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Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan

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Abstract

Subterranean termites have unusual plasticity in their breeding systems. As a result of their cryptic foraging and nesting habits, detailed information on the numbers and types of reproductive individuals in colonies has been difficult to obtain. In this study, we used microsatellite markers to infer the major features of the breeding system of the Formosan subterranean termite, *Coptotermes formosanus*, in southern Japan, where it is believed to have been introduced from China. A total of 30 colonies was sampled from two islands (Kyushu and Fukue) located 100 km apart. Twenty workers from each colony were genotyped at six microsatellite loci. Analysis of worker genotypes within colonies indicated that 27 colonies (90%) were simple (Mendelian) families. The remaining three colonies, all from Kyushu, were consistent with being extended families having begun as simple families but being currently headed by multiple neotenic (secondary) reproductives descended from the original king and queen. Workers from simple families in both populations were significantly inbred (F_{IT} = 0.10 for Kyushu and 0.46 for Fukue) and highly related to their nestmates (coefficient of relatedness, r = 0.59 for Kyushu and 0.77 for Fukue), suggesting that many simple-family colonies were headed by closely related reproductives, especially in the Fukue population. This conclusion is supported by the high coefficient of relatedness between nestmate reproductives in simple-family colonies (r = 0.23 for Kyushu and 0.61 for Fukue) based on genotypes inferred from their worker offspring. There was moderate genetic differentiation (F_{ST} = 0.12) between the two populations, suggesting rather restricted gene flow between them. There was no significant isolation by distance among colonies, as might be expected given the limited dispersal of reproductives, presumably because of the frequent movement of colonies by humans. Finally, there was no evidence of a recent bottleneck, a finding possibly consistent with the more than 300-year history of this species in Japan.

Keywords: breeding system, gene flow, Isoptera, microsatellites, Rhinotermitidae, social organization

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Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is native to the Oriental region, most probably to China. As a result of human-aided transport, this species has become established in many tropical and subtropical regions around the world, including Japan where it was apparently first introduced more than 300 years ago (Mori 1987; Su & Tamashiro 1987; Wang & Grace 1999). As a result of its large colony size, capacity to penetrate a variety of materials, and ability to consume a wide range of wood types, *C. formosanus* is a severe structural pest wherever it occurs.

Like other subterranean termites (Rhinotermitidae), *C. formosanus* has cryptic nesting and foraging habits, consisting of a diffuse network of underground nests and foraging areas interconnected by tunnels. Because the nests and the reproductives they harbour are often difficult to find,

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there is little detailed information concerning the breeding system of this and other subterranean termite species. Based on field observations and laboratory studies of colony growth, Thorne *et al*. (1999) give the following account of colony ontogeny in *Reticulitermes* spp., a scenario that most likely applies to many other subterranean termites, including *C. formosanus* (see e.g. Su & Tamashiro 1987). Colonies are generally founded by a pair of primary (winged) reproductives following a mating flight, resulting in a simple family structure early in colony ontogeny. Later in the colony life cycle, one or both primary reproductives die and these may be replaced by neotenics (non-winged reproductives) produced within the nest. Colonies may contain a few or dozens of neotenics which inbreed in the colony. Moreover, as colonies grow and expand their foraging range, groups of foragers and / or satellite nests may become cut off from the rest of the colony. The resulting nest buds may produce new neotenics from among the workers present, leading to independent colonies. Finally, there is evidence that subterranean termite colonies may, on occasion, fuse or adopt unrelated reproductives (Clément 1981; Jenkins *et al*. 1999a; Bulmer *et al*. 2001; Matsuura & Nishida 2001). Thus the breeding system within any subterranean termite population can be potentially complex, consisting of a number of colony types depending on age, frequency of budding and interaction with neighbouring colonies.

Genetic markers are a powerful way to infer the breeding system of social insects (Pamilo *et al*. 1997; Ross 2001), and there is an increasing number of genetic studies of colony and population genetics of termites (Clément 1981; Reilly 1987; Luykx 1993; Atkinson & Adams 1997; Husseneder *et al*. 1998, 1999, 2002; Thompson & Hebert 1998a,b; Jenkins *et al*. 1999a,b; Bulmer *et al*. 2001; Clément *et al*. 2001; Goodisman *et al*. 2001; Husseneder & Grace 2001a,b). These studies show that there is considerable variation in breeding systems of termites both among and within species. Genetic studies of *C. formosanus* have been particularly difficult because of the low variability of allozyme and mitochondrial DNA markers (Strong & Grace 1993; Broughton & Grace 1994; Wang & Grace 2000). Husseneder & Grace (2001a,b) employed multilocus DNA fingerprinting to distinguish among colonies and to quantify the hierarchical genetic structure in a Hawaiian population of *C. formosanus*. In this study, we used microsatellite markers to characterize the breeding system of two populations of *C. formosanus* in Japan.

Materials and methods

Sample collection

Between 3 August and 5 October 2001, samples of the Formosan subterranean termite *Coptotermes formosanus* were collected from below-ground Sentricon® (Dow AgroSciences) monitoring stations placed around infested structures located in Nagasaki Prefecture in southern Japan (Fig. 1). The colonies formed two populations located 90–100 km apart and separated by water. The Kyushu population consisted of 20 colonies located on the island of Kyushu in and around the city of Nagasaki. The Fukue population was comprised of 10 colonies, nine of which were located on Fukue Island while the 10th was located on a small island nearby. Each monitoring station was sampled only once and the samples were collected from the monitoring devices before any bait had been used on the structure to ensure that the sampled colonies had not been exposed to insecticidal bait prior to sampling. Only a single monitoring station was sampled per structure and the minimum distance between structures was 0.7 km, ensuring that each of the 30 collection points represented different colonies. Several hundred individuals were collected from each point, including large numbers of workers and soldiers, and when present, nymphs and functional reproductives. Live termites were placed directly into 95% ethanol and stored at 4 °C until extraction. Voucher specimens from each population have been deposited in the North Carolina State University Insect Collection.

Studies of subterranean termites generally assume that only foragers from a single colony will be found together in a monitoring station (see e.g. Su 2003). Evidence that this is the case in *C. formosanus* comes from a study using multilocus DNA fingerprinting, in which Husseneder & Grace (2001b) genotyped groups of workers from 17 monitoring stations in Hawaii and found that workers from the same stations shared close genetic affinities but were genetically distinct from those present at other stations. The groups of workers from each monitoring station in the present study had genotypes consistent with close family groups (see below), suggesting that they were members of a single colony.

Microsatellite analysis

Genomic DNA was extracted from individual termite whole bodies using the DNeasy Tissue Kit (QIAGEN Inc.). Microsatellite genotypes for 20 workers and the one recovered primary reproductive pair from each collection point were determined at eight loci by means of polymerase chain reaction amplification according to the methods of Vargo & Henderson (2000). Amplified fluorescence-labelled products were run out on 6.5% polyacrylamide sequencing gels on a Li-Cor 4000 or 4200 automated DNA sequencer.

Microsatellite allele sizes were scored using the program GENE IMAGIR ver. 3.56 (Scanalytics, Inc.). Deviations from Hardy–Weinberg equilibrium for each locus and tests for linkage disequilibrium between all pairs of loci were conducted by means of exact tests using the program GENETIC

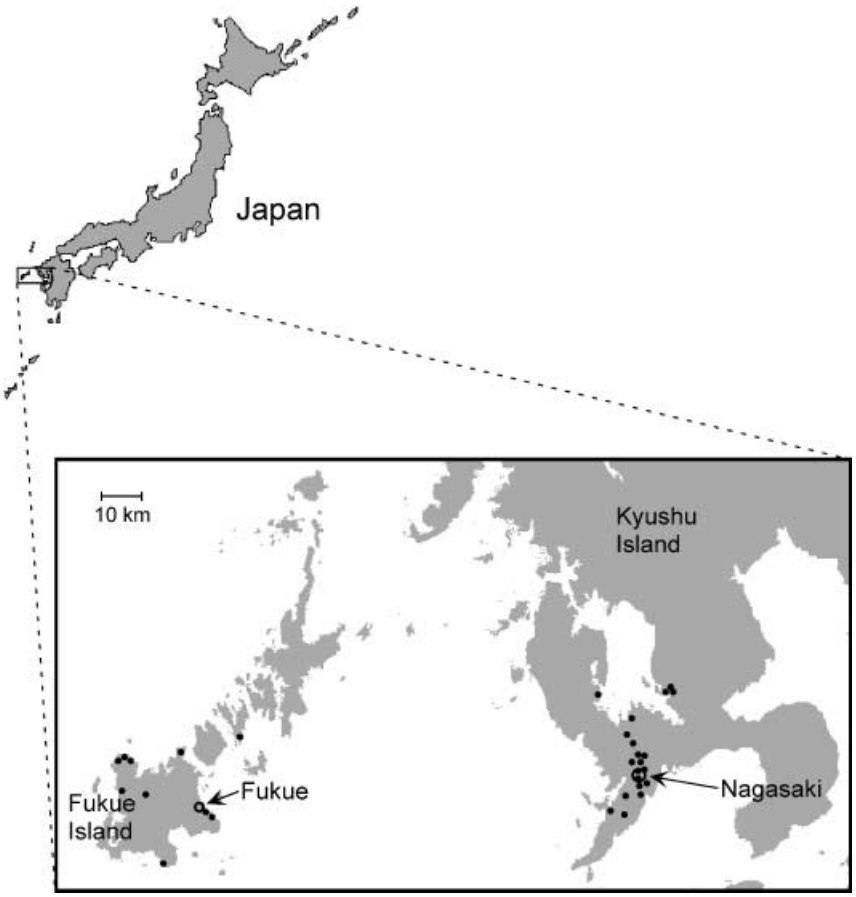

Fig. 1 Location of samples collected from southern Japan.

DATA ANALYSIS version 1.1 (GDA; Lewis & Zaykin 2000) with 3200 shufflings. Because colonies were comprised of close kin (see Results) only a single individual per nest was used for these tests to avoid analysis using nonindependent genotypes. A resampling procedure was performed in which a single individual from each colony was selected at random for a total of 20 replications.

The genotypes of workers and their frequencies were used to classify colonies as simple (Mendelian) families or as extended families. Simple-family colonies are those containing a single monogamous pair of reproductives, which are generally expected to be primary reproductives, but could possibly consist of one primary and one neotenic reproductive, or two neotenics. Colonies were considered simple families if worker genotypes at all loci were consistent with being the direct offspring of a monogamous pair of reproductives, and the observed frequencies of the genotypes did not differ significantly from those expected for simple families as assessed by means of a *G*-test. *G*-values were generated by a goodness-of-fit comparison of the observed frequencies of genotypes to the expected at each locus. For each colony, the locus-specific *G*-values were summed to produce an overall *G*-value. Extended-family colonies were inbred groups containing more than two functional reproductives but which appeared to have descended from simple families. In such cases, the multiple reproductives most likely arose through the production of neotenics within the colony, and these reproductives may or may not coexist with one or both of the original primary reproductives. Colonies were considered extended families if worker genotypes were inconsistent with a single pair of reproductives, or if Mendelian genotypes were present in the colony but their frequencies deviated significantly ($P < 0.05$) from those expected for simple families.

Colony and population genetic structure were investigated by estimating *F*-statistics using the method of Weir & Cockerham (1984) as implemented in the program GDA. Because there was significant differentiation between populations, the analysis were performed for each population separately so that the *F*-values used to infer the population breeding system were not confounded by higher-level genetic structure. The significance of the *F*-statistics was assessed by constructing the 95% confidence intervals (CIs) by bootstrapping over loci with 1000 replications. Values whose 95% CIs did not overlap zero were considered to be significant at the $\alpha = 0.05$ level. In comparing between populations, values with nonoverlapping 95% CIs were considered to be significantly different. We followed the

notation of Thorne *et al*. (1999) using the subscripts I, C and T to represent the individual, colony and total components of genetic variation, respectively. Because the hierarchy we employed is different from those used in conventional studies of population structure, the biological explanation of each component warrants explanation. F_{IT} is the standard inbreeding coefficient, while F_{CT} is similar to F_{ST}, representing genetic differentiation among colonies. F_{IC} is the colony inbreeding coefficient, and although this component has no analogue in solitary organisms, it is expected to be highly sensitive to the number of reproductives and their mating patterns within social groups. Specifically, F_{IC} is expected to be strongly negative for simple families, to increase toward zero with greater numbers of interbreeding reproductives, and to become positive if there is assortative mating among multiple reproductives within colonies or there is mixing of individuals from different colonies. The coefficient of relatedness (r) among nestmate workers within a population was estimated using the program RELATEDNESS version 5.0.8 (Queller & Goodnight 1989). The 95% CIs were obtained by jackknifing over loci. Features of the breeding system of the population were then inferred by comparing these empirically generated values to previous results of computer simulations of possible breeding systems of subterranean termites (Thorne *et al*. 1999; Bulmer *et al*. 2001). In this comparison, we present only a subset of all the possible breeding systems that were simulated, excluding those that were clearly inconsistent with the empirical results, such as mixing of workers from different colonies. Finally, *F*-statistics and the coefficient of relatedness were estimated for the reproductive pairs in simple-family colonies based on their genotypes as inferred from the genotypes of their worker offspring.

Possible isolation by distance within each population was investigated by estimating F_{ST} between pairs of colonies and calculating the Pearson product correlation coefficient between F_{ST} values and physical distance for all pairs of colonies. The two populations were analysed separately because the distance between them was great compared to the distances among colonies within each population. The significance of the correlation coefficients was assessed by means of a Mantel test with 10 000 replications as implemented in the program GENEPOP on the Web version 3.1c (available via http://wbiomed.curtin.edu.au/genepop/; Raymond & Rousset 1995).

To determine whether there was evidence of a detectable genetic bottleneck, the genotypes were tested for heterozygosity excess using the two tests as implemented in the program BOTTLENECK version 1.2.02 (Piry *et al*. 1999). One test, developed by Cornuet & Luikart (1996), uses the sign test to determine if there is a significantly greater proportion of loci with heterozygosity excess than expected for a population at mutation drift equilibrium. The other test, which is considered more powerful and more robust than the sign test (Piry *et al*. 1999), detects significant heterozygosity excess on average across loci using a Wilcoxon sign-rank test. The tests were performed on each of the 20 replicate data sets using both the infinite alleles and the stepwise mutation models.

Results

Genetic diversity and tests for Hardy–Weinberg equilibrium and linkage disequilibrium

Table 1 shows the summary statistics for genetic variability in the populations. Overall, there were between two and 10 alleles per locus, with a mean of six alleles per locus for the six loci retained for subsequent analyses (see below). The number of alleles in the Fukue population was only about half that found in the Kyushu population. Most loci did not deviate significantly from Hardy–Weinberg equilibrium. In the Kyushu population, genotypes of *Cf 8-4* differed significantly from expected in 17 of the 20 re-sampled data sets, and locus *Cf 10-5* deviated significantly from expected in two cases. In the Fukue population, *Cf 4-4* and *Cf 8-4* deviated significantly from expected in nine and five cases, respectively. Because locus *Cf 8-4* deviated significantly from Hardy–Weinberg equilibrium in more than half of the resampled data sets, it was dropped from the analysis. Across all re-sampled data sets, pairs of loci were in significant linkage disequilibrium in 20 (1.8%) of 1120 cases, and in 19 of these cases the linkage disequilibrium involved *Cf 4-9A* and *Cf 4-10* in the Kyushu population, suggesting that these two loci were not independent markers in this population. To avoid the use of nonindependent markers, locus *Cf 4-9A* was also dropped from the analysis. In the one colony in which the primary reproductives were collected and genotyped, all the expected F_1 genotypes were present among the workers and they occurred in the expected ratios, indicating that the six loci used were independently assorting markers suitable for investigating colony and population genetic structure.

Genetic differentiation between populations

There was significant differentiation between the two populations according to both worker genotypes (F_{ST} = 0.123, 95% CIs = 0.055–0.178) and the reconstructed genotypes of reproductives in simple-family colonies (F_{ST} = 0.143; 95% CIs = 0.081–0.190), indicating moderate barriers to gene flow between these two spatially separated populations.

Colony genetic structure

As seen in Table 2, some 90% of the colonies overall had Mendelian genotypes in ratios not significantly different

Table 1 Variability of microsatellite loci

Locus	Kyushu population		Fukue population		Overall	
	No. of alleles	Frequency of most common allele	No. of alleles	Frequency of most common allele	No. of alleles	Frequency of most common allele
Cf 4 : 1 A2-4	9	0.36	2	0.77	10	0.49
Cf 4-4	6	0.71	3	0.41	6	0.57
Cf 4-9A*	2	0.54	2	0.83	2	0.64
Cf 4-10	3	0.55	2	0.81	3	0.64
Cf 8-4†	6	0.27	4	0.56	6	0.28
Cf 10-4	8	0.27	3	0.55	8	0.31
Cf 10-5	6	0.87	2	0.97	6	0.90
Cf 12-4	4	0.85	4	0.78	5	0.83
Mean‡ (± SD)	6.0 ± 2.3		2.7 ± 0.9		6.3 ± 2.4	

Allele frequencies were calculated using the program RELATEDNESS (Queller & Goodnight 1989) using all worker genotypes and colonies were weighted equally.
*Was in significant linkage disequilibrium with locus *Cf 4-10* in 19 of 20 cases in the resampled data sets for the Kyushu population. This locus was dropped from the analysis.
†Deviated significantly from Hardy–Weinberg equilibrium in 17 of the 20 resampled data sets in Kyushu and in five of 20 in Fukue. This locus was dropped from the analysis.
‡Mean calculated for the six loci used in the analyses only.

Table 2 Numbers of simple-family and extended-family colonies within each population

Population	Total no. colonies	Simple-family colonies (%)	Extended-family colonies (%)
Kyushu	20	17 (85.0%)	3 (15.0%)
Fukue	10	9 (90.0%)	1 (10.0%)
Total	30	26 (86.7%)	4 (13.3%)

from that expected for simple families. Included in this group was the colony from the Kyushu population in which the primary reproductives were collected. The genotypes of the reproductives in this colony were consistent with them being the sole parents of the workers analysed. The ability to detect multiple unrelated reproductives in the Fukue population was limited by the lower variability of the loci. However, the fact that all of these colonies had Mendelian genotypes in expected ratios indicates that they were all simple families. The only extended-family colonies (n = 3) were found in the Kyushu population, two of which had nonMendelian genotypes, and one of which had Mendelian genotypes but in ratios differing significantly from those expected. Although four of the six loci had five or more alleles present in the Kyushu population, no more than four alleles at a locus were found in any of these colonies, suggesting that they were headed by multiple neotenics descended from no more than two primary reproductives. This conclusion is further supported by the relatively high values of F_{IT} and nestmate relatedness for these colonies (see below), which are inconsistent with colonies containing multiple unrelated reproductives Thorne *et al*. (1999) and Bulmer *et al*. (2001).

Table 3 shows the estimates of the *F*-statistics and coefficient of relatedness for the worker genotypes in each population. There were considerable differences between the two populations in the *F*-statistics for simple families. In the Kyushu population, workers in the simple-family colonies were moderately inbred (F_{IT} = 0.10), whereas those in the Fukue population were significantly more inbred (F_{IT} = 0.46) based on nonoverlapping 95% CIs. Within the Kyushu population, the extended-family colonies had higher values for all the *F*-statistics and for the coefficient of relatedness than the simple-family colonies, although this difference was significant for the F_{IT}-values only, no doubt the result of the small sample size of the extended families.

Comparison of these empirical results with those of computer simulations of possible breeding systems for subterranean termites (Thorne *et al*. 1999; Bulmer *et al*. 2001) clearly shows that the simple-family colonies from both populations were more inbred than would be expected for a population of colonies headed by monogamous pairs of outbred reproductives (Table 3, case A). The high level of inbreeding suggests that nestmate reproductives in simple-family

Table 3 *F*-statistics and relatedness coefficients for worker nestmates of *Coptotermes formosanus* from southern Japan and values expected for some possible breeding systems of subterranean termites as derived from computer simulations by Thorne *et al*. (1999) and Bulmer *et al*. (2001)

	F_{IT}	F_{CT}	F_{IC}	r
Kyushu				
All colonies (n = 20)	0.161	0.350	−0.290	0.604
(95% CI)	(0.102–0.237)	(0.324–0.385)	(−0.358 to −0.224)	(0.561–0.647)
Simple-family colonies (n = 17)	0.103	0.326	−0.332	0.590
(95% CI)	(0.047–0.173)	(0.288–0.376)	(−0.405 to −0.265)	(0.508–0.672)
Extended-family colonies (n = 3)	0.455	0.449	0.012	0.654
(95% CI)	(0.247–0.745)	(0.271–0.680)	(−0.280–0.462)	(0.416–0.893)
Fukue				
Simple-family colonies (n = 10)	0.461	0.565	−0.237	0.774
(95% CI)	(0.216–0.722)	(0.367–0.770)	(−0.519–0.035)	(0.537–1.011)
Simulated breeding system				
(A) Simple-family colonies headed by outbred reproductive pairs	0.00	0.25	−0.33	0.50
(B) Extended-family colonies with inbreeding among neotenics				
(1) $N_f = N_m = 1$, $X = 1$	0.33	0.42	−0.14	0.62
(2) $N_f = N_m = 1$, $X = 3$	0.57	0.65	−0.22	0.82
(3) $N_f = N_m = 10$, $X = 1$	0.33	0.34	−0.01	0.51
(4) $N_f = 200$, $N_m = 100$, $X = 3$	0.34	0.34	0.00	0.51
(C) Nest budding with interconnected daughter nests				
(1) $N_f = N_m = 1$, $X = 3$, $P = 0.5$	0.66	0.56	0.22	0.68
(2) $N_f = N_m = 1$, $X = 3$, $P = 0.9$	0.66	0.64	0.04	0.77
(3) $N_f = N_m = 100$, $X = 3$, $P = 0.9$	0.43	0.41	0.03	0.58

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; and P represents the proportion of workers coming from one of the two nests when there is mixing.

colonies in both populations were closely related to each other. Indeed, we can gain some insight into relatedness among monogamous pairs of reproductives from the primary reproductive pair that was recovered in one simple-family colony from Kyushu. Nestmate workers in this colony were related by $r = 0.62$ (95% CIs = 0.24–1.00), a value very similar to the average nestmate relatedness for the simple-family colonies in this population. The two primary reproductives in this colony were also closely related ($r = 0.49$, 95% CIs = –0.03–1.01). Thus, in this population, some colonies were headed by closely related primary reproductives, but we cannot exclude the possibility that some colonies may have been headed by monogamous pairs of sibling neotenics. In fact, the empirical values in the more inbred Fukue population closely resembled those expected for colonies founded by outbred reproductives which are subsequently replaced by a single pair of neotenics inbred between one and three generations (Table 3, cases B1 and 2). Since no primary reproductive pairs were recovered in the Fukue population it is not possible to say whether the high levels of inbreeding in this population are mainly the result of colonies being headed by highly related primary reproductives or are the result of many colonies being headed by monogamous pairs of neotenic reproductives.

The high levels of inbreeding and near-zero F_{IC} in the extended-family colonies are suggestive of colonies with numerous neotenic reproductives (Table 3, cases B3 and 4), or colonies with multiple groups of neotenic reproductives located in spatially separated reproductive centres within the colony, as would be expected if there was nest budding with interconnected daughter nests (Table 3, case C). However, the small sample size of this group resulted in large CIs, so that any conclusions regarding the breeding structure of the extended-family colonies is highly tentative.

Analysis of the coefficients of relatedness and *F*-statistics for the reproductives in simple-family colonies in each population indicates that reproductives were highly related in both populations (Kyushu: $r = 0.61$, 95% CIs = 0.13–0.33;

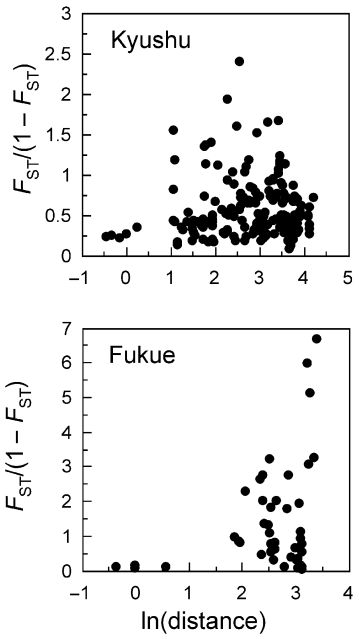

Fig. 2 Isolation by distance analysis for *Coptotermes formosanus* colonies within each of the two study populations. The correlation coefficients were not significant for either of the relationships ($r = 0.01$ and 0.41, $P = 0.47$ and 0.15, Mantel test, for Kyushu and Fukue, respectively).

Fukue: $r = 0.61$; 95% CIs = 0.25–0.98), but significantly more related in the Fukue population ($P < 0.02$, two-tailed *t*-test). The inbreeding coefficients for reproductives within each population (Kyushu: $F_{IT} = 0.131$, 95% CIs = –0.003–0.258; Fukue: $F_{IT} = 0.421$, 95% CIs = 0.089–0.767) were very similar to the values for workers (Table 3).

Isolation by distance

There was no significant isolation by distance within either population (Fig. 2; $r = 0.01$ and 0.41, $P = 0.47$ and 0.15, for Kyushu and Fukue, respectively).

Test for genetic bottleneck

There was no strong evidence that either population has experienced a recent genetic bottleneck. Of the 80 tests performed for each population (20 resampled data sets × two tests × two models for mutation drift equilibrium), there were no cases in the Kyushu population with significant heterozygosity excess. In the Fukue population, there was significant heterozygosity excess in three cases. In all of these cases, it was the Wilcoxon sign-rank test that showed significant heterozygosity excess ($P < 0.05$) using the infinite alleles model of mutation drift equilibrium.

Discussion

These results provide some of the most detailed information to date on the breeding system of *Coptotermes formosanus*. All colonies had genetic structures consistent with being simple (Mendelian) families or being inbred families descended from simple families. Despite the fact that 90% of the colonies were simple families, individuals were highly inbred, suggesting that the reproductive pairs in many of these colonies consisted of individuals that were closely related to each other. This conclusion is supported by the high coefficient of relatedness between reproductives in simple-family colonies based on their inferred genotypes. Such pairing of closely related individuals could arise by one or both of two routes. First, colonies could be headed by primary reproductives that pair with siblings or other close relatives following mating flights. Second, many colonies could be headed by single pairs of neotenic reproductives that are the inbred descendants of the original founding pair. If there were such colonies in the study population, the neotenic pairs must have been in place long enough to have produced all of the workers present at the time of sampling. Without more complete colony census data, it is not possible to distinguish between these possibilities. However, in the one case where the primary reproductive pair was collected, they were as closely related as siblings, demonstrating that pairing of closely related primaries does occur in this population. Nonetheless, the possibility still remains that some of the simple-family colonies were headed by pairs of neotenic reproductives. Interestingly, nestmate reproductives in the Fukue population were on average significantly more related than those in the Kyushu population, either because of a higher frequency of sib-pairings among primary reproductives in Fukue, or because of a greater proportion of colonies headed by monogamous pairs of neotenic reproductives. Unfortunately, it will be difficult to investigate further the types of reproductives in simple-family colonies because reproductives are only rarely encountered because of the cryptic nesting habits of this species.

The higher values for all the *F*-statistics in the extended-family colonies are consistent with these colonies being more inbred than the simple-family colonies, although only the F_{IT} values differed significantly between the two types of colonies, no doubt because of the small number ($n = 3$) of extended-family colonies. An F_{IC} value of close to zero in the extended-family colonies suggests the presence of a high effective number of reproductives (see Table 3, cases B3 and 4), or the presence of spatially separated reproductive centres within colonies (Table 3; case C). There are very few published accounts of the numbers of reproductives collected in colonies of *C. formosanus*, and none for Japan, but what records do exist (reviewed in Myles 1999), indicate that there are often > 15 and as many as 100

neotenics in a single colony. Detailed data from many more colonies are needed for a better understanding of the breeding structure of extended-family colonies in the Kyushu population.

The present results showing that colony members are derived from a small number of closely related reproductives agree with a previous study on the genetic structure of *C. formosanus* in Hawaii. Husseneder & Grace (2001b) used multilocus DNA fingerprinting to investigate the genetic structure of 17 colonies. These authors concluded that colonies were comprised of close family units with moderate levels of inbreeding. However, because of the dominant nature of the multilocus markers used in their study, the authors were not able to determine whether individual colonies were simple families or extended families.

The finding that colonies are sometimes headed by closely related primary reproductives suggests that dispersal by primary reproductives is limited in both populations, but especially so in the Fukue population. If this is the case, one would expect pronounced population viscosity. However, there was no significant isolation by distance among colonies at the spatial scale studied (0.7–70 km) in either population. This could be the result of the occasional transport of infested materials (Ehrhorn 1934; Su & Tamashiro 1987; Jenkins *et al*. 2002) into each of the study populations leading to an infusion of colonies from more distant populations. In studies of introduced populations of *C. formosanus* in New Orleans, LA and Oahu, HI, we also did not find significant isolation by distance among colonies (unpublished data), suggesting a general lack of strong population viscosity in introduced populations of this species. Indeed, given the ease with which colonies are moved by human activity, it would not be surprising if traces of isolation by distance resulting from limited natural dispersal were often erased, even in its native range.

The close genetic affinity among reproductives in simple-family colonies contrasts with the situation in other termites, including other subterranean species. Simple-family colonies of the subterranean species *Reticulitermes flavipes* are headed by outbred primary reproductives in populations in Massachusetts (Bulmer *et al*. 2001) and North Carolina (Vargo 2003). Similarly, Husseneder *et al*. (1999) found that monogamous pairs of reproductives in the African subterranean termite, *Schedorhinotermes lamanianus* are unrelated to each other. The reproductives in simple-family colonies have also been found to be unrelated in other termites, including the termitids, *Nasutitermes nigriceps* (Thompson & Hebert 1998b) and *N. corniger* (Atkinson & Adams 1997), the dampwood termite, *Zootermopsis nevadensis* (Shellman-Reeve 2001), and the dry wood termite *Incisitermes schwarzi* (Luykx 1985). Additional studies are needed in other introduced and native populations of *C. formosanus* to determine if the unusually high relatedness among reproductives in simple-family colonies in Japan is typical of this species or is unique to the study populations.

Although there are only a few estimates for levels of inbreeding in termite populations, the levels found here (F_{IT} = 0.16 and 0.46 for Kyushu and Fukue, respectively) appear to be at the upper end of published reports. This is no doubt the result of the close genetic relatedness among reproductives in the study colonies, possibly combined with several generations of inbreeding in neotenic-headed colonies. Studies of *R. flavipes* reveal a wide range of inbreeding coefficients among different populations. Reilly (1987) estimated F_{IT} = 0.62 in a population in Middle Tennessee, although this value was probably inflated by the uniquely high results given by one of the four loci used in the study. Bulmer *et al*. (2001) reported values of F_{IT} very similar to those found in the present study in two sites in Massachusetts (F_{IT} = 0.34 and 0.27). Unlike the present study population, however, *R. flavipes* colonies in Massachusetts are founded by outbred reproductives, and the high inbreeding coefficients are the result of most colonies being headed by numerous neotenics with several cycles of inbreeding. In another study of *R. flavipes*, Vargo (2003) recently found relatively low levels of inbreeding in central North Carolina (F_{IT} = 0.09), where the frequency of simple-family colonies was much higher than in Massachusetts. In other termites, a range of F_{IT} values has been reported, including F_{IT} = 0.10 in the primitive mastotermitid *Mastotermes darwiniensis* (Goodisman & Crozier 2002), and F_{IT} = 0.04 in the drywood termite *Incisitermes schwarzi* (Luykx 1985). Thus, the level of inbreeding can vary considerably within and among termite species, and this variation may have different causes, including the degree of relatedness among founders, the frequency of neotenic-headed colonies, the numbers of neotenics, and the number of generations of inbreeding among neotenics.

The results suggest moderate levels of genetic differentiation between the two populations (F_{ST} = 0.12 and 0.14 based on genotypes of workers and reproductives, respectively), which were located approximately 100 km apart and separated by water. Alates of this species appear to have limited flight ranges, dispersing less than 0.5 km (Su & Tamashiro 1987). Thus, the populations are much too far apart to expect direct genetic exchange via natural dispersal. The most likely routes of migrants connecting the populations are occasional human-mediated movement of infested material from one population to the other, and /or alate dispersal by a stepping-stone model of gene flow through the smaller islands stretching between Kyushu and Fukue Islands.

The level of differentiation between the two populations studied here is similar to that reported for populations of the Neotropical termitid *Nasutitermes nigriceps* separated 100–200 km apart on the island of Jamaica (F_{ST} = 0.11; Thompson & Hebert 1998b), a spatial scale similar to that

in the present study. Thus, distances of ≥ 100 km over land or water appear to be sufficient for some degree of genetic differentiation among termite populations (see also Goodisman & Crozier 2002).

The tests of Cornuet & Luikart (1996) and Piry *et al*. (1999) did not detect strong evidence of a recent genetic bottleneck in either population, as might be expected given the three centuries or more since *C. formosanus* was introduced to Japan (Mori 1987; Su & Tamashiro 1987; Wang & Grace 1999). The tests for genetic bottlenecks are based on detecting allele deficiency in a population, which in turn is a complex function of time since the bottleneck, the effective size of the colonizing population, the mutation rate of the loci and the number of genes sampled (Cornuet & Luikart 1996). Although the generation time for *C. formosanus* colonies has not been precisely determined, they probably reach maturity in 4–8 years (Su & Tamashiro 1987). If so, 300 years represents some 37.5–75 generations, perhaps a sufficiently large number, along with the high mutation rate of microsatellite loci, to bring the populations into mutation drift equilibrium, thereby eliminating detectable allele deficiencies. Alternatively, the size of the founding population may have been quite large and / or there may have been serial introductions of this species from China or other infested locations. Taken together, the Japanese populations contained 26% fewer alleles than did a native population of *C. formosanus* from Guangdong Province, China (unpublished data) at the six microsatellite loci studied here, a finding consistent with this species having been introduced to Japan from China at some point in the past. Detailed studies of population genetic structure in the native range may help identify potential source populations from which the Japanese populations originated.

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This research is part of a collaborative project on *Coptotermes formosanus*, including studies of variation in breeding systems across native and introduced populations and analysis at the genetic relationships among introduced and native populations. Edward Vargo studies breeding systems, fine-scale and large-scale population genetic structure, and colony spatial organization in subterranean termites. Claudia Husseneder's research program focuses on the molecular biology of termites and biodiversity of their gut microbes Kenneth Crace focuses on termite biology, behavioural ecology, and management.
