

The breeding system and population structure of the termite *Reticulitermes grassei* in Southwestern France

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We assessed colony and population structure in three French populations of the termite *Reticulitermes grassei* using eight polymorphic microsatellite loci. Although most colonies contained the offspring of multiple, highly related replacement reproductives (complex families), some contained the offspring of a single pair of reproductives (simple families), and the proportion of such colonies varied across populations. Populations also showed variability in the numbers of reproductives within complex families; the *F*-statistics of these families in one population were consistent with having upwards of 100 replacement reproductives, while in another population these colonies contained fewer than 10 pairs of reproductives. Colony boundaries in all populations

were well defined, in spite of reports of a widespread breakdown in nestmate recognition and uniclonal populations of *R. grassei* from these regions in France. A second unexpected finding was a lack of significant isolation by distance among colonies within populations, indicating that colony reproduction by budding was rare or absent. The lack of this form of colony reproduction even within populations where it is expected to be common suggested that the propensity for colony budding may not be as common as suggested by the literature.

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Introduction

Detailed knowledge of the genetic structure of populations can yield significant insights into a suite of ecological and evolutionary processes (reviewed in Neigel, 1997; Bossart and Prowell, 1998). For social organisms, genetic structure assumes additional significance not only for the evolution of social behaviour, but also for the evolution of multiple-queen societies, reproductive skew, sex ratio conflict, conflict over growth versus reproduction, and worker reproduction (reviewed in Pamilo *et al.*, 1997; Ross, 2001; Sundström and Boomsma, 2001; Mehdiabadi *et al.*, 2003).

The Hymenoptera, because of their haplodiploid sex determination, formed the bulwark of both Hamilton's (1964) original treatise on kin selection and Trivers and Hare's (1976) seminal work on sex ratio conflict. Therefore, this group has naturally been the focus of most empirical genetic studies on eusocial insect societies (Crozier and Pamilo, 1996). Colony genetic structure in the Hymenoptera exhibits considerably more variation than anticipated (Ross, 2001), and explaining this variation has remained a fundamental goal in the study of these systems (Keller, 1995; Schmid-Hempel and Crozier, 1999; Brown and Schmid-Hempel, 2003). Studies

of genetic structure in the eusocial termites should provide an important foil to much of this work, yet the group has received comparatively little genetic scrutiny. Those studies which have been published confirm some of the expectations of termite colony genetic structure, but also raise new questions. In contrast to the Hymenoptera where supernumerary queens tend to outbreed (Keller, 1995; Ross, 2001), termite colonies headed by multiple same-sex reproductives usually exhibit equal or higher nestmate relatedness to colonies with a single pair of breeders, presumably because new reproductives of both sexes are typically recruited from the offspring of the original king and queen (Reilly, 1987; Luykx, 1993; Bulmer and Traniello, 2002b). However, there are sporadic reports of termite colonies with unexpectedly low nestmate relatedness (Clément, 1981; Jenkins *et al.*, 1999; Goodisman and Crozier, 2002; Bulmer and Traniello, 2002b; DeHeer and Vargo, 2004; Dronnet *et al.*, 2005), and several authors suggested that these have resulted from a breakdown in nestmate recognition. Although relatedness within these colonies is generally still much higher than zero, they still provide an interesting parallel to the existence of highly polygynous 'supercolonies' seen in some ant species, which in some cases have been shown to result from a collapse in recognition ability (Morel *et al.*, 1990; Holway *et al.*, 1998; Giraud *et al.*, 2002; Tsutsui *et al.*, 2003).

The western European termite *Reticulitermes grassei* (= *R. lucifugus grassei*; Clément *et al.*, 2001) is one such species in which both behavioural (Clément, 1986) and genetic data (Clément, 1981) suggested that colonies

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within some geographic regions lack effective nestmate recognition. Work by Clément and colleagues documented geographic variation in several basic elements of colony genetic structure which correlated with variation in the strength of aggression among colonies (Clément, 1981, 1986; Clément and Bagnères, 1998), and in some regions they have been hypothesized to form expansive 'supercolonies' like some invasive ant species (Clément *et al*, 2001). Herein we revisit the colony genetic structure of *R. grassei*, armed with microsatellite markers (Vargo, 2000; Dronnet *et al*, 2004), formal genetic analyses (Weir and Cockerham, 1984; Queller and Goodnight, 1989), and recent simulation models of breeding systems developed specifically for the Isoptera (Thorne *et al*, 1999). Our objective was to obtain a detailed picture of the breeding system of *R. grassei* from several separated populations in southwest France, with specific goals of characterizing geographic variation in breeding system and determining the nature of colony boundaries in these locations. These data allowed us to obtain relevant information on higher level genetic structure among populations of this termite, and also potentially gain insights into mechanisms of new colony propagation and the dispersal potential of winged reproductives. We do note that the taxonomic status of *Reticulitermes* is still in flux (eg, Austin *et al*, 2002), and that *R. grassei* was recently elevated to species status (reviewed in Clément *et al*, 2001). The current work on colony and population genetic structure should thus complement the growing body of work on the life history, systematics, and phylogeography of *Reticulitermes* in Europe (Uva *et al*, 2004; Kutnik *et al*, 2004, in preparation).

Methods

Field collections

During June of 2003 we collected termites from three spatially separated populations (A, B, and C) in southwest France (Figure 1). Population A was located about 5 km west of La Tremblade in La Coubre Forest (In the département of Charente Maritime), Population B was located about 5 km west of Ychoux (Landes), and population C was located about 5 km east of Pissos (Landes). These populations were separated from one another by between 35 and 170 km. Although these populations were somewhat haphazardly distributed across this region, these particular locations had the advantage of having known established termite populations from which we were permitted to collect, and from which preliminary data on genetic structure had been obtained previously (Clément *et al*, 2001; Kutnik *et al*, 2004). Each of these locations consisted of a managed forest, although habitat still varied widely across these populations. Population A was more heavily managed than the other populations and was a relatively open forest with widely spaced mature pines. Population B was a clear cut containing the stumps of mature harvested pines that was bordered by mature forest on all sides. Population C was a young (20–30 years old) stand of closed canopy pine forest in close proximity to several cultivated fields. Within each population, we collected from multiple, spatially separated feeding sites (usually tree stumps), and from each feeding site we obtained at least 40 workers. Although we attempted to

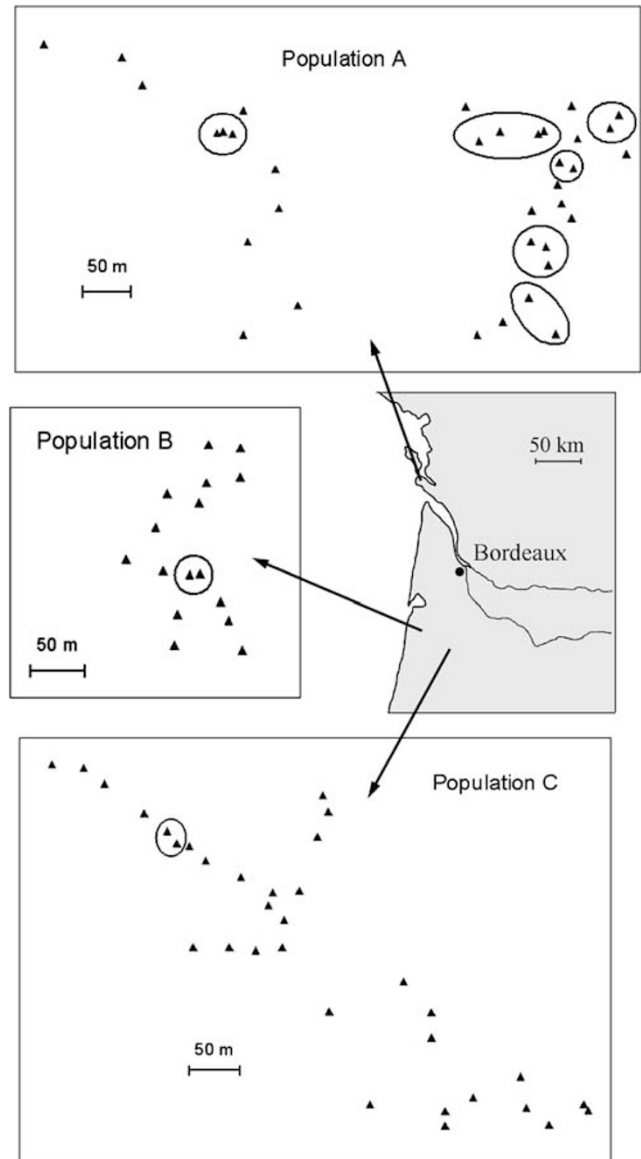


Figure 1 The spatial distribution of collections of *R. grassei* from three populations in southwestern France. Circles surround those collection points, which contained workers belonging to the same colony.

make these collections along a straight line transect, the extreme patchiness of termite feeding locations prevented us from entirely relying upon this more systematic collection method. In order to maximize the numbers of different colonies from which we collected, we typically collected only from those feeding sites which were separated from other such collection points by at least 15 m. In the field we preserved 20–100 termites from each colony in 95% ethanol, and immersed an additional 20 termites in 4 ml of pentane. The former were used for genetic analyses, while the pentane extracts were analysed for their cuticular hydrocarbon profiles. In both cases the samples were stored in the laboratory for approximately 3 months at 4°C until the respective analyses could be performed. We mapped the feeding sites at which we collected termites by measuring the linear distance between consecutively collected locations as well as their angular deviation relative to the magnetic north pole.

Genetic data collection

We isolated DNA from the whole bodies of 20–40 worker termites from each feeding site using the Puregene™ DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN, USA), and resuspended the dried pellet in 300 µl of 1 × TE buffer. We determined the genotypes of these termites ($n = 1940$) at eight polymorphic microsatellite loci. (*Rf6-1*, *Rf15-2*, *Rf21-1*, and *Rf24-2* from Vargo, 2000; *Rs62*, *Rs76*, and *Rs78* from Dronnet *et al*, 2004). The eighth locus, *Rs1* (F 5'-atctctcaagcgaaagtggc-3', R 5'-cgttggagcaacaggac-3'), is a trimeric repeat (GTT) developed for *R. santonensis* simultaneously to those reported in Dronnet *et al* (2004).

Primers *Rf21-1*, *Rf24-2*, and *Rs78* were amplified in a single reaction following the conditions of Vargo (2000), except that the final primer concentrations were 150 µM for *Rf21-1* and 40 µM for both *Rf24-2* and *Rs78*. The remaining loci were amplified in two multiplexed reactions (*Rf6-1* with *Rf15-2* and *Rs1*; *Rs62* with *Rs76*), and both reactions followed Vargo (2000) except for two modifications. The annealing temperature was increased to 57°C in order to eliminate the occurrence of nonsense bands which interfered with scoring, and the primer concentrations were modified in order to equalize the amplification strength of the various loci (*Rf6-1* and *Rs76*: 40 µM, *Rs62*: 50 µM, *Rs1*: 60 µM, *Rf15-2*: 130 µM). PCR products were separated by electrophoresis on 6% polyacrylamide gels run on a Li-Cor 4300 DNA analyzer, and allele sizes determined by comparison to 50–350 bp IRDye700™ or 800 standard (Li-Cor, Inc.).

Data analysis

We first determined the boundaries of colonies within each population by estimating genotypic differentiation between all pairs of feeding sites using Genepop on the web (Raymond and Rousset, 1996). Genepop assessed the significance of differentiation at each locus with a log-likelihood (G)-based exact test, and the overall significance was determined via Fisher's combined probability test. Feeding sites were considered part of different colonies when differentiation between them was significant.

We next obtained estimates of genotypic disequilibrium using Fstat 2.9.3.2 (Goudet, 2001). In order to circumvent possible problems that may arise from the nonindependence of genotypes within colonies, we performed this analysis on 20 resampled datasets, each of which consisted of a single worker randomly drawn from each colony. The overall significance of disequilibrium for each pair of loci within each population was assessed by averaging the *P*-values across all 20 resampled datasets. We also obtained estimates of allele frequencies for each locus using Fstat on these resampled datasets (again, to avoid the problem of nonindependence of genotypes from the same colony), for which we report the average frequency over all data subsets.

We classified colonies into either simple families or complex families, based on their genotype distributions at all eight loci. We defined simple families as those in which the worker genotype distributions did not differ from those expected for a single pair of reproductives. Significant deviation was assessed at each locus with a G-test, and overall significance was determined using Fisher's combined probability test. We defined complex

families as those in which either (1) the workers collectively had too many genotypes at any one locus (> 4) to have a single pair of parents, or (2) the genotype distributions differed significantly from those expected from a single pair of reproductives (as above). Previously we have subdivided complex families into extended-family colonies (those having multiple reproductives which are the descendants of the original founding pair) and mixed-family colonies (those having three or more unrelated reproductives) (DeHeer and Vargo, 2004). However, insufficient marker variability prevented us from differentiating between these two family structures for individual colonies, and we therefore analysed them together here.

We estimated colony level *F*-statistics (Weir and Cockerham, 1984) and nestmate relatedness (Queller and Goodnight, 1989) separately in each population with Fstat 2.9.3.2. Within each population we also estimated these *F*-statistics separately for the simple- and complex-family colonies. Confidence intervals (CI) were generated by bootstrapping over loci, and these results were compared to different models of termite-breeding structure proposed by Thorne *et al* (1999) and Bulmer *et al* (2001). Empirical estimates were considered significantly different from one of the modelled breeding systems when their CI did not overlap the model estimate for at least one of the parameters (F_{IC} , F_{IT} , F_{CT} , or r). We followed Thorne *et al* (1999), for the notation and biological interpretation of the *F*-statistic parameters: F_{IC} is the inbreeding of individuals relative to the colony level, F_{IT} is the inbreeding of individuals relative to the population (and since this is computed independently in each population, this is equivalent to true inbreeding F_{IS}), while F_{CT} represents differentiation among colonies within each population. Statistical differences between *F*-statistic and relatedness point estimates were determined using the permutation method available in Fstat 2.9.3.2.

We determined the likelihood for colony reproduction by budding in each population by assessing isolation by distance among colonies. This was done by examining the correlation between pairwise differentiation (F_{CT}) and the linear distance separating colonies. The significance of this correlation was determined via Mantel tests using the program Fstat. Additionally, we followed the lead of other studies (Bulmer and Traniello, 2002a; DeHeer and Vargo, 2004) by exploring the relationship between colony size and F_{IC} . This colony level inbreeding coefficient is particularly sensitive to the number and distribution of reproductives within colonies: it is strongly negative when there are few reproductives, it approaches zero as the numbers of reproductives increases, but can be considerably greater than zero when a colony contains multiple, independent centres of reproduction (particularly as a result of the mixing of workers between unrelated colonies). If colonies that are more expansive, in fact, do have more reproductives than smaller colonies, we anticipated finding a positive correlation between colony-foraging range and F_{IC} .

We examined possible differentiation among populations by assessing hierarchical genetic structure (Weir, 1996) with the program GDA 1.1 (Lewis and Zaykin, 2001). Significance was determined by bootstrapping over loci, and with this program we also obtained pairwise estimates of differentiation among the three populations.

Results

Collections and identification of samples

We collected termites from 84 different feeding sites spread across all three populations (35, 16, and 33 feeding sites from populations A, B, and C, respectively; see Figure 1). These populations were located in an area where *R. grassei* and *R. santonensis* can sometimes occur in sympatry. Nevertheless, the cuticular hydrocarbon profiles of all samples matched the profiles of *R. grassei* from previous reports (Bagnères *et al*, 1991; Clément *et al*, 2001; Kutnik *et al*, 2004), indicating that this was the only species we obtained in our collections.

Genetic markers

In Table 1 we show the numbers of alleles and the overall expected heterozygosity for the eight loci used in this study. None of the loci showed consistent patterns of genotypic disequilibrium within the three populations used in this study. Out of 4620 possible comparisons for disequilibrium (we tested disequilibrium on each of 20 different datasets consisting of a single worker drawn from each colony), only 67 (1.5%) showed significant departures from equilibrium. Moreover, these appeared to be randomly distributed across possible pairs of loci: the average significance of disequilibrium for any one pair of loci across all 20 datasets was never lower than 0.30.

Colony boundaries

We grouped several sets of feeding sites into the same colony, based upon the exact tests of genotypic differentiation. In populations B and C this was very straightforward: only a single pair of adjacent feeding sites in each population showed no significant differentiation (see Figure 1), while every other pair of sites showed highly significant differentiation ($P < 0.0001$) at two or more loci. The northernmost population (A), on the other hand, showed several ambiguous cases of feeding site differentiation. These arose when delimiting the boundaries of three multisite colonies in population A. One feeding site within these groups of sites was not significantly differentiated from either a second or a third feeding site, but these latter two sites showed slight but significant differentiation from one another at one or two loci. In these cases, we concluded that all three sites were part of a single, spatially structured colony; this would not be unexpected given the complex family structure

(see below) and the large distances over which colonies seemed to forage in this population.

There appeared to be a general difference in the proportion of spatially expansive colonies between the northern population (A) and those further south (B and C; see Table 2). Population A had a greater proportion of colonies occupying multiple feeding sites (seven out of 24 colonies) than did population C (one out of 32 colonies; Fisher's exact test, two-tailed; $P = 0.016$) or the two southern populations (B and C) combined (two out of 47 colonies; Fisher's exact test, two-tailed; $P = 0.006$). Populations A and B (one out of 15 colonies), however, did not differ significantly from one another in the proportion of multisite colonies (Fisher's exact test, two-tailed; $P = 0.121$).

Breeding system and genetic structure

A preliminary analysis indicated that utilizing 20 workers per colony did not provide sufficient statistical power to discriminate simple families from complex families in all cases. Specifically, when the workers in a colony collectively have no more than two alleles at any locus, the expected genotype ratios for the offspring of large numbers of replacement reproductives may be indistinguishable from the expected genotype ratios for a simple family in which both parents are heterozygous at the biallelic loci. Therefore, for colonies with no more than two alleles at any one locus, we genotyped a minimum of 40 workers at all eight loci.

In Table 2 we show the breakdown of colonies into simple and complex families for each site. The sampled colonies in site A did not contain any simple families, and this proportion was significantly lower than the proportion of simple families found either at site B (Fisher's exact test, two-tailed; $P = 0.015$) or at site C (Fisher's exact test, two-tailed; $P < 0.0001$). Although sites B and C exhibited a slight difference in the proportion of simple families, this was not statistically significant (Fisher's exact test, two-tailed; $P = 0.343$).

We failed to detect a strong association between colony-foraging area and colony family structure. None of the simple families ($n = 18$) occupied more than one of the sampled feeding sites, compared to 17% (nine of 53) of the complex family colonies (Fisher's exact test, one-tailed, 0.060). Considering only the complex family colonies, the F_{IC} values for colonies feeding at multiple sampling locations (median 0.015) was not significantly

Table 1 Number of alleles (N_a) and the overall expected heterozygosity (H_e) for the microsatellite loci used in this study

Locus	N_a	H_e
Rf6-1	4	0.26
Rf15-2	2	0.48
Rf21-1	7	0.69
Rf24-2	2	0.48
Rs1	3	0.62
Rs62	2	0.50
Rs76	2	0.38
Rs78	2	0.46
Average	3.0	0.48

Table 2 The numbers of colonies collected in each population, broken down by family structure and whether or not workers were collected from multiple feeding sites

	Single-site colonies	Multiple-site colonies
<i>Population A</i>		
Simple families	0	0
Complex families	17	7
<i>Population B</i>		
Simple families	4	0
Complex families	10	1
<i>Population C</i>		
Simple families	14	0
Complex families	17	1

Table 3 Observed (with 95% CI) and expected colony level F -statistics and nestmate relatedness (r) broken down by population and by family structure

Breeding system	F_{IC}	F_{IT}	F_{CT}	r
<i>Observed</i>				
Pop. A – Complex families ($N=24$)	0.019 (–0.016 to 0.054)	0.294 (0.180–0.408)	0.280 (0.183–0.377)	0.435 (0.321–0.549)
Pop. B – Simple families ($N=4$)	–0.212 (–0.335 to –0.065)	0.330 (0.068–0.545)	0.450 (0.214–0.632)	0.688 (0.393–0.826)
Pop. B – Complex families ($N=11$)	–0.038 (–0.115 to 0.036)	0.297 (0.191–0.375)	0.322 (0.262–0.369)	0.498 (0.434–0.544)
Pop. C – Simple families ($N=14$)	–0.250 (–0.302 to –0.191)	0.080 (0.027–0.136)	0.264 (0.225–0.292)	0.490 (0.431–0.529)
Pop. C – Complex families ($N=18$)	–0.113 (–0.167 to –0.042)	0.312 (0.238–0.394)	0.381 (0.326–0.441)	0.582 (0.519–0.638)
<i>Expected</i>				
a. Primaries, unrelated	–0.34	0.00	0.25	0.50
b. Replacements, $n=1$, $X=1$	–0.14	0.33	0.42	0.62
c. Replacements, $n=1$, $X=3$	–0.22	0.57	0.65	0.82
d. Replacements, $n=10$, $X=3$	–0.02	0.37	0.38	0.56
e. Replacements, $n=100$, $X=3$	–0.00	0.34	0.34	0.51
f. Replacements, $n=10$, $X=3$, cooperatively founded by 3 alates	–0.03	0.27	0.29	0.45
g. Mixing between related colonies, $n=1$, $X=3$, $P=0.90$	0.04	0.66	0.64	0.77
h. Mixing between unrelated colonies, $n=10$, $X=3$, $P=0.80$	0.15	0.37	0.25	0.36

For the simulated breeding systems, n represents the number of pairs of reproductives, X is the number of generations of inbreeding among replacement reproductives, and p represents the proportion of worker mixing between different colonies which have connected tunnel systems.

Table 4 Summary of the breeding systems predominating within each population

	Simple families		Extended families	
	%	Inbred	%	# Reproductives
Population A	0		100	100s
Population B	27	Yes, ~1 generation	73	10s–100s
Population C	44	Sometimes	56	<10

higher than those for complex families collected at only one sampling location (median –0.024) (Kruskal–Wallis test; $P=0.066$).

We give details on the observed and expected (case a–h) colony level F -statistics and nestmate relatedness in Table 3, and we summarize these results in Table 4. Complex families in population A exhibited F -statistics consistent with those expected for colonies having large numbers of replacement reproductives (case e), and differed significantly (based on their 95% CI) from all other breeding systems in at least one of the four genetic parameters (F_{IC} , F_{IT} , F_{CT} , and/or r). Using the same criteria, the complex families in population B differed significantly from all the breeding systems except two (cases e and f), and thus are likely to be headed by large numbers of replacement reproductives (tens to hundreds). In contrast, the genetic parameters for the complex families in population C were suggestive of smaller numbers of reproductives (case b); the average F -statistics therein differed significantly in at least one parameter from every other modelled breeding system. Moreover, when directly compared to population A, complex families in population C exhibited significantly more negative F_{IC} ($P=0.012$), and significantly higher F_{CT} and r ($P=0.012$ and 0.0001, respectively), both of which are expected for colonies with fewer reproductives.

The simple families in population B, although few in number, exhibited average F -statistics consistent with having a single pair of related reproductives (case b). In spite of their substantial levels of inbreeding ($F_{IT}=0.33$) not all these simple families were necessarily headed by a pair of replacement reproductives, because in one of these colonies we collected a pair of colony-founding primary reproductives. The simple families in population C did not have F -statistics consistent with any one breeding system; this situation is likely to arise when colonies are represented by a mixture of breeding systems, with no single type of colony predominating.

We detected no significant correlation between geographic distance and genetic differentiation among colonies within any of the three populations. Population B showed the strongest trend for isolation by distance but even here, the proportion of explained variation was low and only marginally significant ($R^2=3.2$, $P=0.07$). Thus, colonies were no more likely to be related to their neighbours than to more distant colonies, at least over the scale of several hundred meters.

Higher-level structure at the scale of many km, however, was significant. Among-population F_{ST} was 0.105 and significantly greater than zero (95% CI: 0.061–0.140). Most of this among-population differentiation appeared to be due to differences between population A and the two southern populations. Pairwise measures of F_{ST} were significant between A and B and between A and C (0.137 and 0.139, respectively), but negligible between populations B and C (–0.008).

Discussion

We found extensive within- and among-population variation in colony genetic structure among these populations of *R. grassei* (summarized in Table 4). One source of variation was that populations differed in the proportion of colonies with a single pair of reproductives versus those with more than a single king and queen.

Colonies in population A were all complex families, while both populations B and C had a significantly greater proportion of simple family colonies (26.6 and 43.8%, respectively). A second source of variation arose from differences among populations in the numbers of reproductives within the complex family colonies. In populations A and B these colonies exhibited F -statistics consistent with having tens or hundreds of pairs of replacement reproductives, whereas those in population C appeared to contain far fewer (more than one pair but fewer than 10 pairs).

Simple-family colonies in both populations in which they occurred were significantly inbred. In population B the inbreeding coefficient (F_{IT}) was consistent with these colonies being headed by either first-generation replacement reproductives or by primary reproductives which were close relatives. However, the F_{IT} of simple-family colonies in population C was lower than expected for closely related pairs of reproductives, while still being significantly inbred. This intermediate level of inbreeding for the latter population suggests that these simple-family colonies were a mixture of colonies whose workers were the offspring of either unrelated or related reproductive pairs.

The simultaneous occurrence of both simple and complex families in one population is not particularly remarkable. This was expected from their life history (Roisin, 1993; Thorne *et al*, 1999), has already been shown across regional scales in this species (Clément, 1981), and has been described in several other termite species (Thompson and Hebert, 1998; Bulmer *et al*, 2001; Goodisman and Crozier, 2002; Vargo, 2003a; DeHeer and Vargo, 2004). The underlying causes of this variation remain unclear. In the case of the current study, it may be that the differences in habitat across populations could affect the breeding system of termite colonies, for example by altering the age structure of colonies within populations. The fact that the youngest forest also contained colonies with fewer replacement reproductives than other populations might suggest that there is a correlation between colony age (or size) and forest age (or food abundance), but without greater numbers of populations we cannot formally test this association. We do note, however, that such interpopulation variability in family structure is not a universal feature in termites, as some studies have documented very little variation across broad geographic scales (Dronnet *et al*, 2005).

Nevertheless, in spite of some similarities, the current study differs markedly from some previous work in its assessment of patterns of colony reproduction in subterranean termites. The ability of subterranean termites like *Reticulitermes* to generate replacement reproductives with relative ease has often been cited as a characteristic conducive to colony reproduction by budding, whereby workers and replacement reproductives initiate new colonies in close proximity to their natal nest (Myles and Nutting, 1988; Thorne *et al*, 1999). However, we found no evidence for genetic isolation by distance among colonies within populations, and therefore conclude that colony reproduction by budding was relatively rare in the populations that we studied. Previous work on *R. flavipes* has also failed to find this footprint of colony budding (Vargo, 2003b; DeHeer and Vargo, 2004), but this could be because populations of this latter species are populated by colonies initiated by

primary reproductives, which initiate colonies after dispersal flights. The same caveat, however, cannot be made for the current results in *R. grassei*.

Lastly, we can draw some conclusions regarding the dispersal of colony-founding kings and queens based on the analyses of structure within and among populations. The lack of isolation by distance among colonies suggests that most new colonies are initiated by winged kings and queens during swarms, and that the dispersal of those individuals that successfully initiate new colonies is typically greater than the distance separating colonies. Across larger spatial scales, on the other hand, we did find significant structure. Populations showed significant differentiation from one another ($F_{ST} = 0.105$), most of which resulted from differences between the northern population, A, and the two southern populations. Although the genetic and physical distances separating these three populations are suggestive of isolation by distance over large spatial scales (with the greatest differentiation between pairs of populations separated by the greatest distances), we had insufficient numbers of populations to test this rigorously.

We note that the colony genetic structure, which we describe here, is quite different from that described by Clément for *R. grassei* from this region in France (Clément, 1981; reviewed in Clément *et al*, 2001). First, Clément described the population of *R. grassei* from the pine forest in La Coubre (the same location as our population A) as consisting of mostly (82%) simple-family colonies, whereas we found that all the colonies from this forest were extended families. Second, more recent studies by Clément and colleagues (Clément, 1986; Clément *et al*, 2001) have combined observations on worker aggression and the previous genetic work (Clément, 1981). They hypothesize that colonies in the region south of La Coubre (encompassing our populations B and C) are 'open societies' which exchange both reproductives and workers during the summer months when aggression is on average low, and that this entire region should be considered one extensive supercolony (Clément *et al*, 2001). In contrast, our results here demonstrate that open societies are at best rare in the populations we have studied: the majority of collection points were strongly differentiated from one another and average nestmate relatedness (r) was not significantly lower than 0.50.

The differences between the current study and that of Clément (1981, 1986) could reflect a genuine change in colony genetic structure that has occurred over the last 25 years. If, for example, the colonization and ageing of termite colonies parallels that of the forest in which they reside, the termite colonies may have grown and replaced their founding kings and queens with numerous secondary reproductives within the last 25 years. Although this hypothesis is at least plausible for population A, which consisted of mostly older forest, it cannot explain the putative reversion within populations B and C from unicolonial populations into those consisting of distinct, multiple colonies. Moreover, it is much simpler to argue that the observed differences have resulted because Clément (1981) based most of his conclusions on only two allozyme loci, and also did so without the aid of modern techniques in the analysis of colony genetic structure (eg, Thorne *et al*, 1999). Even with eight polymorphic microsatellite loci, we could not

always differentiate between simple and extended families until we had analysed at least 40 workers and, on average, we could differentiate between any two colonies at only three of our eight loci. Lastly, subsequent behavioural work has in fact found a more complicated pattern of among-colony aggression than did Clément (1986), suggesting that these previous conclusions about colony boundaries do not apply to this entire region. Working only a short time after this original work, Bagnères (1989) found three distinct differences: (1) that colonies were generally more aggressive towards one another than reported by Clément (1986), (2) that this aggression exhibited extensive between-colony variation (half of the pairwise interactions were highly aggressive while the other half showed intermediate to low aggression), and (3) that aggression did not vary in a seasonal pattern.

Detailed studies of colony and population structure in the Isoptera should continue to yield important insights into the sociogenetic organization of these societies. We have demonstrated here that the number of reproductives and their relatedness to one another show substantial within- and among-population variability in *R. grassei*. Nevertheless, the high genetic relatedness within colonies indicates that colony boundaries can remain distinct even when nestmate recognition appears weak or variable when assayed in the laboratory (see also Bulmer and Traniello, 2002b; DeHeer and Vargo, 2004). These patterns provide an interesting contrast to the breakdown in nestmate recognition which correlates with the near-zero nestmate relatedness (Ross, 1993) or even unicoloniality seen in some hymenopteran systems (Holway et al, 1998; Giraud et al, 2002). Our lack of support for colony reproduction via budding was also somewhat unexpected, and also contrasts with what has been described in some highly polygynous ant societies (Chapuisat et al, 1997; Pirk et al, 2001; Fournier et al, 2002). This could reflect a greater ongoing connectivity between daughter nests in subterranean termites when compared to polygynous ants, although more studies are clearly needed to assess the generality of this pattern.

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