Colony Social Organization and Population Genetic Structure of an Introduced Population of Formosan Subterranean Termite from New Orleans, Louisiana

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ABSTRACT The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an invasive species in many parts of the world, including the U.S. mainland. The reasons for its invasive success may have to do with the flexible social and spatial organization of colonies. We investigated the population and breeding structure of 14 *C. formosanus* colonies in Louis Armstrong Park, New Orleans, LA. This population has been the focus of extensive study for many years, providing the opportunity to relate aspects of colony breeding structure to previous findings on colony characteristics such as body weight and number of workers, wood consumption, and intercolony aggression. Eight colonies were headed by a single pair of outbred reproductives (simple families), whereas six colonies were headed by low numbers of multiple kings and/or queens that were likely the neotenic descendants of the original colony (extended families). Within the foraging area of one large extended family colony, we found genetic differentiation among different collection sites, suggesting the presence of separate reproductive centers. No significant difference between simple family colonies and extended family colonies was found in worker body weight, soldier body weight, foraging area, population size, or wood consumption. However, level of inbreeding within colonies was negatively correlated with worker body weight and positively correlated with wood consumption. Also, genetic distance between colonies was positively correlated with aggression levels, suggesting a genetic basis to nestmate discrimination cues in this termite population. No obvious trait associated with colony reproductive structure was found that could account for the invasion success of this species.

KEY WORDS microsatellite genotyping, breeding system, Isoptera, Rhinotermitidae

THE FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki, is an invasive species that has been introduced from its native range in southeast China and southern Asia to many parts of the world, including the U.S mainland and Hawaii, where it is a highly destructive pest of wooden structures and trees. One of the first detections of *C. formosanus* on the U.S. mainland was in 1966 in New Orleans, LA, at which time buildings and trees were heavily infested, indicating it had been present for several years already (Spink 1967). Since that time, *C. formosanus* has spread through a large part of the southern United States and as of 2001 was found in at least 10 states from North Carolina and Tennessee south to Florida on the East Coast, throughout the Gulf Coast into Texas, and in California (Woodson et al. 2001).

Although a number of termite species have become established in nonnative areas, *C. formosanus* is one of only a few termite species that can be considered truly invasive, i.e., widely established and locally dominant with severe economic and/or ecological impact. The reasons for the exceptional success of *C. formosanus* as an invader are not clear, but one possibility is that certain features of its colony structure facilitate the invasion of new areas, as is characteristic of several invasive ants (Holway et al. 2002, Tsutsui and Suarez 2003). Two of the most notorious ant invaders, the red imported fire ant, *Solenopsis invicta* Buren, and the Argentine ant, *Linepithema humile* Mayr, have undergone population genetic changes during or after their introductions, leading to shifts in social behavior and colony structure that favor their invasiveness (Holway et al. 2002, Tsutsui and Suarez 2003). Specifically, in at least some parts of their introduced ranges both species exhibit greater unicoloniality (formation of large multinest colonies) than in their native ranges. Unicolonial societies are characterized by reduced in-

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traspecific aggression, high levels of polygyny (the presence of multiple reproductive queens within a colony), and colony reproduction by budding (initiation of a new colony by one or more queens and workers who leave their natal colony), all of which can help in the success of introduced populations. There have been very few studies of colony social organization in introduced populations of *C. formosanus* and none in native populations, so it is not yet possible to say whether introduction events have affected colony structure in this species and what role any such changes may have played in the invasive success of this species.

Like other subterranean termites, *C. formosanus* has a complex life cycle involving changes in colony breeding structure and spatial organization. Colonies generally begin as simple families headed by two primary (alate-derived) reproductives who pair during mating flights (King and Spink 1974, Su and Tamashiro 1987, Thorne 1998, Thorne et al. 1999, Raina et al. 2003). Eventually, the primary king and/or queen will be supplemented or replaced by neotenics (non-alate-derived reproductives) from within the colony, leading to inbred extended families. As colonies grow, they expand their foraging range with underground tunnels, some of which may contain satellite nests, reaching up to 50 m or more from the main nest (King and Spink 1969, Su and Tamashiro 1987). It is likely that groups of foragers and/or satellite nests sometimes form buds in which they are physically separated from the rest of the colony and subsequently generate neotenics from existing workers to become independent colonies. In addition, there is evidence from *C. formosanus* and other species that subterranean termite colonies can occasionally fuse or adopt unrelated reproductives (Su and Scheffrahn 1988a, Jenkins et al. 1999, Bulmer et al. 2001, Clément et al. 2001, Matsuura and Nishida 2001, DeHeer and Vargo 2004). Thus, there is great potential for variation among *C. formosanus* populations in colony breeding structure, depending on age structure of the colonies, dynamics of colony–colony interactions, local ecological conditions, and population genetic structure. To date, genetic studies of introduced populations from only two areas have been published. In Hawaii, Husseneder and Grace (2001) found that all 17 colonies studied consisted of genetically distinct family units, and in Japan Vargo et al. (2003a) found that 27 of 30 colonies contained a single pair of reproductives, whereas the remaining three colonies contained multiple related reproductives.

As part of a larger project investigating the breeding structure of various introduced and native populations of *C. formosanus*, we examined here the genetic structure of an introduced population in Louis Armstrong Park, New Orleans. The park has a long history of *C. formosanus* activity dating back to a documented introduction in 1973 of an infested stage that was placed inside the park's Performing Arts Center (Scott and Scott 1996), which originated from Camp Leroy Johnson, one of several military bases credited with introducing *C. formosa*nus from Asia to New Orleans after World War II (La Fage 1987). This termite population in Louis Armstrong Park has been the focus of an intensive recent study of population and colony structure and colony–colony dynamics (Messenger and Su 2005a, b; Messenger et al. 2005). Mark–release recapture methods, i.e., releasing dyed termites to determine colony identity, allowed delineation of 14 foraging areas (Messenger and Su 2005a). Each of these foraging areas was shown to consist of genetically distinct groups based on multilocus DNA fingerprinting and was therefore considered to belong to different colonies (Husseneder et al. 2003a).

The goal of this study was to provide a comprehensive view of the population genetics and social organization of the Louis Armstrong Park population of *C. formosanus* in relation to specific features of colonies and to behavioral interactions among colonies. To achieve this objective, we used microsatellite genotyping (Vargo and Henderson 2000) to describe the population genetic structure and the social organization of the 14 colonies. In addition, we connected the sociogenetic system of each colony, i.e., the breeding structure and the level of inbreeding within colonies to a number of colony characteristics previously reported by Messenger and Su (2005a, b), such as worker body weight, soldier body weight, size of foraging area, size of the foraging population, annual wood consumption rate, and levels of intercolony aggression. This study, which provides the most detailed analysis to date of many key attributes of an introduced population of this pest, represents an important step toward understanding the exceptional success of this termite species as an invader.

Materials and Methods

Definitions. We use the following definitions throughout this article. Termites were sampled from *collection sites*, i.e., distinct inground foraging stations, stakes, logs, or trees. In a previous study using mark–release–recapture (Messenger and Su 2005a), collection sites were connected to 14 *foraging areas*, i.e., areas within which dyed termite workers were found to intermix, indicating they shared the same set of interconnected foraging tunnels. To call groups of termites sharing a foraging area a distinct *colony*, they have to form a functional unit of social interactive individuals and a distinct genetic unit (Thorne et al. 1999). The latter we confirmed by showing that termites from different collection sites within the same foraging area genetically group together, yet separate clearly from termites from neighboring foraging areas (Husseneder et al. 2003a). Colonies may consist of one or several interconnected *nests*, i.e., reproductive centers that contain a single pair or multiple reproductives and brood. This may lead to genetic structure among spatially separated areas within colonies. Colonies may vary in their social structure; some colonies may be headed by a single pair of reproductives (*simple family colonies*, or *Mendelian colonies*), some colonies may be headed by multiple kings and queens (*extended family colonies*, or *non-Mendelian colonies;*

Thorne et al. 1999, Vargo et al. 2003a). Termites in the park were considered to belong to the same gene pool and therefore to the same *population* because distances between collection sites in Louis Armstrong Park lay well within the flight ability (up to 892 m) of alates (Messenger and Mullins 2005).

Collections and DNA Extraction. Louis Armstrong Park is located in New Orleans, a region heavily infested with Formosan subterranean termites. The 12.75-ha park is densely populated with termite colonies adjacent to each other. Foraging areas ranged from 83 to 1,634 m^2 (Fig. 1a). At least 50 workers were collected from one collection site at the center of the foraging area of each of the 14 colonies of Louis Armstrong Park between 1999 and 2000 by using artificial inground stations. From the largest colony (AP1), which occupied a foraging area ≈500 m^2 larger than any other colony, termites were collected from five collection sites within the same foraging area to test for intracolonial genetic structure (Fig. 1b). Specimens were stored in 95% ethyl alcohol until extraction of DNA. DNA was extracted from individual termites using the DNeasy tissue kit (QIAGEN, Valencia, CA).

Microsatellite Genotyping. We scored 18–35 individuals of each colony at eight microsatellite loci (Table 1), which were described previously for *C. formosanus* by Vargo and Henderson (2000). A detailed description of the polymerase chain reaction (PCR) conditions and genotype scoring procedures can be found in Vargo and Henderson (2000). Because individuals within colonies are related and thus genetically nonindependent, we used only one individual per colony, i.e., when testing for linkage disequilibrium between loci and for the presence of a genetic bottleneck.

General Statistics, Tests for Linkage Disequilibrium, and Recent Bottleneck. General descriptive statistics, such as numbers of alleles per locus, observed versus expected heterozygosity, and allele frequencies were calculated for each colony and locus using the program GDA (Lewis and Zaykin 2000; Tables 1 and 2). To test for linkage disequilibrium between pairs of loci, *G*-statistics based on log-likelihood ratios were calculated and summed over all samples (FSTAT; Goudet 1995, 2001). To test for significance of association between genotypes of all pairs of polymorphic loci, randomized data sets were created (2,400 permutations); genotypes at each pair of loci were combined at random, and the log-likelihood ratio *G*-statistic was calculated for each randomized data set. The value of *P* was estimated from the proportion of permutations that were greater or the same as the observed. None of the 28 pairs of loci showed significant linkage. Thus, all eight polymorphic microsatellite loci were assumed to assort independently.

Table 1. Descriptive statistics of the eight microsatellite loci

Locus		Allele size (kb)	No. alleles	Frequency of the most common allele	*Ho*	*He*
1	Cf 4:1A2-4	194, 191, 188, 185	4	0.46	0.57	0.61
2	Cf 4-4	248, 239, 230	3	0.54	0.21	0.54
3	Cf 10-4	173, 170	2	0.50	0.29	0.51
4	Cf 12-4	191, 182, 173, 146	4	0.68	0.57	0.52
5	Rf 6-1	172, 163	2	0.80	0.36	0.30
6	Cf 4-10	245, 242, 236	3	0.46	0.71	0.59
7	Cf 10-5	308, 296, 281	3	0.50	0.71	0.64
8	Cf 4-9A	302, 299, 287	3	0.50	0.71	0.61
Mean			3.00	0.56	0.52	0.54
SD			0.76	0.12	0.20	0.11

Locus designations follow Vargo and Henderson (2000). Genotypes were scored for one randomly chosen individual per colony.

Table 2. Descriptive statistics of the 14 colonies of Louis Armstrong Park

Colony	*n*	*A/locus*	*Ho*	*He*	*r*
AP1	21.38	2.13	0.44	0.34	0.49
AP2	22.00	2.13	0.62	0.42	0.55
AP3	23.00	1.75	0.26	0.24	0.72
AP4	22.38	1.88	0.37	0.36	0.47
AP5	22.50	2.50	0.69	0.56	0.15
AP6	22.38	2.13	0.51	0.38	0.63
AP7	20.63	2.13	0.56	0.46	0.37
AP8	20.63	2.25	0.56	0.42	0.57
AP9	20.75	2.13	0.70	0.47	0.46
AP10	22.25	2.00	0.48	0.42	0.44
AP11	20.38	2.00	0.52	0.37	0.72
AP12	20.00	1.75	0.29	0.28	0.66
AP14	20.50	2.00	0.48	0.39	0.52
AP15	21.75	1.88	0.36	0.33	0.65
Mean simple family	21.77	2.07	0.50	0.40	0.53*
SD	0.85	0.20	0.11	0.07	0.14
Mean extended family	21.07	2.02	0.47	0.37	0.60*
SD	1.04	0.22	0.17	0.10	0.15
Mean all	21.47	2.05	0.49	0.39	0.55*
SD all	0.97	0.20	0.14	0.08	0.18

Simple family colonies are shown with gray background, and extended family colonies with black background. *n*, mean number of individuals across all loci; *A/locus*, the number of alleles per locus; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *r*, relatedness coefficients within colonies.

* Means for *r* were calculated according to Queller and Goodnight (1989). SD values are calculated from the standard errors derived from jackknifing over loci (1000 replications).

We determined whether the population had gone through a recent genetic bottleneck based on the fact that bottlenecks reduce allele numbers faster than heterozygosity, so that observed heterozygosity is greater than the heterozygosity expected from allele numbers. Calculations of expected heterozygosity depend on the model of mutation (Infinite Allele model, Stepwise Mutation model). Tests were performed with both mutation models as implemented in the program BOTTLENECK (Piry et al. 1999).

Population Structure, *F*-Statistics. Colonies were tested for significant differentiation using log-likelihood *G*-statistics by using FSTAT (Goudet 2001). *P* values were obtained through permutations of the multilocus genotypes between each pair of colonies and standard Bonferroni corrections were applied. Pairwise genetic distances (F_{CT}; see below) between colonies were visualized using principal coordinate analysis (NTSYSpc, 2.11, Applied Biostatistics Inc., Setauket, NY). To assess isolation by distance F_{CT} values were correlated to geographical distance using

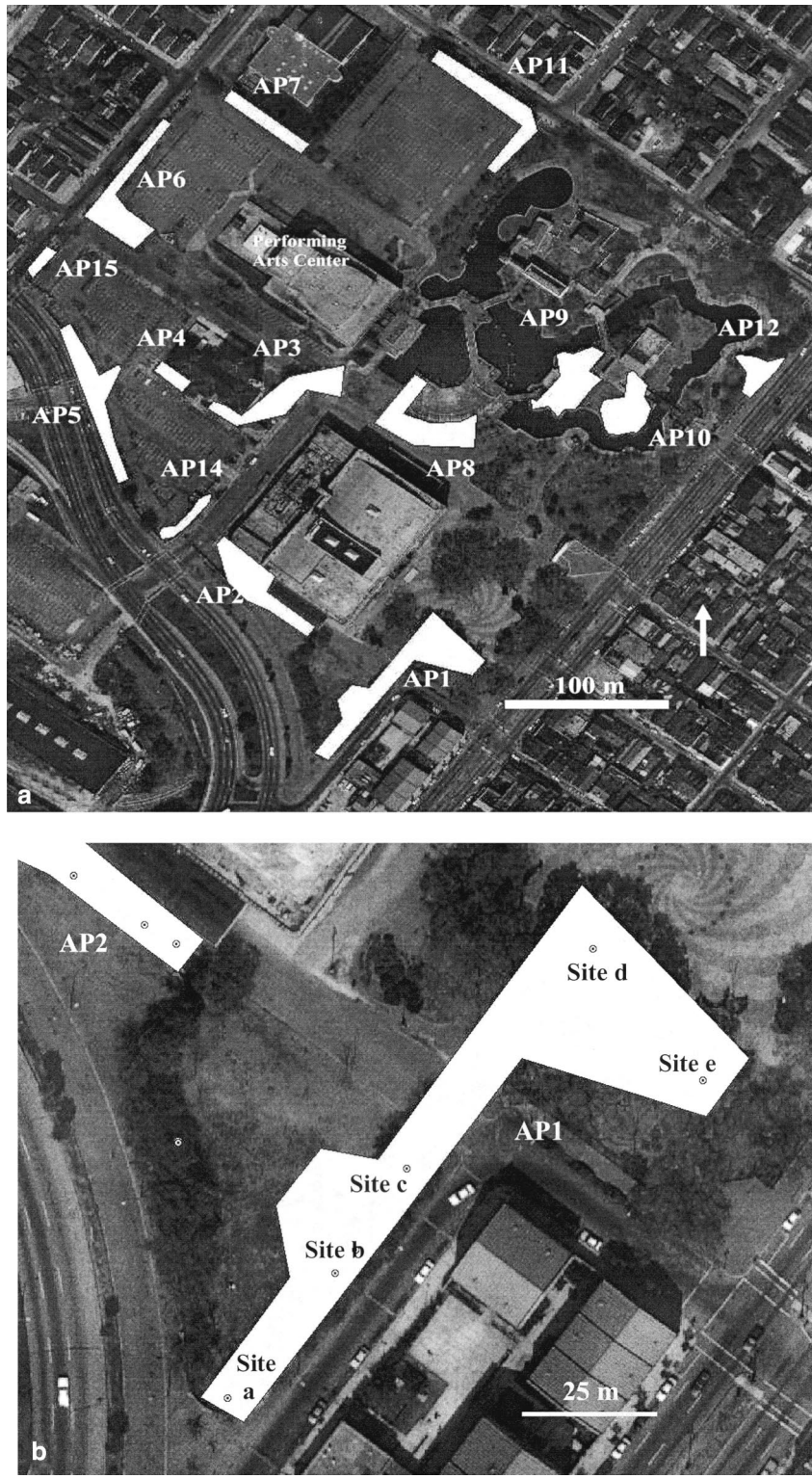

Fig. 1. (a) Map of the 14 foraging areas in Louis Armstrong Park determined by mark–release–recapture. (b) Detailed map of the five collection sites from foraging area AP1.

Pearson's correlation coefficient and the correlation was subsequently tested for significance using a Mantel test (1000 iterations, GENEPOP on the Web; Raymond and Rousset 1995).

To analyze the population genetic structure and inbreeding at the levels of the colony and population, hierarchical *F*-statistics were calculated using the methods of Weir and Cockerham (1984) as implemented in GDA (Lewis and Zaykin 2000). To assess the significance of the *F*-statistics, 95% confidence intervals (CI) were constructed by bootstrapping over loci. *F* values were considered significantly different from zero if their confidence intervals did not span zero. When two *F* values were compared, they were considered significantly different when their 95% CI did not overlap each other.

Traditionally, in nonsocial insects F_{IT} signifies the coefficient of inbreeding for individuals relative to the total population and can be divided into F_{ST} (the coefficient of inbreeding of subpopulations relative to the total population) and F_{IS} (the coefficient of inbreeding of individuals within subpopulations). Because of the strongly hierarchical genetic structure in social insect populations (Ross 2001), it is reasonable to treat colonies as subpopulations (Thorne et al. 1999, Bulmer et al. 2001). Thus, F_{IT} in social insects is analogous to the standard inbreeding coefficient in nonsocial populations, 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 reflects most clearly the colony's breeding system (Thorne et al. 1999; Bulmer et al. 2001; Bulmer and Traniello 2002a; Vargo 2003a, b; Vargo et al. 2003a; DeHeer and Vargo 2004).

Colony Social Organization and Genetic Structure. We tested whether colonies were headed by a single pair of reproductives (simple family colonies) or multiple reproductives (extended family colonies) based on the frequencies and classes of worker genotypes (Vargo 2003a, Vargo et al. 2003a). Colonies were considered simple families if the classes of genotypes of the workers were consistent with those expected for the offspring of a single pair of parents, and if the frequencies of the observed genotypes did not differ significantly from the expected Mendelian ratios. Deviations from Mendelian ratios were determined between observed and expected genotype frequencies at each locus by a *G*-test for goodness-of-fit. An overall *G*-value for each colony was obtained by summing all the *G*-values across all eight loci. Colonies were considered extended families when they had more genotypes than possible for the offspring of a monogamous pair or the observed frequencies of the genotypes deviated significantly from those expected in simple families ($P < 0.05$, *G*-test).

To further determine degrees of inbreeding within colonies, we assessed average nest mate relatedness (*r*) for workers (RELATEDNESS 5.0.8, Queller and Goodnight 1989, 95% CI were obtained by jackknifing over loci) and calculated *F*-statistics by treating colonies as subpopulations as described above. The inferred reproductive structure together with estimated values of relatedness and inbreeding were compared with the values predicted by simulations of a variety of possible reproductive systems for subterranean termites (Thorne et al. 1999, Bulmer et al. 2001).

From the worker genotypes present in each of the simple family colonies, the genotypes of the parents were reconstructed, and *F*-statistics for the reproductives were estimated from the inferred genotypes. Pairwise relatedness between the parents was calculated using SpaGeDi 1_1b (Hardy and Vekemans 2002). To test whether parents were putative siblings, i.e., prior nest mates, we used likelihood calculations in KINSHIP 1.3.1 (Goodnight and Queller 1999, available at http://www.gsoftnet.us/GSoft.html). The null hypothesis was that pairs were unrelated ($r = 0$), whereas the alternate hypothesis was that pairs were nest mates ($r = 0.55$ as determined by the average nest mate relatedness within all colonies; Table 2). Significance at the level of $P = 0.05$ was determined by log likelihood tests with 10,000 series of pairs created at random.

Results

General Statistics, Linkage Disequilibrium, and Bottleneck. Each colony in the Louis Armstrong Park population was represented by termites from one central collection site per foraging area as we have previously shown that different collection sites from the same foraging area belong to one genetic unit, i.e., the same colony (Husseneder et al. 2003a). To analyze the colony organization, we genotyped 390 individual worker termites from the 14 colonies at eight microsatellite loci. Each of the eight microsatellite loci was polymorphic with two to four alleles observed across the 14 colonies surveyed (Table 1).

Mean observed heterozygosity for all eight loci across all 14 colonies was 0.52 (SD = 0.20) and corresponded well with the expected heterozygosity 0.54 (SD = 0.11; Table 1). We tested each locus for heterozygote excess or deficit, i.e., deviations from Hardy-Weinberg equilibrium. None of the eight loci showed significant heterozygote excess. Only *Cf 4-4* showed significant heterozygote deficiency ($P = 0.006$; *P* for a 5% significance level = 0.003 after Bonferroni correction). The most likely cause of heterozygote deficiency at only one locus is the presence of null alleles. However, the genotypic distribution in the data set with at least 18 individuals genotyped per locus did not indicate the presence of null alleles. In fact, in eight colonies *Cf 4-4* showed no deviation from Mendelian ratios and in six colonies the locus was monomorphic. No individual was found in which this locus did not amplify consistently (null allele homozygote). Therefore, the slight heterozygote deficit observed was not due to the presence of null alleles.

There was significant evidence of a recent genetic bottleneck in the Louis Armstrong Park population for both the infinite alleles model of mutation-drift equilibrium ($P = 0.004$) and the stepwise mutation model

Table 3. *F*-statistics and relatedness coefficients for *C. formosanus* workers of 14 colonies from Louis Armstrong Park, New Orleans, and expected values for different breeding systems derived from computer simulations

	F_{IT}	F_{CT}	F_{IC}	r
All colonies (n = 14) (95% CI)	0.13 (0.03 to 0.25)	0.31 (0.25 to 0.40)	−0.28 (−0.38 to −0.21)	0.55 (0.47 to 0.64)
Simple family colonies (n = 8) (95% CI)	0.12 (−0.01 to 0.26)	0.30 (0.24 to 0.38)	−0.27 (−0.36 to −0.16)	0.53 (0.46 to 0.62)
Extended family colonies (n = 6) (95% CI)	0.16 (0.03 to 0.32)	0.34 (0.26 to 0.46)	−0.28 (−0.33 to −0.23)	0.60 (0.49 to 0.70)
Simulated breeding system				
(A) Simple family colonies with				
(1) outbred reproductive pairs	0.00	0.25	−0.33	0.50
(2) inbred pairs $N_f = N_m = 1, X = 1$	0.33	0.42	−0.14	0.62
(B) Extended family colonies with inbreeding among multiple neotenics				
(1) $N_f = 2, N_m = 1, X = 1$	0.26	0.35	−0.14	0.55
(2) $N_f = N_m = 10, X = 1$	0.33	0.34	−0.01	0.51
(3) $N_f = N_m = 10, X = 3$	0.37	0.38	−0.02	0.56
(4) $N_f = 200, N_m = 100, X = 3$	0.33	0.34	−0.00	0.50
(C) Pleometrosis				
$N_f = 1, N_m = 2$	0.00	0.19	−0.23	0.38
(D) Inbreeding, then mixing of unrelated workers from two colonies (1:4)				
$N_f = N_m = 10, X = 3, p = 0.8$	0.37	0.25	0.15	0.36

For the simulated breeding systems, X represents the number of generations of production of replacement reproductives within a colony; N_f and N_m represent the number of replacement females and males, respectively, produced per generation (Thorne et al. 1999, Bulmer et al. 2001).

(P = 0.008, one-tailed Wilcoxon sign-rank test for heterozygote excess).

Social Organization of Colonies. Permutation tests of the distribution of genotypes between pairs of colonies showed significant differentiation between each pair of colonies at the 5% level. Each pair of colonies was distinguished from each other by at least two and up to 13 private alleles, i.e., alleles only occurring in one of the colonies, but not the other (mean = 6.71, SD = 2.41, n = 91). Thus, the genetic differentiation found in a previous study based on multilocus DNA fingerprinting (Husseneder et al. 2003a) was confirmed; the 14 colonies of Louis Armstrong Park were genetically distinct. No significant correlation between genetic distance (F_{CT}) and geographic distance was found (Pearson's r = 0.08, $P > 0.20$, Mantel test).

To assess whether colonies were headed by a single pair (simple families) or by multiple reproductives (extended families), we analyzed genotypes of at least 18 workers per colony. Eight colonies (57%) had genotypes and genotype frequencies consistent with the presence of a single pair of reproductives and were thus considered to be simple families. Six colonies (43%) had genotypes or genotype frequencies inconsistent with simple families, indicating the presence of multiple same-sex reproductives in an extended family (Table 2).

The *F*-statistics and relatedness values with their 95% confidence intervals estimated from the worker genotypes across all loci and colonies are shown in Table 3, along with values derived from computer simulations by Thorne et al. (1999), Bulmer et al. (2001), and Vargo (2003a, b). Overall, F_{CT} was large (0.31) and confirmed the genetic differentiation between colonies. Workers within colonies were moderately inbred on the level of the local population (F_{IT} = 0.13) and showed highly negative F_{IC}-values (F_{IC} = −0.28), consistent with low numbers of reproductives within colonies. Nestmates were closely related to each other (r = 0.55).

F-statistics and relatedness analyses considering the eight simple families and six extended family colonies separately yielded values not significantly different from the overall population and each other. Simple family colonies showed genetic differentiation, and low inbreeding within colonies with intracolonial relatedness not significantly different from 0.5 as did extended family colonies (Table 3). No significant differences were found comparing simple family colonies versus extended family colonies in genetic differentiation (F_{CT}), nor in the degrees of inbreeding and relatedness (F_{IC} and r) within colonies (two-sided P values >0.20; permutation test, FSTAT).

The F values for simple family colonies were not significantly different (based on the overlapping 95% CI) from those of the computer simulations for colonies headed by a pair of outbred reproductives (Table 3, case A.1). The empirical values for extended family colonies did not provide a clear match with any of the simulated breeding systems, but the highly negative F_{IC} value is suggestive of a low number of reproductives, on the order of fewer than 10 (Table 3). Nevertheless, certain breeding systems could be excluded, such as a high number of neotenics inbreeding over at least three generations (case B.3), pleometrosis (case C), and mixing of unrelated workers at collection sites (case D).

Reproductives of simple family colonies were not significantly inbred based on the genotypes inferred from their worker offspring (F_{IT} = 0.02, 95% CI = −0.14–0.11). Overall, reproductive pairs were less inbred than workers but the difference was not significant (F_{IT} = 0.13, 95% CI = 0.03–0.25). Relatedness between the reproductives within pairs (r = 0.11, SD = 0.41, n = 8) was slightly higher than the population background derived from pairwise compari-

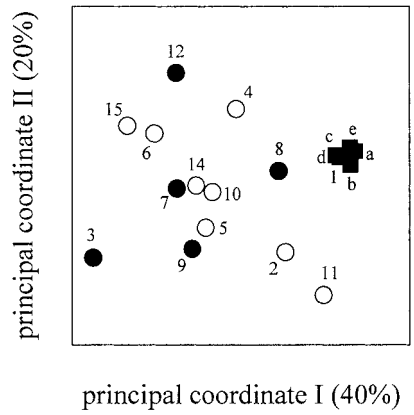

Fig. 2. Ordination of the genetic distances ($F_{CT}/(1 - F_{CT})$) between 14 colonies of Louis Armstrong Park (circles) and termites collected from five collection sites (a–e) across the foraging area of colony AP1 (squares). Open symbols indicate simple family colonies; filled symbols indicate extended family colonies. The first two principal coordinates account for 60% of the total variance. Note that different colonies which seem to be close in the multivariate plot separate clearly in the third dimension.

sons between reproductives from different colonies ($r = -0.08$, SD = 0.36, $n = 112$, two-tailed Mann–Whitney U, $P = 0.13$); however, this difference was not significant due to the small number of single pairs and the large standard deviation of the relatedness values. The latter reflects the wide range of the relatedness values between pairs (range −0.75–0.62). Only in colony AP15 were the parents putative siblings, i.e., they most likely originated from the same colony (10,000 simulations, $P = 0.05$).

Genetic Differentiation within Colonies. We found small but significant genetic differentiation among termites from five different collection sites within the foraging area of the spatially expansive colony AP1. Significant differentiation in the genotypic distribution occurred between six of the 10 pairings of collection sites (Fisher method, χ^2 = infinity, df = 14–16, $P < 0.001$). The detected genetic differentiation among termites from different collection sites of AP1 ($F_{CT} = 0.02$, SD = 0.01, $n = 10$) was significantly smaller than the differentiation among groups of termites from different colonies (average $F_{CT} = 0.31$, SD = 0.09, $n = 91$; Fig. 2, two-tailed Mann–Whitney U, $P < 0.001$). This result is consistent with all the termites within AP1 belonging to a single colony with slight differentiation among spatially separated portions of the foraging area.

Correlation of Genetic, Colony Characteristics and Behavioral Data. Previously, Messenger and Su (2005a, b) reported colony characteristics and aggression of termite colonies in Louis Armstrong Park. Colony characteristics were described for 12 colonies, and aggression was described for 11 of the colonies investigated in the current study. We correlated their data with our results concerning social organization and genetic distance.

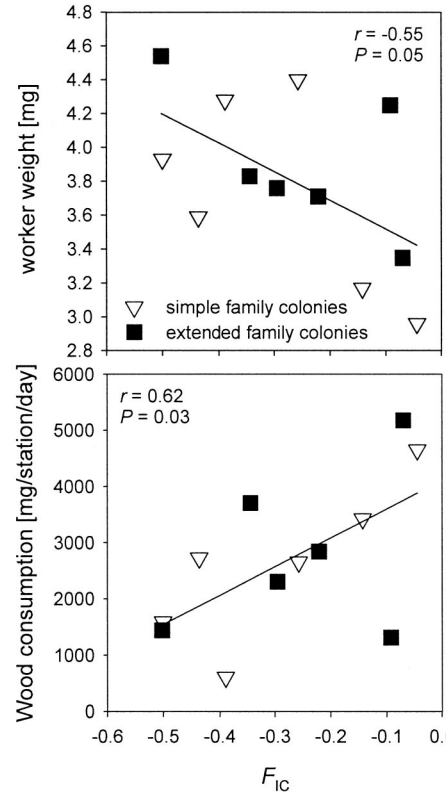

Fig. 3. Correlation of inbreeding levels within colonies (F_{IC}) with worker body weights and total wood consumption.

No significant difference in worker and soldier weight, in wood consumption, foraging population, and foraging area (reported in Messenger and Su 2005a) was found between simple family colonies and extended family colonies (two-tailed Mann–Whitney U, $P > 0.20$). No significant correlation was found between the colonies' soldier weight, size of the foraging area, or population size (Messenger and Su 2005a) and the inbreeding coefficient within colonies (F_{IC}). However, F_{IC} was significantly negatively correlated with worker weight ($r = -0.55$, $P = 0.05$) and positively correlated with wood consumption ($r = 0.62$, $P = 0.03$; Fig. 3). A weak but significant positive correlation occurred between the pairwise genetic distance between colonies $F_{CT}/(1 - F_{CT})$ and the level of aggression determined by Messenger and Su (2005b) in both no choice tests in petri dishes (Mantel test, 55 pairings, 999 permutations, $r = 0.31$, $P = 0.01$) and 48 h mortality in arenas ($r = 0.35$, $P = 0.02$).

Discussion

The present results on the genetic structure of the *C. formosanus* colonies in Louis Armstrong Park together with previous studies of colony foraging areas, colony census data and intercolony aggression (Husseneder et al. 2003a; Messenger and Su 2005a, b;

Messenger et al. 2005), provide the most comprehensive view to date of the population genetics, breeding system, ecology, and behavior of an introduced population of *C. formosanus.* Our genetic analyses showed that termite colonies in Louis Armstrong Park were approximately equally divided between simple and extended families. One would expect colonies with multiple reproductives to have greater population size and thus occupy larger territories and consume more wood than simple family colonies with just one reproductive pair (Thorne et al. 1999, Grube and Forschler 2004). Our results did not support this prediction. Although the two colonies with the largest foraging areas were extended families (AP1 with 1,634 m^2, and AP8 with 1,186 m^2; Messenger and Su 2005a), we found no significant difference overall between simple families and extended families in the three variables associated with colony size: size of the worker population, size of foraging area, or quantity of wood consumed.

This lack of association between family type and characteristics linked to colony size may be due in part to the difficulty of measuring the latter accurately, especially the size of the worker population and total amount of wood consumed by colonies. For practical reasons, colony population size can only be estimated by the indirect method of mark–release–recapture because excavation of colonies for direct colony census is not feasible given their large underground foraging areas and the destructive and time-consuming nature of the procedure. Mark–release–recapture can lead to biased estimates of the actual number of workers in a colony if there is intracolony differentiation, because this violates one of the main assumptions of this method, that of equal distribution and free interchange of individuals within the entire foraging area (Evans et al. 1999). In fact, we did find significant genetic differentiation among collection sites within the most expansive colony, AP1, indicating that, in at least this one colony, the mark–release–recapture technique probably did not provide an accurate estimate of colony size. Wood consumption was determined from artificial monitoring stations, which likely comprised only a few of a much larger number of other food sources used by the study colonies. Thus wood consumption, as measured by Messenger and Su (2005a), may depend as much or more on the amount of other food materials to which a colony has access as the number of individuals in the colony. Of these three variables likely to be associated with colony size, the most accurately measured was probably foraging area, but still we found no difference between simple and extended families. Recently, DeHeer and Vargo (2004) also reported a lack of association between colony breeding structure and size of foraging area in *Reticulitermes flavipes* Kollar, suggesting that the presence of multiple neotenics in this species is not associated with more expansive foraging areas either. However, among extended family colonies of *R. flavipes,* both DeHeer and Vargo (2004) and Bulmer and Traniello (2002a) found a positive association between foraging area and level of inbreeding within colonies (F_{IC}), suggesting that within this colony type, larger colonies have more reproductives and possibly spatially separate reproductive centers. No such correlation was found in the current study for *C. formosanus,* possibly because competition among neighboring colonies might have limited expansion of foraging areas of extended families (Messenger and Su 2005b), or the extended family colonies in our study were comparatively young as suggested by the fact that they were not significantly more inbred than simple families (see below).

Our results may provide some insight into the factors underlying the considerable variation among *C. formosanus* colonies in the size of workers, a phenomenon noted by others (Su and Scheffrahn 1988a) but for which no explanation had been previously offered. Although we found no significant difference between simple and extended families in size of workers, across both colony types combined there was a significant negative correlation between the level of inbreeding within colonies (F_{IC}) and worker body size, as measured by weight. The colony inbreeding coefficient also was positively correlated with wood consumption. Together, these results suggest that colonies that are more inbred have smaller workers and consume more food. A negative relationship between worker size and wood consumption also was reported by Su and La Fage (1984). One possible explanation for why more inbred colonies might have smaller workers is that inbreeding negatively affects development, resulting in slightly stunted growth. It is of interest to note that such a negative effect of inbreeding on growth rates and adult body size has recently been reported in the subsocial spider *Stegodyphus lineatus* Latreille (Bilde et al. 2005), suggesting this may be a widespread consequence of inbreeding, at least in arthropods. However, the assumption that smaller worker size is a sign of inbreeding depression and thus associated with a negative effect on the termite colony, is inconsistent with previous findings suggesting that termite colonies with smaller workers are actually more vigorous and therefore more active foragers (Shimizu 1962, Grace et al. 1995). More studies on the mechanisms linking worker size, inbreeding, and breeding structure of termite colonies are needed.

In a previous study, pairwise aggression tests between 11 of the Louis Armstrong Park colonies revealed variable levels of agonism (Messenger and Su 2005b). These aggression levels were weakly but significantly correlated with genetic distances among colonies determined in the current study, suggesting there is some genetic component to colony labels leading to colony mate recognition and discrimination (Beye et al. 1997). Genetically based discrimination cues have been suggested for other termite species (Adams 1991, Husseneder et al. 1998, Kaib et al. 2004), but two previous studies with *C. formosanus* failed to detect a genetic influence on levels of intercolonial aggression (Husseneder and Grace 2001, Florane et al. 2004). The reasons for the discrepancy between the current study and the previous work are not clear,

but there are several mutually compatible possibilities. First, there could simply be variation among populations in the level of intraspecific aggression (Su and Haverty 1991) and the extent to which genetic factors influence behavior toward foreign conspecifics such that genetically based odor cues are more important in influencing agonism in some populations. Second, there could be differences between studies in the types of genetic markers used to assess genetic relationships among colonies. Using multilocus DNA fingerprinting, Husseneder and Grace (2001) did not find a significant correlation between intercolonial aggression and genetic similarities in a population of 17 colonies from Oahu, HI. However, multilocus DNA fingerprinting may not be as precise as the microsatellite markers used in the current study for quantifying genetic distances (Husseneder et al. 2003b). Small sample size may account for the lack of a significant relationship between aggression levels and genetic similarities as measured by microsatellite markers among only four colonies from Louisiana as recently reported by Florane et al. (2004).

Another possible reason why we found a relationship between genetic similarity and agonistic behavior not detected in previous studies may have to do with the detailed nature of the current study. Our study concerned a small area with neighboring colonies, which probably encountered each other frequently in the field. The high frequency of these encounters might have led to aggression mediated by genetic cues between familiar neighboring colonies, which may not occur in colonies so distant that they would never meet under natural conditions. Encounter-induced hostility has been described in the ants *Cataglyphis fortis* Forel (Knaden and Wehner 2003) and *Pristomyrmex pungens* Mayr (Sanada-Morimura et al. 2003). This phenomenon, which is the opposite of the dear-neighbor concept, also has been reported in other social insects, including the nest building termite, *Nasutitermes corniger* Motschulsky (Dunn and Messier 1999). However, no evidence for increased aggression among neighboring colonies was found in the eastern subterranean termite *R. flavipes* (Bulmer and Traniello 2002b), suggesting that this is not a universal phenomenon in termites. Further detailed studies of the genetic relationships among colonies and levels of aggression are needed to determine whether the positive relationship found in the current study is general in this species. Of particular importance will be studies of native populations which are genetically more diverse than introduced populations (see below).

Genetic diversity in the Louis Armstrong Park population, as measured by allele number per locus, was low (2.9) compared with most other populations of *C. formosanus* investigated so far, but it was similar to a neighboring population from City Park, New Orleans (3.1, $n = 17$ colonies, unpublished data). Three other introduced populations had more alleles per locus on average, Oahu, HI (3.9, $n = 12$ colonies; unpublished data), Rutherford County, North Carolina (3.5, $n = 8$ colonies; unpublished data), and Kyushu, Japan (6.0, $n = 20$ colonies; Vargo et al. 2003a), whereas two introduced populations had slightly lower allele numbers: Fukue, Japan (2.7, $n = 10$ colonies; Vargo et al. 2003a) and Charleston, SC 1.9, $n = 25$ colonies; unpublished data). As expected, a native population from China had the highest number of alleles per locus (6.4, $n = 13$ colonies; unpublished data).

Reduced genetic diversity can be caused by genetic drift and/or a recent genetic bottleneck, i.e., a sharp reduction in population size (Cornuet and Luikart 1996, Piry et al. 1999). Such a drop in effective population size often occurs when a species is introduced to a new area (founder effect). Not surprisingly, we detected a recent genetic bottleneck in the Louis Armstrong Park population, which was reportedly founded only 30 yr ago from wood brought in from a nearby military base (Scott and Scott 1996). Thus, the Louis Armstrong Park population was likely established by a very small population that had undergone at least two recent bottlenecks, the first during its introduction to New Orleans and the second upon its introduction to the park from a nearby population. In addition to its introduction history, a bottleneck also might reflect the continuous effort to control termite populations in New Orleans, leading to frequent reductions in population size. We also have found evidence of a recent bottleneck in two other introduced populations on the U.S. mainland (City Park, $n = 17$ colonies; and Charleston, $n = 25$ colonies), but not in a third population (Rutherford County), which consisted of only eight colonies (unpublished data). In contrast, in a previous study we did not detect a recent bottleneck in two introduced populations in Japan, where *C. formosanus* has been established for some 300 yr (Vargo et al. 2003a). The lack of a trace of a recent bottleneck in these Japanese populations is not unexpected considering that the heterozygosity excess caused by loss of rare alleles during a bottleneck, which forms the basis of the tests used, is expected to persist for only a couple of dozen generations (Cornuet and Luikart 1996).

F-statistics conducted to describe the small scale population structure of Louis Armstrong Park termites revealed moderate inbreeding on the population level ($F_{IT} = 0.13$). Because of the hierarchical nature of the analysis in our study and in other similar studies, F_{IT}, when estimated for a single population, is equivalent to the standard inbreeding coefficient F_{IS} applied to solitary breeders. The degree of total inbreeding varies within and among subterranean termite species and depends on the relatedness among founders, the frequency of colonies headed by neotenics, the number of reproductives within neotenic-headed colonies, and the number of generations of inbreeding within colonies. The level of inbreeding in the Louis Armstrong Park population studied here is very similar to that found in a nearby population from City Park ($F_{IT} = 0.08$; unpublished data), despite the fact that the latter population had a higher frequency of simple family colonies (82 versus 57% in Louis Armstrong Park). Similar levels of inbreeding also have been found in introduced populations in Charleston ($F_{IT} =$

0.14; unpublished data), in which 48% of 25 colonies were simple families, and in Kyushu ($F_{IT} = 0.16$; Vargo et al. 2003a), in which 85% of 20 colonies were simple families. A population of eight colonies in Rutherford County of which six were simple families, was found to have a higher level of inbreeding ($F_{IT} = 0.24$; unpublished data). The most inbred population found to date is that of Fukue, Japan, in which 10 colonies, all simple families, had an inbreeding coefficient of $F_{IT} = 0.46$. The high level of inbreeding in this population was primarily due to the high degree of relatedness of nestmate reproductives heading simple family colonies ($r = 0.61$). Similar variation in levels of inbreeding has been reported for native populations of *R. flavipes*, where values of F_{IT} ranged from 0.62 in Tennessee (Reilly 1987), 0.34 and 0.27 in Massachusetts (Bulmer et al. 2001) where 0 and 38% of colonies were simple families, to 0.05–0.12 in North Carolina (Vargo 2003a, b; DeHeer and Vargo 2004), where ≈75% of colonies were simple families. Two populations of *Reticulitermes santonensis* (Feytaud) in France, which most likely are *R. flavipes* that was introduced from the United States and became established there >100 yr ago (Clément et al. 2001, Jenkins et al. 2001, Austin et al. 2002, Marini and Mantovani 2002, Luchetti et al. 2004, Uva et al. 2004, Ye et al. 2004), were moderately to highly inbred ($F_{IT} = 0.17$ and 0.39; Dronnet et al. 2005); both of these populations consisted entirely of extended family colonies (Dronnet et al. 2005).

Eight (57%) of the 14 colonies in Louis Armstrong Park showed Mendelian distribution of genotypes and were therefore simple family colonies headed by a monogamous pair. The proportion of simple family colonies falls into the rather large range for other introduced *C. formosanus* populations that have been studied: Oahu, 30% of 20 colonies (Vargo et al. 2003b); Charleston, 48% of 25 colonies (unpublished data); Rutherford County, 67% of eight colonies (unpublished data); City Park, 82% of 17 colonies (unpublished data); Kyushu, 85% of 20 colonies; and Fukue, Japan, 100% of 10 colonies (Vargo et al. 2003a). In contrast, a native population of 14 colonies in Guangdong, China, consisted exclusively of extended families (C. H., E.L.V., and J.K.G., unpublished data). Thus, apart from having at least some simple family colonies, there seems to be considerable variation among different introduced populations in the reproductive structure of colonies. It is not clear whether such differences might be due to variation in age structure of colonies, ecological conditions, or genetic composition.

The pairs of reproductives in simple family colonies in the current study were outbred ($F_{IT} = 0.02$), and on average no more related to each other than to individuals in the population as a whole. Only one of the eight simple family colonies was headed by putative siblings, i.e., former nest mates. Together with the fact that relatedness of tandem running dealated pairs during swarming in the French Quarter, New Orleans, was not significantly different from the population background (C.H., unpublished data), these results suggest that mating is independent of the reproductives' relatedness and does not involve kin recognition. Similarly, simple family colonies in some other subterranean termites are predominantly headed by unrelated primary reproductives, e.g., *R. flavipes* in Massachusetts (Bulmer et al. 2001) and North Carolina (Vargo 2003a, b; DeHeer and Vargo 2004), and *Schedorhinotermes lamanianus* Sjöstedt in Kenya (Husseneder et al. 1999). The only exception to these general findings so far was the unusually high relatedness between pairs of reproductives in simple family colonies in a Japanese population in Fukue ($r = 0.61$; Vargo et al. 2003a). Additional genetic and ecological studies of introduced and native populations of *C. formosanus* are needed to determine the extent to which colonies are founded by unrelated reproductives and what environmental and/or genetic factors promote pairing of close relatives during colony founding.

The prevalence of simple family colonies headed by outbred pairs in the Louis Armstrong Park population studied here indicates a relatively young population, because the production of neotenic replacement reproductives occurs later in the colony life cycle (Thorne 1998, Thorne et al. 1999). Thus, an area with a high proportion of colonies headed by single pairs may indicate that it was recently colonized by swarming termites, resulting in a population of uniformly young colonies. Alternatively, it could mean that simple family colonies of *C. formosanus* in the population are fairly short-lived so that fewer than half the established colonies live long enough to produce neotenics, as has been proposed for populations of *R. flavipes* in North Carolina (Vargo 2003a). Furthermore, the fact that extended family colonies of Louis Armstrong Park were not significantly more inbred than their simple family counterparts suggests that these colonies also were relatively young, headed by reproductives that were only a generation or two removed from the founders. In contrast to the present findings on *C. formosanus* in Louis Armstrong Park, extended families in Kyushu (Vargo et al. 2003a), City Park (unpublished data), Charleston (unpublished data), and Rutherford County (unpublished data) clearly showed higher degrees of inbreeding than simple family colonies in the same population. This suggests that extended family colonies in these other populations contain more neotenics and/or neotenics inbreed for more generations than those in Louis Armstrong Park.

Nearly half the 14 colonies (47%) studied here were headed by multiple reproductives as inferred from the classes and frequencies of genotypes among worker nest mates. *F* values and the coefficient of relatedness indicated that the multiple reproductives did not arise from pleometrosis (multiple same-sex reproductives cooperating in colony founding), which would lead to lower F_{IT} and relatedness values than those observed (Table 3, case C), nor from colony fusion, which would result in positive F_{IC} values and relatedness lower than 0.5 (Thorne et al. 1999, Bulmer et al. 2001). Thus, colonies with multiple reproductives were true extended families consisting of the neotenic descen-

dants of monogamous pairs of founders. This seems to be generally true for this species as it has been found in six other introduced populations (Vargo et al. 2003a, b; unpublished data). The closest match between the empirical *F*-statistics and relatedness values obtained here with those predicted for various types of extended families is for colonies headed by the least number of multiple neotenics possible, that is, two female and one male neotenic who are the direct descendants of the founding pair (Table 3, case B.1). Even so, the empirical values for levels of inbreeding in individuals relative to the total population (F_{IT}) and for individuals relative to their own colonies (F_{IC}) are somewhat lower than the predicted values but only significantly so in the latter case (based on non overlap of the 95% CI). The reason for such low *F* values in extended family colonies is not clear, but one possibility is that at least some colonies contain a mixture of primary founders and their neotenic offspring resulting in colonies less inbred than those headed by neotenics only. The presence of both primary reproductives and neotenics in colonies may reflect the beginning of a turnover from the first generation of founders to the second generation of replacement reproductives. Another possibility is that such colonies have very few neotenics but have unequal reproduction so that a single pair within each colony produces most of the offspring. Distinguishing between these possibilities will require further studies combining more detailed genetic analyses with nest censuses.

Mature colonies of *C. formosanus* may contain millions of individuals with foraging areas extending >3,500 m^2 (Su and Scheffrahn 1988b). The occurrence of extended family colonies with multiple reproductives coupled with the tendency of this species to produce satellite nests, could lead to genetic differentiation within colonies (Roisin and Pasteels 1986, Su and Tamashiro 1987, Husseneder et al. 1998). Separate breeding units within a colony could have important implications for the sociogenetics and management of termites. We detected subtle genetic differentiation among collection sites within the foraging area of the most expansive colony. The magnitude of differentiation was substantially smaller than the differentiation between colonies and was due to differences in allele and genotype frequencies rather than to differences in genotypes or alleles.

The occurrence of spatially separate reproductive centers could potentially lead to colony reproduction by budding, should connections among reproductive centers become severed. It is thought that multiple-site nesting termites, which includes many subterranean termite species such as *C. formosanus* (Shellman-Reeve 1997), efficiently exploit spatially heterogeneous resources by increasing the number of colonies through budding (Clément 1981, Roisin and Pasteels 1986, Husseneder et al. 1998, Thorne 1998). However, detailed genetic studies on a small scale capable of detecting budding are few, and those that have been conducted, e.g., on *R. flavipes* (Bulmer and Traniello 2002a, Vargo 2003a, DeHeer and Vargo 2004), have failed to find evidence of frequent colony reproduction by budding. That budding may occur in *C. formosanus* is suggested by results from a Japanese population, in which Vargo et al. (2003a) found a positive F_{IC} value in extended family colonies, a finding consistent with nest budding with interconnected daughter nests (Thorne et al. 1999). However, the fact that all 14 colonies in the current study were genetically distinct and had discreet foraging areas and the fact that there was no significant isolation by distance suggest that colony reproduction by budding is not common in the Louis Armstrong Park population. Perhaps budding, if it occurs at all in this species, is limited to the rare, large colonies with spatially separate reproductive centers, and is therefore difficult to detect. Longer term studies in which the foraging areas and genetic structure of individual colonies are tracked over time are needed to fully investigate the possibility of colony reproduction by budding in *C. formosanus* and other subterranean termites.

Like other subterranean termites, *C. formosanus* possesses a great deal of plasticity in social organization, providing for the possibility of shifts in social organization after introductions to new areas that could favor invasiveness. Although colony reproductive structure has yet to be fully characterized in native populations, the present results together with those on other introduced populations suggest there have not been widespread changes in colony breeding structure common to introduced populations. Rather, introduced populations seem to consist of genetically and spatially distinct colonies comprised of close family groups founded by monogamous pairs of primary reproductives, albeit with considerable variation in the frequency of extended families and levels of inbreeding. In this respect, introduced populations of *C. formosanus* differ from those of many invasive ants, which frequently exhibit unicoloniality and the attendant traits of polygyny, colony reproduction by budding and reduced intraspecific aggression (Holway et al. 2002, Tsutsui and Suarez 2003). More extensive studies are needed to determine whether the observed variation in colony reproductive structure among introduced populations of *C. formosanus* is related to the severity of different invasive populations, and whether this variation is associated with the age of populations, local ecological conditions, or genetic composition. In light of current results, the key to understanding the invasion success of *C. formosanus* may have as much to do with other features of its biology that are characteristic of invasive species generally, such as relatively broad diet (Lai et al. 1983), ability to use a variety of nesting sites, and tolerance for disturbed habitats, than with particular features of its colony social organization. Continued work on the genetics, ecology and behavior of native and introduced populations of this destructive pest will help shed light on why *C. formosanus* is such a successful invader.

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