) and Messenger and Su (2005), and in Hawaii by Lai (1977). Thus, two colonies in this study are among the most expansive *C. formosanus* colonies reported to date. Although the most spatially expansive colony we found was an extended family, the Charleston colony that spanned 133 m was a simple family. To the extent that foraging area reflects colony population size, the second largest colony among those studied here had only a single queen. These results confirm previous findings for *C. formosanus* in Louis Armstrong Park, New Orleans (Husseneder et al. 2005) and for *R. flavipes* in North Carolina (DeHeer and Vargo 2004), indicating that the presence of multiple neotenics is not necessarily associated with larger foraging areas and population sizes as is sometimes assumed (e.g., Grube and Forschler 2004).

There was no significant isolation by distance in any of the populations once the large water barrier separating the two groups of colonies in Charleston was taken into account. In a study of two introduced Japanese populations on a slightly larger scale, Vargo et al. (2003a) also did not find significant isolation by distance. Isolation by distance is expected if dispersal is relatively limited over the spatial scale studied, either because of short range mating flights by alates in the absence of inbreeding avoidance and/or frequent colony reproduction by budding. This lack of strong population viscosity suggests that budding is not common and that during mating flights reproductives disperse relatively far over the spatial scale studied or they actively engage in avoiding relatives when forming tandem pairs. Alternatively, no strong population structure is expected if human-mediated dispersal plays an important role in population expansion. Studies of the spatial distribution of *C. formosanus* in New Orleans (La Fage 1987) and Charleston (Chambers et al. 1988) indicate a rather patchy occurrence in these areas, consistent with limited natural dispersal and frequent spreading by human transport of infested materials from one part of the city to another. Thus, the absence of strong local population structure in these two older populations is not unexpected.

Results of the bottleneck tests provide strong evidence that the Charleston population underwent a strong genetic bottleneck on the introduction of *C. formosanus* to this area. The small number of alleles present is consistent with this population originating from very few colonies. Neither the tests for heterozygosity excess nor the mean ratio of the number of alleles to the range in allele sizes detected significant bottlenecks in the City Park or Rutherford County populations. The reason for the lack of detection in these two recently introduced populations is unclear. It may be that the sample sizes were rather minimal for these tests, especially in the Rutherford County population, where the number of colonies ($n = 8$) was below the minimum of 10 recommended for the heterozygosity excess tests (Piry et al. 1999). Husseneder et al. (2005) recently detected a bottleneck in a population of 14 *C. formosanus* colonies from Louis Armstrong Park, New Orleans, using the test for heterozygosity excess. However, a study of Japanese populations, where this species was introduced >300 yr ago, did not detect a recent bottleneck (Vargo et al. 2003a), presumably because there has been sufficient time since the introduction to Japan to erase the heterozygosity excess characteristic of recently bottlenecked populations (Piry et al. 1999).

The Charleston population, with a maximum of three alleles per polymorphic microsatellite locus and an average of fewer than two, is the least variable population of *C. formosanus* so far studied. Even the relatively recent Rutherford County population with only eight colonies had considerably more genetic variability. Other populations that have been studied include two Japanese populations with an average of 6.3 and 2.7 alleles per locus, respectively (Vargo et al. 2003a), Louis Armstrong Park, New Orleans, with 2.9 alleles (Husseneder et al. 2005), and Oahu, HI, with 3.9 alleles (C.H., E.L.V., and J.K.G., unpublished data). To the extent that allele number reflects the effective size of the founding population, Charleston seems to have been founded by the least number of colonies, possibly only a single colony. Alternatively, it could have been founded by several colonies introduced from a previously bottlenecked population from Asia or elsewhere.

Another main finding of our study is the distinct introduction history of the three studied populations. Results of the unweighted pair-group method with arithmetic average clustering indicated strong differences between the Charleston and City Park populations ($F_{ST} = 0.30$), Charleston and Louis Armstrong Park population ($F_{ST} = 0.24$), and the Charleston and Rutherford County populations ($F_{ST} = 0.36$), suggesting different introduction histories for Charleston and the other three populations. These results confirm those of an earlier study using allozymes suggesting at least two separate introductions of *C. formosanus* to the U.S. mainland (Korman and Pashley 1991, Wang and Grace 2000a, b). In contrast, City Park, Louis Armstrong Park, and Rutherford County were much more genetically similar ($F_{ST} = 0.05–0.16$), suggesting a close association between these three populations. However, by comparing the alleles present in each of these populations, we can exclude the possibility that the Rutherford County population could have originated solely from the City Park population. This is because the alleles present in Rutherford County were not simply a subset of those in City Park. In fact, of the 44 total alleles present in both populations, there were 12 (27%) private alleles, 7 of which resided in Rutherford County. Moreover, it is unlikely that the Rutherford County population originated simply from other populations in or around New Orleans, because the Louis Armstrong Park population closely resembles the City Park population. It is possible that the Rutherford County population may have originated from another nearby population from outside New Orleans or may have resulted from two or more colonies coming from different populations within the United States. Based on cytochrome oxidase II sequence data, Jenkins et al. (2002) concluded that four

colonies from Atlanta, GA, likely originated from Louisiana, most probably having been transported by railroad ties. A similar route of introduction was likely for the Rutherford County population, although from an unknown location, because of the close association between this small population and the abandoned railroad line (Fig. 3).

The colonies studies here, as well as those in other introduced populations studied previously, are close family groups that retain their distinctness even in the face of high population densities. Thus, the invasion success of *C. formosanus* cannot be attributed to a breakdown in nestmate recognition and an ensuing shift to unicoloniality in introduced populations, a major factor associated with highly invasive ants (Holway et al. 2002, Tsutsui and Suarez 2003). Nor would it seem that the exceptional success of *C. formosanus* as an invader is caused by any one dominant form of social organization, given the high variability in colony breeding structure among introduced populations. Perhaps the invasion success of this species is related to the plasticity of the breeding structure allowing populations to adapt to local ecological conditions. However, it may be that this species has such a superior competitive ability in many non-native areas because of ecological release that it can invade successfully despite the variability in breeding structure. Future studies investigating the breeding structure in additional populations, particularly within the native range, and the nature of competitive interactions with rival species in both the native and introduced ranges should shed more light on the factors underlying the repeated success of this important invader.

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