

# Biology of Subterranean Termites: Insights from Molecular Studies of *Reticulitermes* and *Coptotermes*

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## Key Words

Rhinotermitidae, population genetics, molecular ecology, microsatellites, caste determination, breeding structure

## Abstract

Molecular genetic techniques have made contributions to studies on subterranean termites at all levels of biological organization. Most of this work has focused on *Reticulitermes* and *Coptotermes*, two ecologically and economically important genera. DNA sequence data have significantly improved our understanding of the systematics and taxonomy of these genera. Techniques of molecular biology have provided important new insights into the process of caste differentiation. Population genetic markers, primarily microsatellites, have furthered our understanding of the life history, population biology, community ecology, and invasion biology of subterranean termites. Recent results on the behavioral ecology of subterranean termites reveal a picture different from long-held views, especially those concerning colony breeding structures and foraging ranges. As additional molecular tools and genomic resources become available, and as more subterranean termite researchers incorporate molecular techniques into their approaches, we can expect accelerating advances in all aspects of the biology of this group.

## INTRODUCTION

Subterranean termites (Rhinotermitidae) are a large and important group of social insects. They are the most widely distributed family of termites, occurring throughout the tropical, subtropical, and temperate regions of the world (32). They are especially abundant in temperate areas, where their biomass can approach that of many tropical termites (15, 39). In addition, they frequently attack human-made structures, exerting a major economic impact estimated to be as high as \$11 billion per year in damage and control costs in the United States alone (119).

Subterranean termites occupy an important evolutionary position within the Isoptera. Termites are typically divided into the higher termites (Termitidae), containing some 80% of all species, and the lower termites, represented by the remaining six families. Of the lower termites, the Rhinotermitidae are the most derived (59). *Reticulitermes* and *Coptotermes* are especially important as transitional taxa between the lower and higher termites for three reasons. First, new phylogenetic analyses (5, 59, 75) show that the clade containing these genera is likely the sister group of the Termitidae, indicating that *Reticulitermes* and *Coptotermes* share an especially close affinity with the higher termites. Second, these two genera contain more species than any other genus of subterranean termites and are among the most species-rich genera of all the lower termites (63). Third, they exhibit features intermediate between lower and higher termites (59), such as feeding habits typical of lower termites, nesting habits intermediate between the single-site nesting of more basal lower termites (in which colonies use a single piece of wood as both nesting site and food source) (116) and the central-site nesting of higher termites (in which colonies use multiple sources of food away from the nest site) (116), as well as the presence of a true worker caste, a trait primarily associated with higher termites. Thus, understanding basic features of the life history, behavior, and ecology of *Reticulitermes* and *Coptotermes* can provide insights into the evolution and remarkable radiation of the higher termites.

Termites have attracted increasing attention from entomologists. This is especially true of subterranean termites, where a search of the worldwide literature shows that between 2000 and the date of this review there have been more papers published on this group (934) than during all of the previous century (694). Although aspects of the biology of this group have been the subjects of many excellent reviews, these have tended to be regionally and/or taxonomically limited in nature. Some more recent reviews of this group include summaries of the biology of *Reticulitermes* spp. (130) and *Coptotermes formosanus* (122, 142), the life histories of termites in general (116), the evolution and development of termite castes (109), and the gut symbionts of wood-feeding termites (16).

Molecular genetic methods are providing exceptional new insights into the biology of subterranean termites. In addition to elucidating such basic processes as development and caste differentiation, molecular techniques give us a window into the breeding structure, as well as colony and population dynamics, that has remained elusive owing to the cryptic nesting and foraging habits characteristic of these species. Here, we review some of the progress that has been made using molecular methods in the areas of taxonomy, caste differentiation, breeding structure, behavioral biology, and community ecology. We focus on *Reticulitermes* and *Coptotermes* because these genera have been the subject of more than three-quarters of these studies, and because these genera contain the most economically important termites in many parts of the world, especially temperate and subtropical regions.

## SYSTEMATICS AND PHYLOGEOGRAPHY OF *RETICULITERMES* AND *COPTOTERMES*

### Taxonomy

Subterranean termites pose many taxonomic challenges at the species level and above. The taxonomy of both *Reticulitermes* and *Coptotermes*

**Table 1** Species status of *Reticulitermes* in North America

Species	Distribution	Status	Reference(s)
<i>R. flavipes</i>	Throughout eastern and central United States	Valid	(11)
<i>R. arenincola</i>	Sandy soils near the Great Lakes	<i>Nomen dubium</i>	(4)
<i>R. virginicus</i>	Throughout eastern and central United States	Valid	(4)
<i>R. hageni</i>	Throughout eastern and central United States	Valid, but may be species complex	(44)
<i>R. malletei</i>	Eastern United States	Valid	(11)
<i>R. tibialis</i>	Western and Midwestern United States	Valid, but may be species complex	(24, 44)
<i>R. hesperus</i>	Western United States	Valid, but may be species complex	(24, 44)
<i>R. okanaganensis</i>	Pacific Northwest	Valid	(124)

is far from settled. At the present time, these two genera are by far the largest within the Rhinotermitidae, with 75 and 71 described species, respectively, accounting for nearly half of all subterranean termite species among the 15 recognized genera (63). The large number of species in these two genera is due largely to a plethora of new species descriptions that have appeared in China over the past 60 years (31). These genera are in need of careful monographic revisions, with particular emphasis on Oriental forms.

Existing taxonomic keys are spotty in coverage, both in terms of taxonomic breadth and geographic region, and the characters used to distinguish species are often too variable to provide reliable determinations (125). The recent application of molecular genetic data, especially in combination with cuticular hydrocarbon composition, morphological characters, and flight phenologies, has helped clarify the taxonomy of these genera.

The past five years have seen significant changes in the taxonomy of *Reticulitermes* in the United States, where there are currently seven recognized species (Table 1). On the basis of primarily molecular data, these changes include the probable synonymy of one species (*R. arenincola* is considered to be *R. flavipes*) (4) and the addition of two new species: *R. malletei* (4) in the eastern United States and *R. okanaganensis* in the Pacific Northwest (124). From all the available evidence, there are likely other undescribed species, especially within *R. tibialis* and *R. hageni*, both of which appear to be species complexes (24, 44).

Compared to the still unsettled situation in the United States, the taxonomic status of *Reticulitermes* seems well resolved in Europe, where there are currently seven recognized taxa: *R. balkanensis*, *R. grassei*, *R. banyulensis*, *R. urbis*, *R. lucifugus lucifugus*, *R. l. corsicus*, and the introduced *R. flavipes* (= *R. santonensis*) (22, 134). These taxonomic designations have been supported by a number of studies using DNA sequence data (13, 81, 82, 134).

What little taxonomic work has been done on *Coptotermes* suggests this genus is in serious need of revision. The widespread and destructive *C. gestroi* was apparently described as several different species that have recently been synonymized (66, 144), including *C. havilandii* and *C. vastator*, previously recognized as invasive pest species. In Australia, where there are six currently described *Coptotermes* species, there are likely several more unrecognized species (74). We can certainly expect to see many taxonomic changes in this genus in the future as greater attention is given to this widespread and economically important taxon.

### Molecular Tools for Species Identification

The application of molecular genetic techniques to clarify species relationships has provided PCR-based tools for species identification. PCR-restriction fragment length polymorphism methods have been developed for distinguishing among *Reticulitermes* species in the south-central United States (125) and for differentiating *C. formosanus* from other species

of *Coptotermes* (126). The advantages of such diagnostic methods are (a) they remove much of the ambiguity of identification based on morphological keys, (b) they can be used with any caste or developmental stage, and (c) they can be performed on a single individual. These techniques have allowed for more extensive studies of species ranges, for detection of species introduced into locations outside their native ranges, and for the determination of the relative abundance of species in particular geographic areas (7–10, 125). For example, the relative abundance of *Reticulitermes* species in the eastern and central United States has received much recent attention. Three species, *R. flavipes*, *R. virginicus*, and *R. bageni*, are sympatric over much of this region (118). In addition, they often occur together with *R. mallei* (4) in the eastern United States, *R. tibialis* in the central United States, and the introduced *C. formosanus* throughout much of the southern portions of their ranges (120). Studies of samples collected across the range (7–9, 27, 92, 101) show varying species compositions, with *R. flavipes* occurring most commonly, ranging from 74% to 90% of all samples, and *R. virginicus* and *R. bageni* present at much lower frequencies. The one clear exception to this pattern was a South Carolina coastal site, where *R. bageni* was the most common species, occurring at a slightly higher frequency (43%) than *R. flavipes* (37%) (141).

Little is known about the determinants of species diversity in subterranean termite communities, or the factors that contribute to coexistence of species that are apparently so similar in their ecological roles and life histories. Future studies of relative species abundance, combined with population genetic characteristics such as gene flow and dispersal, will provide insights into both environmental factors and population processes that influence species richness in subterranean termite communities.

### Phylogeography and Diversification

Phylogeography, the study of historical processes and their effects on species distributions

(14), has great potential to elucidate the evolutionary relationships among subterranean termite taxa, the processes leading to speciation, and the factors determining current distributions. So far only *Reticulitermes* spp. in southern Europe and the Middle East have received attention in this regard (6, 20, 22, 68, 80–82, 85). Results of these studies suggest that there were four refugia scattered through southern Europe and the Middle East during the last glacial maximum, each harboring one or more species or subspecies. The northward expansion and radiation of populations from these refugia fit reasonably well with the current distributions of species and subspecies throughout southern Europe, especially if one assumes that the rate of mitochondrial DNA evolution in this group has occurred at 10 times the rate normally assumed for insects, as appears to be the case (80). Our understanding of the taxonomy and evolutionary history of the *Reticulitermes* spp. would benefit from similar analyses in other parts of the world.

## DEVELOPMENT AND CASTE DIFFERENTIATION

### Castes

Termites are unique among the social insects because they undergo incomplete metamorphosis and display a remarkably complex and diversified caste polyphenism (109). Within each mature colony, morphologically differentiated castes (workers, soldiers, reproductives) and undifferentiated immatures cooperate in a highly integrated manner. This functional network of behaviorally and morphologically specialized individuals is the cornerstone of the advanced eusociality characteristic of termites.

The terminology regarding caste, especially in the lower termites, is complicated and somewhat controversial. Here we follow the nomenclature of Thorne (128). There appears to be considerable variation in the developmental pathways within the large and diverse family Rhinotermitidae (109), but *Reticulitermes* and *Coptotermes* share many similarities in their

caste patterns. In principle, larvae develop into workers or nymphs. Nymphs develop either into alates with wings and eyes (imagoes) that disperse and become primary colony founders, or they develop into brachypterous (second form) neotenic reproductives with rudimentary wings and no eyes that do not disperse but supplement or replace the reproductives within the colony. Workers can transform into apterous (third form) neotenic reproductives with no wings and eyes, remain workers, or become presoldiers that molt into soldiers. Diagrams depicting the various developmental pathways within colonies of subterranean termites have been presented for *Reticulitermes* spp. (70, 145), *C. formosanus* (106), and *C. lacteus* (110).

As with other types of polyphenisms, caste determination is the result of the interaction of endogenous and exogenous factors during critical stages in development (96). Although little progress has been made in identifying exogenous factors involved in caste determination in termites, morphogenic hormones, primarily juvenile hormone (JH), play a pivotal role in caste determination (46, 96). With the new tools of molecular biology, such as expressed sequence tag (EST) libraries, DNA arrays, quantitative PCR, and gene silencing via RNA interference, termite researchers are now able to investigate the molecular mechanisms modulating the action of JH, as well as elucidate the downstream effects of JH and the genomic network under its control. In addition, molecular studies are providing important new insights into gene expression levels associated with caste-specific morphogenesis in general.

### Soldier Differentiation

A number of studies have shown that in both *Reticulitermes* and *Coptotermes* relatively high JH levels in workers will induce them to molt into soldiers (33, 84, 100, 113). On the basis of gene expression profiles and gene silencing experiments (114, 115, 145–147), Zhou et al. (147) proposed that hexameric proteins play a central role in regulating soldier caste determination by modulating the availability of JH in

the hemolymph. A first approach to determine how JH action modulates expression of caste-specific genotypes is to identify genes whose expression levels change in response to JH action during caste differentiation. Zhou et al. (147) identified various genes associated with morphogenesis that were up- or downregulated in response to silencing of a hexamerin gene, including genes involved in signal transduction, transcription, translation, and cytoskeletal structure. These genes are likely part of the hexamerin-controlled JH-dependent gene network that regulates soldier differentiation.

Zhou et al. (147) proposed a model in which JH production is influenced by extrinsic and intrinsic factors. According to the model, various intrinsic factors modulate hexamerin levels that in turn attenuate the effects of JH. The possible extrinsic factors affecting JH production include both environmental and social stimuli. Intrinsic factors that may affect JH production and/or hexamerin levels are nutritional status, allatostatins (143), sex, and developmental stage. The link between caste differentiation and nutritional status is especially intriguing given the role of hexamerins as both storage proteins and putative JH binding proteins that may regulate caste polyphenism in the eusocial wasp *Polistes metricus* (49), suggesting a widespread role for hexamerins in social insect caste determination.

### Differentiation of the Reproductive Caste

Although the process of reproductive caste differentiation, either in the form of imagoes or neotenics, has received less attention than soldier determination, it is likely that many of the same processes are involved (96). Elliott & Stay (33) found elevated JH titers in the differentiation of both soldiers and apterous neotenics from workers in *R. flavipes*, but individuals apparently destined to become soldiers had higher JH levels than those developing into neotenics. Although both apterous and brachypterous neotenics readily develop in colonies of *Reticulitermes* lacking functional reproductives

(93, 102, 103), neotenic castes are not formed in laboratory colonies of *C. formosanus* devoid of active reproductives (106). Raina et al. (106) have proposed that nymph development and subsequent neotenic differentiation in this latter species require a yet undetermined nymph induction factor produced by reproductives.

To shed light on the genomic network involved in differentiation of the reproductive caste, Scharf et al. (115) identified genes that were differentially expressed in nymphs and reproductives. A gene coding for a hexamerin protein showed highest expression in nymphs and neotenic reproductives and is thus assumed to play a role in reproductive differentiation (115), possibly by modulating the availability of JH in the hemolymph. Following the general model of insect development, low JH titers during critical JH-sensitive periods in development almost certainly regulate differentiation into reproductives in subterranean termites, although the number and timing of these critical periods are likely to differ between development into primary reproductives and development into neotenic castes (96).

### Genetic Caste Determination

Differentiation into primary reproductives in *R. speratus*, at least in the laboratory, may have a sex-linked genetic component (45). According to a proposed model, the production of primary reproductives occurs exclusively in colonies headed by neotenic castes, but so far this has not been confirmed in field colonies of *R. speratus* or other *Reticulitermes* spp. Existing data on *R. flavipes* and *R. virginicus* do not support genetic caste determination as an important mechanism regulating alate production in these species, because alates are not produced mainly or exclusively by neotenic-headed colonies in the field (17, 28). Although the possibility of genetic caste determination in subterranean termites is intriguing, additional studies are needed to confirm that this occurs under field conditions.

Although we still have a very rudimentary understanding of the genetic and physiological mechanisms influencing development and caste

determination in subterranean termites, there have been several important and promising advances in this field. It is clear that the application of molecular tools together with reliable bioassays have shed new light on the processes regulating caste polyphenism.

### ALATE DISPERSAL AND COLONY FOUNDATION

In general, colonies of subterranean termites are founded by monogamous pairs of reproductives following large synchronous mating flights that occur in response to climatic conditions during species-specific times of the year (61, 98). After the mating flight, individuals land on the ground, shed their wings, and begin the process of searching for partners. Partner-finding in some subterranean termite species, including *Reticulitermes* spp., is facilitated by a sex pheromone emitted by the female, whereas in other species, such as *C. formosanus*, females do not appear to produce a chemical attractant (99, 107). After shedding their wings, partners run in tandem with the female in the lead. As soon as a suitable nest site is found, pairs move underground or into wood to mate and reproduce. There is high mortality and thus intense selection during the founding phase.

### Partner Selection

There are a number of reasons to believe that both male and female termites should be selective in their choice of mates. First, termites mate for life and perform intensive biparental care. Second, large body size and/or weight can be advantageous in mate selection, because the first generation of larvae is entirely dependent on the fat reserves of the founding pair until foragers emerge to provide the young colony with nutrition (67, 88, 117). Third, genetic characteristics of potential partners, such as genetic diversity and relatedness, may influence colony fitness. For example, males of *C. formosanus* are more likely to pair with females exhibiting higher levels of heterozygosity (55). However, tandem pair formation in *C. formosanus* appears

to be random with respect to kinship (55), as was reported for the Japanese subterranean termite, *R. kanmonensis* (67), and *R. flavipes* (28). The lack of evidence supporting kin discrimination during colony founding in subterranean termites is surprising considering the mounting evidence of negative effects of inbreeding in many plants and animals (64) and in subterranean termites (28, 36, 54), but data on more species, especially those that undergo short-range dispersal flights, are needed before we can rule this out as a mechanism promoting outbreeding in this group.

### Dispersal Distances

One possible mechanism that could promote outbreeding is long-range dispersal of alates reducing the likelihood that nestmates encounter each other during tandem pair formation. Flight distances appear to vary among species of subterranean termites, ranging from a few meters to 1 km or further, and likely have been underestimated in some cases (61, 98). Genetic studies of alates from mass swarms of *C. formosanus* in New Orleans, Louisiana, suggest alates can fly at least 1 km (57). Dispersal distance in this species is sufficient to guarantee mixing of up to 29 colonies within a swarm aggregation in areas of high population density; under conditions of random mating up to 90% of alate pairings would be among non-nestmates (57).

Studies of gene flow in *Reticulitermes* suggest that alate dispersal distances can be sufficient to promote outbreeding in some species, e.g., *R. virginicus* (28), yet insufficient in others, leading to the probable pairing of related primary reproductives during colony founding, e.g., *R. bageni* (138, 141). In a study of *R. flavipes*, some 20% of founding pairs were composed of likely siblings, but few of these successfully established colonies, presumably because of inbreeding depression during colony foundation (28). The apparent variation in the tolerance to the potential effects of inbreeding in closely related sympatric species merits further investigation.

### Sex-Biased Alate Production by Colonies

Another potential mechanism promoting outbreeding is sex-biased alate production by colonies, in which individual colonies invest predominantly in alates of one sex (61). Such bias may occur as a function of colony breeding structure. Experimentally orphaned colonies of Australian *Coptotermes* spp. that were subsequently headed by inbreeding neotenics produced almost exclusively males (72, 111). Genetic analysis of swarming alates of *C. formosanus* found that male alates in most swarm aggregations were significantly more inbred than females (57), a finding consistent with predominant male production in neotenic-headed (inbred) colonies. One factor promoting female alate production in outbred colonies in this species could be sexual selection favoring heterozygous females during mate selection (55), as mentioned above. In *R. virginicus*, DeHeer and Vargo (28) inferred that female alates were produced primarily in inbred colonies on the basis of lower levels of heterozygosity compared to males. The extent of sex-biased alate production and its possible role in promoting outbreeding in subterranean termites deserve further study.

### COLONY DEVELOPMENT AND BREEDING STRUCTURE

After establishment by the founding pair, colony growth is initially slow, reaching a population size after one year of about 30 to 50 individuals in the case of *Reticulitermes* spp. (130) and 20 to 90 in *C. formosanus* (107, 122). During this initial phase, the colony is a simple family composed of a monogamous pair of reproductives and their offspring. Eventually one or both of the primary reproductives senesces or dies, and these are replaced by neotenics, either apterous or, more commonly, brachypterous forms that develop from within the colony (95, 130), producing an extended family colony. The number of neotenics in these extended family colonies can vary from a few to several

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**Colony breeding structure:** number and degree of relatedness of the reproductive individuals within colonies

**Simple family colony:** group of cohabiting individuals produced by a monogamous pair of reproductives, usually the primary founders

**Extended family colony:** group of cohabiting individuals produced by multiple inbred neotenic reproductives descended from the original founding pair

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dozen, and these colonies can undergo several generations of inbreeding. In addition to simple family and extended family colonies, it has been assumed that colonies often fuse to create genetically complex groups consisting of mixed families (21, 87, 132).

Owing to the cryptic nesting habits of this group, it has been difficult to conduct extensive studies of colony breeding structure. However, this has changed with the application of highly sensitive molecular genetic markers (58), combined with population genetic modeling of subterranean termite breeding systems (17, 132). The main approach to investigating colony breeding structure is to genotype groups of foragers at numerous genetic loci using codominant markers, such as microsatellites (27, 58, 136). The genotypes of the individuals are then subjected to pedigree analysis to determine family structure.

Once the family structure of a colony is determined, more detailed information about the relatedness of reproductives, degree of inbreeding within the colony, and the numbers of reproductives within extended family colonies can be inferred from the coefficients of inbreeding and relatedness (17, 132). There is significant variation in all aspects of colony breeding structure both within and among species (**Table 2**). In most cases, colonies are either simple families or extended families, with simple families more common in many populations.

### Simple Family Colonies

Colony breeding structure has now been characterized in populations of six species of *Reticulitermes*, with four species represented by multiple populations (**Table 2**). In most species and most populations, the majority of colonies—often 75% or more—are simple families. One major exception is the European species *R. grassei*, in which simple families comprised a minority of colonies in all four populations studied, with one population containing no simple families at all. The other conspicuous exceptions are (*a*) populations of *R. flavipes* in Massachusetts, at the northern edge of the range of this species

where only about 25% of the colonies were simple families, and (*b*) populations of *R. flavipes* in France, where this species was introduced and no simple families have been found.

There is both inter- and intraspecific variation in the degree to which the kings and queens heading simple family colonies are related. Colonies of *R. flavipes*, *R. virginicus*, and *R. hesperus* appear to be headed by unrelated reproductives, whereas the reproductives in simple family colonies of *R. hageni* and *R. mallei* appear to be related (101, 138, 141), most likely because primary reproductives often pair with relatives during colony foundation (138). The European species *R. grassei* exhibits variation among populations, with closely related reproductives in one French population and largely unrelated reproductives in a Portuguese population (**Table 2**).

Studies of *Coptotermes* have largely concerned introduced populations of *C. formosanus*, in which considerable variation in colony breeding structure has been found (**Table 2**). The proportion of simple families varies from nearly 100% in two Japanese populations (139) to no simple families present in a population from the native range in southern China (54). However, in six of nine *C. formosanus* populations studied to date, simple families were more common than extended families. The degree of relatedness between the kings and queens heading simple families varies from essentially zero in New Orleans to full siblings ( $r = 0.6$ ) in one Japanese population (53, 139, 140). In the only other *Coptotermes* species studied to date, the Australian mound-building *C. lacteus*, all 39 colonies examined genetically (127) were simple families headed by slightly related reproductives.

### Extended Family Colonies

Extended families can vary both in the numbers of neotenics present, from a few to dozens, and in the number of generations of inbreeding they have undergone. Of particular importance in inferring details regarding the breeding structure of extended families is the coefficient of

**Table 2** Summary of colony breeding structures in *Reticulitermes* spp. and *Coptotermes* spp. termites as inferred by microsatellite markers except where noted

Species/Population ( <i>N</i> = no. colonies)	Simple families		Extended families		Mixed families		Overall <i>F<sub>IT</sub></i>	Reference(s)
	Percent	Reproductives related <sup>a</sup>	Percent	<i>F<sub>IC</sub></i> <sup>b</sup>	Inferred no. neotenic	Percent		
<i>Reticulitermes flavipes</i>								
Central North Carolina <i>N</i> = 319	78.4%	–	19.7%	–0.209	<10	1.9%	0.052	(27, 101, 135, 136, 138)
Charleston, South Carolina <i>N</i> = 18	72.2%	–	22.2%	–0.140	<10	5.6%	0.030	(141)
Eastern Massachusetts <sup>c</sup> <i>N</i> = 22	27.3%	–	59.1%	0.097	>100	13.6%	0.289	(17)
Central Tennessee <sup>c</sup> <i>N</i> = 48	NR	NR	NR	0.260 <sup>d</sup>	NR	NR	0.680 <sup>a</sup>	(108, 132)
Paris, France <sup>d</sup> <i>N</i> = 12	0%	N/A	100%	0.032	>100	0%	0.386	(30)
Oléron Island, France <sup>e</sup> <i>N</i> = 14	0%	N/A	100%	–0.001	>100	0%	0.168	(30)
<i>Reticulitermes hageni</i>								
Raleigh, North Carolina <i>N</i> = 15	86.7%	++	13.3%	–0.257	<10	0%	0.357	(101, 138)
Charleston, South Carolina <i>N</i> = 21	95.2%	+	4.8%	N/A	<10	0%	0.140	(141)
<i>Reticulitermes malleet<sup>f</sup></i>								
Duke Forest, North Carolina <i>N</i> = 13	53.8%	+	46.2%	–0.257	<10	0%	0.190	(138)
<i>Reticulitermes virginicus</i>								
Raleigh, North Carolina <i>N</i> = 8	75.0%	–	25.0%	–0.332	<10	0%	0.037	(101, 135)
Charleston, South Carolina <i>N</i> = 4	100%	–	0%	N/A	N/A	0%	–0.04	(141)
<i>Reticulitermes hesperus</i>								
Northern California <i>N</i> = 30	73.3%	–	26.7%	–0.185	<10	0%	0.081	(23)
<i>Reticulitermes grassei</i>								
Southwestern France, population A <i>N</i> = 24	0%	N/A	100%	0.019	>100	0% <sup>h</sup>	0.294	(26)

(Continued)

Table 2 (Continued)

Species/Population ( <i>N</i> = no. colonies)	Simple families		Extended families		Mixed families		Overall <i>F<sub>IT</sub></i>	Reference(s)
	Percent	Reproductives related <sup>a</sup>	Percent	<i>F<sub>IC</sub></i> <sup>b</sup>	Inferred no. neotenic	Percent		
Southwestern France, population B <i>N</i> = 15	26.7%	++	73.3%	-0.038	10-100	0% <sup>h</sup>	0.306	(26)
Southwestern France, population C <i>N</i> = 32	43.7%	+	56.3%	-0.113	<10	0% <sup>h</sup>	0.210	(26)
Central Portugal <i>N</i> = 15	33.3%	-	53.4% <sup>i</sup>	-0.310	<10	13.3%	-0.020	(97)
<i>Coptotermes lacteus</i>								
Southern Australia <i>N</i> = 38	100%	+	0%	N/A	N/A	0%	NR	(127)
<i>Coptotermes formosanus</i>								
New Orleans, Louisiana <sup>e</sup> <i>N</i> = 46	57.0%	-	43.0%	-0.147	<10	0%	0.159	(53, 56, 140)
Charleston, South Carolina <sup>d</sup> <i>N</i> = 25	48.0%	+	52.0%	-0.058	10-100 <sup>g</sup>	0%	0.139	(140)
Rutherford County, North Carolina <sup>e</sup> <i>N</i> = 8	75.0%	+	25.0%	-0.127	<10	0%	0.239	(140)
Kyushu, Japan <sup>e</sup> <i>N</i> = 20	85.0%	+	15.0%	0.012	>100	0%	0.161	(139)
Fukue, Japan <sup>e</sup> <i>N</i> = 10	100%	++	0%	N/A	N/A	0%	0.461	(139)
Oahu, Hawaii <sup>e</sup> <i>N</i> = 19	36.8%	+	63.2%	-0.10	<10	0%	0.32	(54)
Guangdong Province, China <i>N</i> = 12	0%	N/A	100%	-0.14	<10	0%	0.18	(54)

<sup>a</sup>-, coefficient of relatedness (*r*) not significantly different from zero; +,  $0 < r < 0.25$ ; ++,  $r > 0.25$ .

<sup>b</sup>Strongly negative *F<sub>IC</sub>* (< -0.14) suggests low numbers of reproductively active neotenic (fewer than 10), whereas values close to zero suggest many neotenic (10 to 100).

<sup>c</sup>Allozyme markers used to infer breeding structure.

<sup>d</sup>Estimated by Thorne et al. (132).

<sup>e</sup>Introduced population.

<sup>f</sup>Originally reported as the Duke Forest population of *R. hageni*.

<sup>g</sup>Low power to distinguish extended from mixed families.

<sup>h</sup>Authors refer to extended families as pleometrotic families.

<sup>i</sup>May exhibit a degree of assortative mating in some colonies.

NR, not reported.

inbreeding in individuals relative to their colony ( $F_{IC}$ ), a statistic that is especially sensitive to the numbers of reproductives present (17, 27, 29, 97, 132).

The  $F_{IC}$  values for extended families from a number of populations are given in **Table 2** along with the inferred numbers of functional neotenics. There was variation among populations of the European species, *R. grassiei*, suggesting large numbers of neotenics in some populations and low numbers in others (**Table 2**). In North American *Reticulitermes* species, extended families in most populations contained relatively few reproductives, and these were likely the direct offspring of the founding pair (17, 132). These conclusions are strikingly similar to results from laboratory colonies in which the reproductive composition was censused (79). Laboratory studies of *R. flavipes* (42, 76) have shown that neotenics may sometimes coexist alongside primary reproductives. Husseneder et al. (51) found a primary king together with 25 female neotenics in the African subterranean termite *Schedorhinotermes lamanianus*, demonstrating that primary and neotenic reproductives of other species do sometimes occur alongside each other in the field.

A couple of populations are worth special mention. First, colonies of *R. flavipes* in France (previously called *R. santonensis*) differ radically from those studied so far in the native range. Detailed studies of populations in Paris and northwestern France (30) found only highly inbred colonies headed by many neotenics (**Table 2**). These invasive populations will be discussed in more detail later. Second, an older study of *R. flavipes* in central Tennessee using allozymes (108) reported extremely high levels of inbreeding ( $F_{IT} = 0.680$ ), almost twice as high as the next most inbred native population (see **Table 2**). The reasons for the large discrepancy between this population and the many other populations studied are not known.

In introduced populations of *C. formosanus*, the inferred numbers of reproductives in extended family colonies vary considerably.

Colonies in populations from New Orleans, Louisiana; Charleston, South Carolina; and Rutherford County, North Carolina, had levels of inbreeding indicative of low numbers of neotenics ( $<10$ ) (2, 53, 140), whereas colonies from Japan and Hawaii were more inbred, suggesting higher numbers of reproductives (54, 139). Whether these differences are due to the inherent genetic structure of introduced populations or are responses to local ecological conditions is not known. Extensive studies of native populations of this species from mainland China are needed to determine how the number of reproductives in extended family colonies varies in natural environments and how these numbers compare with introduced populations. One sample of 12 colonies from a native population in Guangdong Province consisted entirely of extended families presumably headed by relatively few neotenics (54). The inferred number of neotenics in these colonies is consistent with data from nest excavations in China (142), in which the number of neotenics in colonies was generally fewer than 20.

Within extended family colonies, genetic substructuring can occur through a couple of processes. First, the presence of spatially separated groups of reproductives with little or no interbreeding between them coupled with limited movement of workers from their natal nest can lead to genetic differentiation within the colony. In an expansive colony of *C. formosanus*, Husseneder et al. (53) found evidence of substructure among foragers feeding on monitoring stations located 25 to 100 m apart. Similar results were reported for a colony of the African subterranean termite *S. lamanianus* (50). In a large introduced colony of *R. flavipes* in France, foragers occurring further apart were genetically more differentiated than those occurring closer together (30).

Another process that can lead to differentiation among foraging groups is kin-biased foraging. In *S. lamanianus*, groups of foragers collected away from the nest were more closely related than workers taken from the nest center (62), which suggests that workers sorted

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**$F_{IC}$** : coefficient of inbreeding in individuals relative to their colony

**$F_{IT}$** : coefficient of inbreeding in individuals relative to their population

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**Mixed-family colony:**  
group of cohabiting  
individuals produced  
by multiple unrelated  
reproductives

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themselves into kin groups while foraging. It is not known whether genetic substructuring, either through kin-biased sorting or as a result of spatially separated reproductive centers, is widespread in subterranean termites, but it is likely to occur mainly in species with expansive colonies. Studies of several species, especially North American *Reticulitermes*, show that colonies are often localized, so opportunities for genetic substructuring within colonies of these species are most likely limited (27, 101, 141).

### Geographic Variation in Breeding Structure

Populations spanning much of the eastern seaboard of the United States show strong clinal variation in colony breeding structure in *R. flavipes*, with a greater proportion of extended family colonies and higher levels of inbreeding in northern populations (137). Studies of *R. grassei* in France and Portugal, although more limited in scope, indicate a similar trend in increasing levels of inbreeding from north to south (Table 2) (26, 97). The apparent geographic variation in these species suggests that colony breeding structure is responsive to local ecological conditions, and that these conditions vary in a gradual manner along latitudinal gradients. One of the big challenges in subterranean termite biology will be to determine the ecological factors that shape colony breeding structure, especially those factors that may select against inbreeding. Studies in additional subterranean termite species focusing on clinal variation in colony breeding structure similar to that found in *R. flavipes* and *R. grassei* should prove particularly fruitful.

### Mixed-Family Colonies and Colony Fusion

Among subterranean termites, mixed-family colonies in the field have so far only been demonstrated in *R. flavipes* (27, 29, 101, 138) and *R. grassei* (97). Several mechanisms can potentially lead to mixed families, but colony

fusion is the only mechanism that has been documented in subterranean termites (27). Pleometrotic association of multiple same-sex reproductives is another means, but to date this route has been found only in some termitids (3, 43). Results of field (29) and laboratory (37) studies indicate that the presence of multiple unrelated groups of reproductives in fused colonies of *R. flavipes* is generally rather short-lived; over time reproduction in fused colonies is usually restricted to individuals from just one of the original source colonies.

The factors underlying colony fusion are not clear. Matsuura & Nishida (87) proposed that colonies with numerous nymphs preparing to molt into alates would be more likely to accept individuals from foreign colonies, but this hypothesis has not been rigorously tested in the field. In a study of mixed-family colonies in North Carolina and South Carolina, DeHeer & Vargo (29) showed that individuals originating from different families had identical or nearly identical mtDNA haplotypes but were unrelated at nuclear microsatellite loci. These results suggest some maternally inherited factor underlying colony fusion, but the nature of this factor is not known. Because mixed-family colonies have low genetic relatedness, and therefore lower inclusive fitness of colony members, the factors influencing colony fusion, including similarities in mtDNA haplotype, are worthy of further study.

### Colony Longevity, Breeding Structure, and Effects of Inbreeding

The proportions of simple families in a population can provide insights into its age structure. Assuming that a population has reached a stable age distribution, the presence of a high proportion of simple family colonies, as we find in most *Reticulitermes* spp. populations studied to date, suggests that most colonies in these populations do not survive past the death of one of the primaries. The life span of primary reproductives in the field is not known for any species, but in a laboratory study of 30 *R. flavipes* colonies, reproductives began to

die after 6 years but some were still alive after 11 years (78). Long-term demographic studies are needed to determine how long colonies live with both primary reproductives present, and how long they survive once neotenic are produced.

Because relatively few colonies in many populations of subterranean termites do not survive the death of the primary reproductives, there may be a cost associated with reproduction by neotenic. The production of neotenic appeared early in the evolution of the Isoptera and is thought to confer many advantages (129). Principal among these is that neotenic reproduction gives workers the option of differentiating into reproductives in their natal colony and inheriting existing resources, thereby foregoing the risks of embarking on mating flights (95, 116, 129). A major consequence of neotenic reproduction is elevated levels of inbreeding. The possibility of inbreeding depression in subterranean termites has received little attention to date.

Recent results from dampwood and subterranean termites give somewhat mixed views regarding the possible importance of inbreeding depression in termites. Sibling founding pairs of the dampwood termite, *Zootermopsis angusticollis*, survived better than pairs in which males and females were unrelated (112). Similarly, sibling founding pairs of *C. formosanus* had higher survivorship than pairs of unrelated individuals, but the survivors in the latter group had higher growth rates (36). Other studies have shown a cost to inbreeding in termites through increased susceptibility to some diseases (19) and reduced survivorship of colonies founded by related primary reproductives in the field (28). In addition, some studies report an effect of inbreeding (77) or numbers of reproductives (54) on worker size in subterranean termites, although possible fitness consequences of these differences are unknown. The costs and benefits associated with neotenic reproduction should be addressed in future research, including both ecological factors related to breeding structure and potential physiological and behavioral consequences of inbreeding.

## Colony Reproduction by Budding

Although it has often been assumed that subterranean termite colonies frequently reproduce by budding (95, 116, 130, 132), in which a portion of a colony splits off or becomes isolated from the natal nest and functions as an independent colony, results from a number of studies on several species do not support this view. If common, budding should lead to high population viscosity in which colonies located near each other are genetically more similar than colonies further apart. Yet, several fine-scale studies of *Reticulitermes* species (17, 26, 27, 136, 138, 141) and *C. formosanus* (53, 140) have failed to find such a relationship. However, Husseneder et al. (50) did find evidence for budding in a population of *S. lamanianus*, suggesting that this mode of reproduction may occur in some subterranean termite species.

## Parthenogenetic Reproduction

Parthenogenetic reproduction occurs in the laboratory in *R. virginicus* (48) and *R. speratus* (86). In the latter species, the mechanism of parthenogenetic reproduction has been identified (86). However, to date there are no clear cases of parthenogenetic colonies reported from field populations of any species, so the significance of this mode of reproduction under natural conditions remains uncertain.

## COLONY-FORAGING AREA AND POPULATION DENSITY

The application of the mark-release-recapture (MRR) technique to connect spatially separated groups of foragers to the same colony (69) was an important advance in delimiting colony-foraging areas and has been used extensively over the past three decades (122, 130). The use of molecular markers, such as highly variable microsatellite markers, offers many advantages over MRR for assigning workers to colonies, for determining the limits of colony-foraging areas, and for determining the numbers of colonies in an area (101). Chief among the advantages

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**Mark-release-recapture (MRR):** method used to delimit colony-foraging areas and sometimes for estimating colony population size

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**Table 3 Foraging ranges of *Reticulitermes* spp. and *Coptotermes* spp. as determined by genetic markers**

Species	Location (N)	Linear foraging distance (m) <sup>a</sup>	Foraging area (m <sup>2</sup> )	Reference(s)
<i>Reticulitermes flavipes</i>	North Carolina (169)	1–26	1–174	(101)
	North Carolina (122) <sup>b</sup>	1–85	NR	(135, 136, 138)
	North Carolina (29)	1–12	1–96	(27)
	South Carolina (18) <sup>b</sup>	1	NR	(141)
	Massachusetts (22)	1–76	1–800	(17)
	France (26)	1–320	1–90,000	(30)
<i>R. bageni</i>	North Carolina (15)	1–11	1–21	(27, 101)
	North Carolina (3) <sup>b</sup>	1	1	(138)
	South Carolina (21) <sup>b</sup>	1	NR	(141)
<i>R. malleteri</i> <sup>c</sup>	North Carolina (13) <sup>b</sup>	1	1	(138)
<i>R. virginicus</i>	North Carolina (8)	1–50	1–318	(27, 101)
	North Carolina (4) <sup>b</sup>	1–122	NR	(135, 138)
	South Carolina (4) <sup>b</sup>	1–125	NR	(141)
<i>R. hesperus</i> <sup>d</sup>	California (30)	1–15	NR	(23)
<i>R. grassei</i>	France (71)	1–70	NR	(26)
	Portugal (15)	1–10	NR	(97)
<i>Coptotermes formosanus</i>	Louisiana (13) <sup>e</sup>	40–175	83–1,634	(90, 91)
	North Carolina, South Carolina, Louisiana (115) <sup>b</sup>	1–144	NR	(2, 53, 56, 140)

<sup>a</sup>Colonies found in only one station or feeding site were assumed to have a foraging range of 1 m.

<sup>b</sup>Study not specifically designed to map colony foraging areas.

<sup>c</sup>Originally reported as the Duke Forest population of *R. bageni*.

<sup>d</sup>Species was not specified but occurs in area where *R. hesperus* is common.

<sup>e</sup>Used both mark-release-recapture and genetic methods to delimit colony foraging areas.

N, number of colonies studied; NR, not reported.

of using genetic markers are (a) studies can be done faster with less effort, (b) far fewer foragers are required for determining colony identity, and (c) studies can be conducted over a period of years without losing the ability to identify colonies. Thus, more colonies can be studied over a larger area and over a period of months or years, allowing for extensive long-term studies of colony dynamics and colony-level effects of termiticide treatments.

### Colony Foraging Area

Here, we summarize what has been learned about the foraging ranges of *Reticulitermes* and *Coptotermes* since 2001 using genetic markers (Table 3). The picture has changed significantly since the last reviews done more

than a decade ago, before the application of molecular methods (122, 130). For example, it now appears that small, localized colonies are the norm for many *Reticulitermes* species. Conspicuous exceptions to small foraging areas in *Reticulitermes* spp. are colonies of *R. virginicus*, which frequently forage over 100 linear meters (135, 138, 141), and introduced populations of *R. flavipes* in France that can cover thousands of square meters (30). Similarly, colonies of *C. formosanus* in introduced areas are often expansive, extending >100 linear meters (90, 140).

It has been generally assumed that the large worker populations and expansive foraging ranges attained by colonies of some subterranean termite species can only be achieved by the reproductive output of multiple female

neotenic reproductives (42, 130). Although the very large colonies of *R. flavipes* in France, which are among the largest subterranean termite colonies known, are headed by numerous neotenic (30), these are introduced populations and do not appear to be representative of natural populations. Studies of natural populations of *R. flavipes* and *R. virginicus* show that colonies headed by a single queen (simple family) do not differ in the size of their foraging areas from colonies headed by multiple queens (extended families) (27, 101, 138, 141), suggesting that colony family structure does not influence the size of the worker population. Similar results reported for an introduced population of *C. formosanus* suggest that the presence of multiple queens in colonies does not necessarily allow for larger colony size in this species either (53). Support for the lack of larger colony size in colonies headed by neotenic also comes from a recent laboratory study by Long et al. (78). There was no difference in colony size (either all individuals or numbers of workers) between laboratory-established colonies containing their original primary reproductive pairs and colonies headed by neotenic (78).

### Population Densities

Data from molecular studies in which individual colonies are identified are now accumulating, showing that colony densities can be high in some areas. A study of forests in central North Carolina (27) found densities of up to 300 *Reticulitermes* spp. colonies per hectare, consisting overwhelmingly of *R. flavipes*; these are among the highest densities recorded for any termite species in any ecosystem (73). Colony densities can be high in urban environments as well. On residential properties in North Carolina, Parman and Vargo (101) found an average population density of 62 colonies per hectare, with a maximum of 185 colonies per hectare, over 90% of which were *R. flavipes*.

Relative abundance is likely to vary with habitat and geographic location. In an undisturbed site in Massachusetts, near the far northern edge of the range of *R. flavipes*, Bulmer et al.

(17) found a much lower population density than that found in North Carolina—only about seven colonies per hectare. The lower colony density in this northern population is associated with a higher frequency of inbred colonies.

Colony density of *C. formosanus* in a park in New Orleans was 1.5 colonies per hectare (53, 90). This is similar to the 1.0 colonies per hectare for this species reported for a park in Charleston (140). The lower colony densities of this species compared with *Reticulitermes* spp. are consistent with the larger colonies it forms, with foraging ranges often exceeding 100 linear meters (90, 122, 140).

Intraspecific interactions among colonies undoubtedly play an important role in determining colony density. Recent studies show that colonies of *R. flavipes* and *C. formosanus* in relatively undisturbed sites appear to form territories that are remarkably stable over a period of years (27, 90) with little or no infringement by neighboring colonies. Further evidence supporting territorial interactions comes from studies in which colonies were removed by baiting. In areas of relatively high population density, the territories of eliminated colonies are quickly invaded by neighboring colonies in both *R. flavipes* (135) and *C. formosanus* (56, 91).

The apparently weak intraspecific agonism displayed by *R. flavipes* (18, 104) and by introduced populations of *C. formosanus* (52, 89, 121) suggests that some mechanism other than aggressive behavior is responsible for intraspecific territoriality, at least in some cases. This is in marked contrast to many termite species that show strong intraspecific agonism (131), including the African subterranean termite *S. lamanianus* (50).

### Use of Genetic Markers for Applied Studies in the Field

The use of molecular markers to identify large numbers of individual colonies and track them over time allows for more rigorous field evaluations of insecticide treatments than was previously possible (35, 56, 135). By comparing the genotypic profiles of colonies present before

and after treatment, we can determine whether termites that reinfest treated areas or bait stations are remnants of targeted colonies, invading neighboring colonies, or previously undetected hidden colonies. For example, studies of *C. formosanus* found that foraging areas of colonies eliminated by baiting with chitinase inhibitors were often reinvaded, and that the source of reinfesting termites was either known neighboring colonies or hidden colonies (56, 91). Similar results were obtained in baiting studies with *Reticulitermes* spp. (135). Although reinfestation after treatment is common in areas with high colony density, reinfestation rates decline with repeated treatment (56, 135), indicating such treatments have a population-level effect, at least on a small scale.

The use of genetic markers in applied studies also allows us to compare the breeding structure of colonies present before and after treatment to determine whether family type or the degree of inbreeding influences treatment success (56). To date, studies have indicated that breeding structure of colonies does not affect treatment success, because all treated colonies of both *Reticulitermes* and *Coptotermes* were eliminated regardless of family type (56, 135). In one study, new colonies appearing within treated areas were primarily simple families, whereas a disproportionate number of hidden *C. formosanus* colonies taking over vacated foraging areas were extended families (56), suggesting that simple families and extended families differ in their response to vacated territories.

## INVASION BIOLOGY OF SUBTERRANEAN TERMITES

A number of termites have been introduced and have become established in new locations, but in only a few cases can these be considered truly invasive in the sense that they have significant ecological and economic impact in their introduced ranges. Termites that have most often been introduced and have become established in new areas are drywood termites (Kalotermitidae) and subterranean termites (Rhinotermitidae) (38). Among the subterranean termites, species of *Coptotermes* and *Reticulitermes* are the most common and the most destructive. The Formosan subterranean termite, *C. formosanus*, is considered among the 100 worst invasive species (40). *Coptotermes gestroi* has been introduced into several places around the world (60). *R. flavipes* is well established in Europe (11) and South America (123).

The use of molecular markers can provide powerful tools for identifying the source populations of introduced species (83). DNA sequence data established that populations of *R. santonensis* in France and South America were introduced populations of *R. flavipes* (11, 123), a native of eastern and central United States. In addition, *R. flavipes* has been introduced into areas of North America north of the native range (34, 65). The precise locations of populations within the United States serving as the sources of introduced populations have not been identified. Attempts have been made to identify the source populations and routes of introduction of invasive populations of *C. formosanus* (12) and *C. gestroi* (60), but small sample sizes and low variation in the mitochondrial genes used in these studies render conclusions from this work tentative at best (94). Studies using highly variable markers, such as microsatellites, should provide greater power in identifying likely source populations (25, 105) and the routes of introduction of invasive subterranean termites.

A major area of inquiry in invasion biology concerns the factors that make some species successful invaders. The attributes of successful ant invaders have received considerable attention (47). Among the most prominent features of many invasive ants is the breakdown of colony boundaries resulting in large unicolonial populations that become ecologically dominant within introduced ranges. The behavioral changes in introduced populations most likely result from reduced genetic variation associated with introduction events, resulting in a homogenization of the cues used by ants to distinguish nestmates. This reduced ability to recognize

nestmates. This reduced ability to recognize

nestmates in turn leads to fusion of colonies into large aggregations (133).

Do invasive subterranean termites share any of the unicolonial characteristics of invasive ants? The evidence to date shows a mixed picture. French populations of *R. flavipes* exhibit reduced genetic variation compared with native populations at both microsatellite loci (30, 136, 141) and mitochondrial genes (11). The populations in France form exclusively large, highly polygamous extended family colonies (30), and this appears to be true of populations introduced into North America as well (34, 41). This is in contrast to colonies in the native range that tend to be primarily simple families with localized foraging areas. Thus, there are some intriguing parallels between these introduced populations of *R. flavipes* and those of some invasive ants. It is of interest to know whether other introduced populations of *R. flavipes*, such as those in Chile (123), also form expansive, highly polygamous colonies.

Studies of introduced populations of *C. formosanus* also show reduced genetic variation compared with native populations at microsatellite loci (53, 54, 139, 140). Despite little or no agonistic behavior among colonies in the introduced range (52, 89, 121), invasive populations of this species do not form large, unicolonial societies. Rather, they form genetically distinct colonies that are either simple families or secondarily polygamous (extended) families derived from simple families. In contrast, the dozen colonies characterized to date from the native range were all polygamous (54). Thus, although the data are still limited, it appears that introduced populations of *C. formosanus* are not characterized by higher numbers of reproductives than native populations. This may not be true of all *Coptotermes* spp., however. Three species of mound-building *Coptotermes* native to Australia, *C. lacteus*, *C. acinaciformis*, and *C. frenchi*, all form almost exclusively simple families in Australia but colonies in New Zealand, where they have been introduced, contain many neotenics (71).

## CONCLUSIONS

There is growing interest among scientists in the biology of subterranean termites. Molecular techniques have begun to make significant contributions to nearly all areas of subterranean biology. Progress in some areas, such as systematics and taxonomy, the molecular basis of caste differentiation, and colony breeding structure, will largely depend on continued application of molecular methods. In other areas, such as foraging ecology, population dynamics, and community ecology, molecular techniques can provide important information on colony identity, allowing for much more detailed and comprehensive studies than would otherwise be possible. Recent molecular ecological studies are already changing long-held views about the breeding structure and dynamics of subterranean termite colonies. In addition, the use of molecular markers is playing an increasingly important role in applied studies to assess colony-level effects of termiticide treatments in the field.

Although molecular techniques will grow increasingly important in studies of subterranean termite biology and management, the results generated by these methods will have greatest utility as part of a multidisciplinary approach. For example, a more complete understanding of the mechanisms regulating caste differentiation will involve integrating molecular tools with physiological methods, chemical ecological approaches, and behavioral studies. Molecular genetic markers have proven invaluable for elucidating colony breeding structure and how it varies within and among species. But understanding the factors underlying this variation will require ecological studies of the biotic and abiotic factors that shape colony breeding structure. Studies of colony-colony dynamics and relative species abundance will need to combine molecular markers for colony identifications with ecological, behavioral, and demographic approaches to understand the factors determining population dynamics and species richness. Thus, in our view, the future of subterranean termite

biology is one of an interdisciplinary approach, with molecular techniques occupying a central position. The development of additional tools, especially in genomics and proteomics, will lead to many new possibilities. We can look forward to rapid advances in all areas of subterranean termite biology in the coming years.

### SUMMARY POINTS

1. *Coptotermes* and *Reticulitermes* are two of the most species-rich genera of lower termites, but they are in need of careful taxonomic revision using both morphological and molecular methods.
2. Recent studies have provided important insights into the molecular processes underlying caste differentiation, especially in soldier development in *Reticulitermes*, in which hexamerins play a key role in modulating the activity of JH.
3. Although it is well known that termites often inbreed, recent evidence suggests that inbreeding depression may occur in some subterranean termite species with possible consequences for mate choice and colony breeding structure.
4. Dispersal and sex-biased alate production can promote outbreeding in some subterranean termite species, but to date there is no evidence for kin discrimination during partner selection.
5. Molecular markers provide a powerful tool for inferring the breeding structure of subterranean termite colonies. There is considerable variation within and among species in the relative frequencies of simple and extended family colonies, but many populations are composed of mainly simple families. Mixed-family colonies, which can form through colony fusion, appear to be rare.
6. Colonies of most species of *Reticulitermes* studied to date have fairly limited foraging ranges, usually less than 10 linear meters, and colony densities can be quite high, reaching up to 300 per hectare in some populations of *R. flavipes*. Colonies of introduced populations of *R. flavipes* are a notable exception, with foraging ranges up to 100 m or more, rivaling the expansive colonies often formed by *C. formosanus*.
7. Colonies of *Reticulitermes* and *Coptotermes* appear to be territorial with well-established boundaries, but the mechanisms by which these boundaries are established and maintained have yet to be identified.
8. Species of *Reticulitermes* and *Coptotermes* are among the most important and destructive invasive termite species. Current studies are using genetic markers to identify source populations and to investigate the factors underlying their invasion success.

### DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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**145. Important paper identifying a gene of major influence on caste determination in a termite.**

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