

Tunnel Orientation and Search Pattern Sequence of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT Foraging behavior of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was studied in the laboratory by using two-dimensional foraging arenas containing multiple foraging sites. Within each arena, 16 foraging sites were arranged in a uniform grid pattern and foragers were introduced into the arena through a central initiation site. Chi-square analysis determined the frequency of tunnels was uniformly distributed around the perimeter of the initiation site but became significantly skewed toward the foraging sites at a distance where the foraging sites could be encountered. Tunnel distribution was similar whether wood was present or absent at the foraging sites, suggesting that foragers respond to structural anomalies in the substrate rather than simply to the presence of food. Also described is the generalized sequence of events as foragers tunnel throughout the arenas.

KEY WORDS *Coptotermes formosanus*, foraging behavior, search pattern, subterranean termites

OVER THE PAST decade the rationale of subterranean termite control with repellent chemical barriers has come under scrutiny, and an alternative paradigm of population suppression has emerged. This approach to termite control relies on nonrepellent baits or insecticide treatments that do not stimulate behavioral defenses or avoidance. With the advent of these nonrepellent treatments, an understanding of termite foraging and tunneling behavior in the subsurface soil environment has become increasingly important. Much of the knowledge of termite foraging behavior has been gained through either direct gallery excavation (Ratcliffe and Greaves 1940, Greaves 1962, King and Spink 1969) or studies on baited plots by using spatio-temporal and mark-release-recapture methods (LaFage et al. 1973, Su et al. 1984, Grace et al. 1989, Jones 1990). Although these methods have provided valuable insights into the ecology and behavior of subterranean termites, they offer no direct information on tunnel formation during territory expansion.

Several laboratory studies that used two-dimensional surfaces have added to our understanding of foraging behavior by creating an artificial window into the cryptic subterranean environment of termites. Using two large vertical Plexiglas sheets to create a two-dimensional tunneling environment, Robson et al. (1995) described the colony-level search pattern of *Reticulitermes flavipes* (Kollar) as nonrandom, suggesting that in the absence of resource cues exploratory tunnels divide a search area evenly to minimize redundancy. Reinhard et al. (1997) conducted similar studies with *Reticulitermes santonensis* De Feytaud

and found that foragers constructed a "regularly branched net" of trails that systematically covered all directions of the territory in the absence of wood cues. Arena tunneling studies conducted on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, have shown that available food size influences the search tunnel network created by foragers in amount of branching and length of tunnels (Hedlund and Henderson 1999).

We further explore *C. formosanus* tunneling behavior in the current study. Spatial analysis of digital images was used to elucidate tunneling as foragers pioneer a new area of substrate containing multiple foraging sites, and to compare tunneling in the presence and absence of food resources.

Materials and Methods

Arena Design. Foraging trials were conducted in laboratory arenas consisting of three layers (85 by 85 cm) of clear acrylic (2.5 mm in thickness) with the middle layer cut as a frame (5 cm in width) to create a space (75 by 75 by 2.5 cm) between the two outer layers (Fig. 1A). Silica sand (40-100 mesh, Fisher, Pittsburgh, PA) and distilled water ($\approx 18\%$ by weight) were added to the middle space to serve as the foraging substrate. The top layer of the arena had holes (3.1 cm in diameter) drilled every 20 cm in a grid pattern with one large hole (8.2 cm in diameter) drilled in the center. Plastic vials (48.1 ml) with the bottoms removed were inserted into the smaller holes to serve as foraging sites. These sites were either left empty or contained wafers (2.5 by 2.0 by 0.5 cm) of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco

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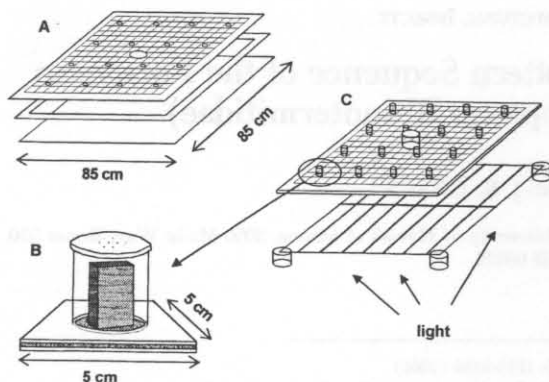


Fig. 1. Laboratory foraging arena. (A) Acrylic squares with sand filled middle layer. (B) Foraging sites. (C) Fluorescent lighting from below.

(Fig. 1B). A plastic jar (capacity 500 ml) with the bottom removed was placed in the large center hole. This center site was the termites' point of entry, or initiation site, into the arena where termites were added and began tunneling. The initiation site contained two moistened, 9 cm-diameter filter paper discs to provide both a substrate and food for the termites. The entire arena was kept in a dark room and backlit from beneath with fluorescent lighting (Fig. 1C) so that digital photographs of the tunnels could be taken from above for computer analysis.

Protocol. Termites were collected from four different field sites and are referred to here as colonies A, B, C, and D. Colonies A (Miller Hall), B (Gilmore Hall), and D (Kuykendall Annex) are located on the campus of the University of Hawaii at Manoa. Colony C is found on the windward side of Oahu at the University of Hawaii agricultural experiment station in Waimanalo. Two arenas were used for each colony, with one arena containing Douglas fir wafers in each foraging site and the other arena containing only empty foraging sites. Fifteen hundred termites (90% workers and 10% soldiers) were added to each arena. Tunneling in the arenas was monitored for a period of 48 h. Digital photographs (D-600 liter digital camera, Olympus, Melville, NY) were taken every 3 h for the first 24 h to distinguish between entry and exit tunnels at the four foraging sites closest to the initiation site. During the second 24 h, as tunneling proceeded outward from the center region of the arena, photographs were taken every 6 h. The arenas were housed in a facility with limited temperature control; therefore, temperature of the indoor ambient environment fluctuated concurrently with the outside daily temperature. Indoor temperatures reached a mean high of 26°C in the late afternoon and a mean low of 22°C in the early morning.

Analysis. ArcView 3.2 GIS software (ESRI, Redlands, CA) was used to process images and digitize tunnels. Tunnels were classified relative to the site they originated from. Tunnel classification allowed the sequence of tunnel development and any patterns leading to site discovery to be followed. A site was classified as primary, secondary, or tertiary according to the origin of the tunnel that first intercepted it. For

example, a site intercepted by a tunnel that originated from the initiation site is termed primary, and a site intercepted by a tunnel that originated from a primary site is termed secondary. To examine the distribution and orientation of tunnels leaving the initiation site and reaching the four nearest foraging sites, the 48-h photograph was overlaid with circles marking the perimeter of the initiation site and the perimeter where the first four foraging sites could be encountered. The circles were divided into 20 slices with each slice occupying an arc on the circle perimeter equal to the span of an individual foraging site. The slices were then grouped into five sectors, such that each sector contained slices from four opposing directions in the circle (Fig. 2). The intersection of tunnels crossing both perimeters were marked in each sector and totaled (Table 1). Chi-square analysis (SPSS for Windows 9.0; SPSS, Chicago, IL) was used to compare the frequency of tunnels in each sector.

Results

Figure 3 illustrates through a series of digitized snapshots the general pattern of events observed over 48 h as the termites tunneled outward from the initiation site in an arena. These events are summarized as follows: 9 h, foragers begin tunneling outward from the initiation site, with tunnels emanating out in a pattern similar to the spokes of a wheel (not all initial tunnels are begun at the same time nor proceed at the same rate); 15 h, when a tunnel intercepts a foraging site, foragers are recruited to that area and tunneling increases in the localized area of the site and decreases in areas where foraging sites have not been encountered; 30 h, upon recruitment to a discovered site, foragers create new search tunnels, which radiate out in the wheel-spoke pattern (most of these search tunnels are abandoned if nothing in the vicinity of the new site is discovered, but one or two main tunnels will continue out in the general direction from where they originated); 48 h, this pattern is repeated upon contact with every new site, when tunnels reach the boundaries of the arena they follow the edge with little deviation, and the majority of tunneling activity continues along these edges until the arena is circumnavigated; and 4 wk, daily tunneling activity decreases after the first few days, but eventually foraging termites construct tunnels into the remaining vacant areas of the arena until the majority of the substrate is explored. The final tunnel pattern after 48 h for all colonies in both treatments is shown in Fig. 4. No observable differences were evident between colonies or within colonies by treatment.

Points where tunnels crossed the initiation site perimeter and the perimeter at which foraging sites could be encountered were pooled from all colonies according to treatment (Fig. 1). Assuming that tunnels show no directional preference, the expected frequency of tunnel crossing points per sector, defined as $\Sigma n/k$ where n = number of points and k = the number of sectors, should be equal (Batschelet 1965). The inner perimeter tunnel distributions in arenas with

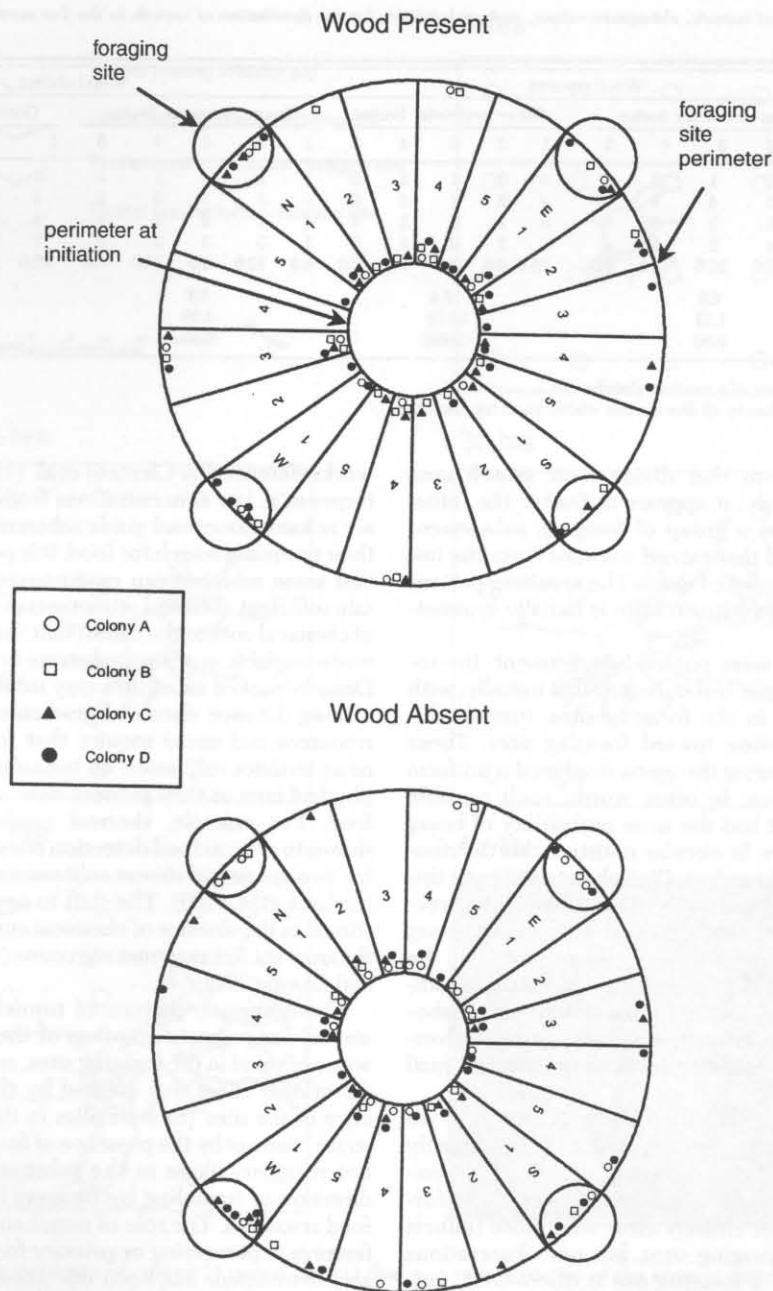


Fig. 2. Points where tunnels exit the perimeter of the initiation site and cross the perimeter of the first four foraging sites. The two perimeters are divided into 20 slices that comprise five sectors. Slices of the same number constitute one sector.

and without wood were not significantly different ($\chi^2 = 1.31$, $df = 4$, $P = 0.86$, and $\chi^2 = 2.36$, $df = 4$, $P = 0.67$, respectively). Tunnel distribution was significantly affected by the presence of foraging sites in arenas with and without wood ($\chi^2 = 34.76$, $df = 4$, $P < 0.001$, and $\chi^2 = 31.75$, $df = 4$, $P < 0.001$, respectively). Inspection of frequencies at the outer perimeters indicated how the tunnel distribution was affected. Sector 1, the sector containing foraging sites, had three to

four times more tunnels than the other sectors (Table 1).

Discussion

Our observations on the sequence of tunneling events by groups of foragers support the findings of Robson et al. (1995), working with *R. flavipes*, that subterranean termites show a significant tendency to

Table 1. Number of tunnels, chi-square values, and probabilities for the distribution of tunnels in the five sectors from all colonies in both treatments

| Colony | Wood present | | | | | | | | | | Wood absent | | | | | | | | | |
|---------------------------|------------------------|-----|------|------|-----|------------------------|-----|-----|-----|-----|------------------------|------|-----|------|-----|------------------------|-----|-----|-----|-----|
| | Inner perimeter Sector | | | | | Outer perimeter Sector | | | | | Inner perimeter Sector | | | | | Outer perimeter Sector | | | | |
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| A | 4 | 0 | 1 | 2 | 0 | 4 | 0 | 1 | 2 | 0 | 1 | 2 | 2 | 5 | 2 | 6 | 0 | 1 | 2 | 1 |
| B | 1 | 3 | 4 | 4 | 2 | 4 | 2 | 1 | 2 | 0 | 2 | 3 | 1 | 0 | 2 | 4 | 0 | 1 | 1 | 1 |
| C | 4 | 1 | 3 | 3 | 2 | 5 | 1 | 1 | 2 | 0 | 2 | 2 | 2 | 2 | 1 | 5 | 2 | 1 | 0 | 2 |
| D | 3 | 4 | 2 | 2 | 4 | 8 | 1 | 2 | 1 | 0 | 1 | 3 | 3 | 5 | 3 | 7 | 0 | 2 | 2 | 2 |
| Obs. Total | 12.0 | 8.0 | 10.0 | 11.0 | 8.0 | 21.0 | 4.0 | 5.0 | 7.0 | 0.0 | 6.0 | 10.0 | 8.0 | 12.0 | 8.0 | 22.0 | 2.0 | 5.0 | 5.0 | 6.0 |
| Exp. Total ^{a,b} | 9.8 | | | | | 7.4 | | | | | 8.8 | | | | | 8.0 | | | | |
| χ^2 | 1.31 | | | | | 34.76 | | | | | 2.36 | | | | | 31.75 | | | | |
| P | 0.86 | | | | | <0.001 | | | | | 0.67 | | | | | <0.001 | | | | |

^a Under the hypothesis of a random distribution.

^b Expected total applies to all five sectors within each treatment.

tunnel in directions that divide their search area evenly. In our study, it appears that after the initial tunnel is begun by a group of foragers, subsequent tunnels are started that extend outward from the initiation site to unexplored areas. The resulting pattern of tunnels from the initiation site is radially symmetrical.

When all trials were pooled by treatment, the results of the chi-square test indicated that initially, with or without wood in the foraging sites, tunnels displayed no orientation toward foraging sites. These initial tunnels entering the arena displayed a uniform circular distribution. In other words, each possible tunnel entry point had the same probability of being chosen by termites. In circular statistics, this distribution is considered random (Batschelet 1965). In this context the word "random" is used to mean nondirectional, and refers to the alignment of a pooled group of tunnels from multiple foraging bouts relative to the compass points of the foraging arena. Initial nondirectional searching also has been described in laboratory tests on *Constrictotermes cyphergaster* (Silvestri), a common tree-nesting termitid in Central Brazil (Souto et al. 1999).

Approximately 10 cm from the initiation site, we found, however, that tunnels deviated significantly from this nondirectional pattern. This difference consisted of more tunnels being aligned toward the foraging sites. It is not entirely clear why more tunnels intercepted the foraging sites, but our observations suggest that once a foraging site is discovered, tunneling activity increases in that general area and decreases or ceases in other areas where termites have not encountered a site. Therefore, some tunnels outside of the zones containing foraging sites were abandoned before reaching the foraging site perimeter. This explanation is supported by the observation that once foragers discovered a site, recruitment to that site occurred and a new search pattern was begun from that site. This systematic series of events created an apparent orientation toward sites and a nonrandom pattern in the resulting experimental gallery system.

Reinhard et al. (1997) attributed systematic search by termites in a vermiculite substrate and in open air to perceived volatiles emanating from wood. Earlier

work referenced by Clement et al. (1988) showed that terpenes at low concentrations from *Pinus* wood can act as kairomones and guide subterranean termites in their tunneling search for food. It is possible, however, that some subterranean environments may not contain sufficient chemical attractants or allow circulation of chemical cues to the extent that they diffuse and are made available in a loose substrate or open-air setting. Densely packed substrates may inhibit the formation of long distance chemical gradients leading to food resources and could require that foraging subterranean termites rely more on tunneling strategies and physical cues as they pioneer new areas in search of food. For example, thermal gradients have been shown to serve as food detection cues in mesic habitats for two species of desert subterranean termites (Ettershank et al. 1980). The shift to a reliance on tactile stimuli in the absence of chemical cues is also made by the army ant *Neivamyrmex nigrecens* (Cresson) (Topoff and Lawson 1979).

The systematic pattern of tunneling that we observed was present regardless of the presence or absence of wood in the foraging sites, suggesting that the directional effect was created by the physical presence of the sites (as anomalies in the tunneling substrate) and not by the presence of food. Thus, physical heterogeneity alone in the substrate can affect the direction of tunneling by foragers in search of new food resources. The role of nonchemical topographic features in pioneering or primary foraging in an open air environment has been documented in some ant and termite species (Klotz and Reid 1992, Jander and Daumer 1974), but little is known about their effect in a subterranean setting. In addition, to a positive tunneling response to the foraging sites, we observed that upon encounter with the edge of the arena, *C. formosanus* concentrated tunneling at the edge until the arena was circumnavigated. It is uncertain, however, whether foragers associate a substrate anomaly with food; or if the anomaly simply makes tunneling easier. In this case the hollow spaces could have provided more area for tunnel initiation and free volume for substrate deposition, and the firm surface of the edges possibly facilitated tunnel excavation. The carpenter ant, *Camponotus pennsylvanicus* (DeGeer), uses phys-

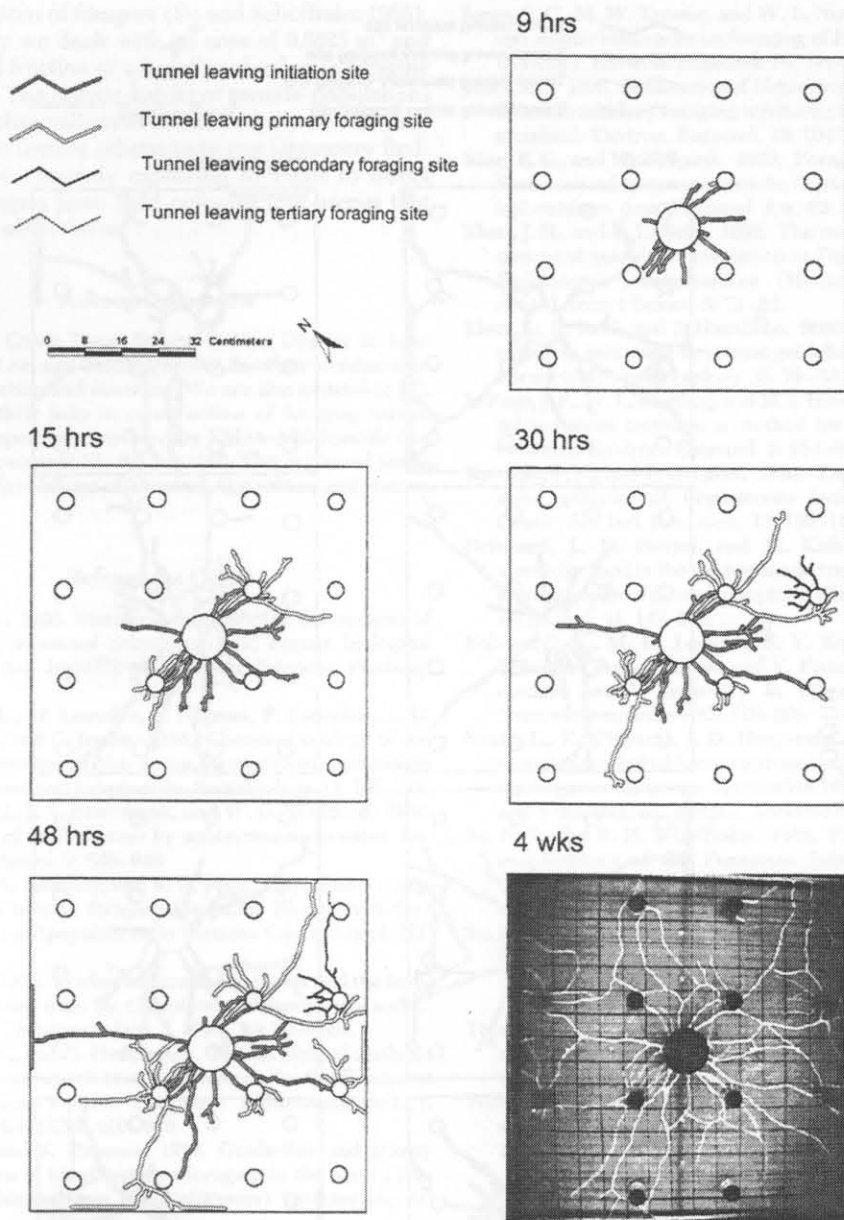


Fig. 3. Tunneling sequence of colony D (wood absent) that was representative of the general pattern observed in all colonies (4 wk is actual photograph of arena). Sites are classified as primary, secondary, or tertiary according to the origin of the tunnel that first intercepted it (i.e., a site intercepted by a tunnel that originated from the initiation site is termed primary, and a site intercepted by a tunnel that originated from a primary site is termed secondary).

ical components of its environment such as structural guidelines to increase "locomotory efficiency" and thereby saves on time and energy in foraging (Klotz et al. 2000).

An additional explanation for this sequence of tunnel patterns is that it is possibly an adaptation for locating food resources with a clumped distribution. That is, when foragers find one site, they expect more to be in the vicinity. Williams (1977) cited exploratory "runways" constructed in the immediate area of a

discovered food source by several species of subterranean termites and attributed this to adaptation to an environment with discontinuous distribution of food resources. Field studies testing the systematic discovery of foraging sites, however, are rare and inconclusive. Site discovery, with respect to distance between sites (1.5 m), by the desert subterranean termite, *Heterotermes aureus* (Snyder), was determined to be a random process (Jones et al. 1987). Our study suggests that systematic discovery can occur, but at site inter-

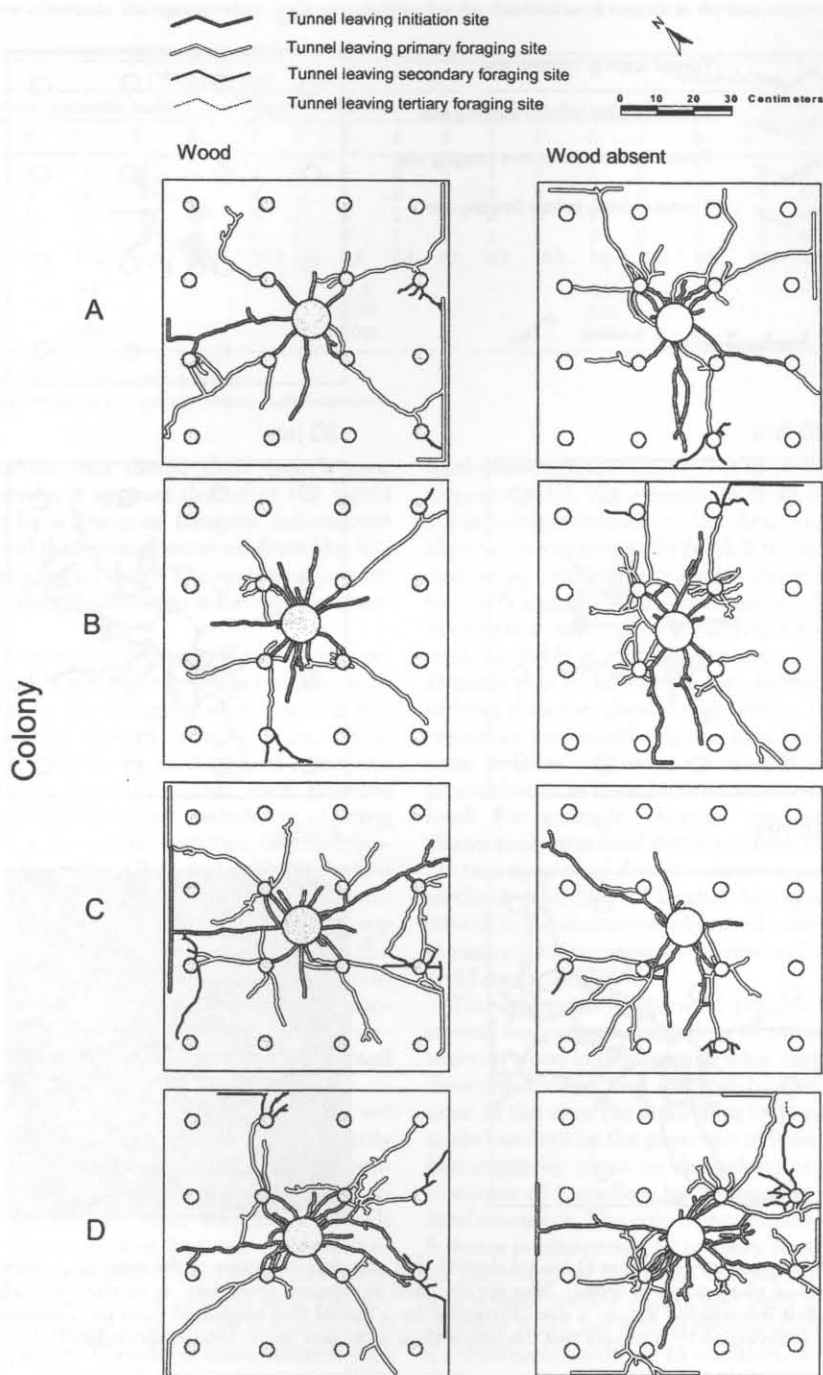


Fig. 4. Digitized tunnel patterns of all colonies and treatments.

vals much smaller than are typically tested in the field. In terms of commercial application, the systematic pattern we observed also lends support for the use of auxiliary baiting stations around an active baiting site. It should be noted however, that the majority of search tunnels around a newly discovered site were usually abandoned after no further sites in the im-

mediate vicinity were found, suggesting that timing of placement from the active site may be an important factor in the use of auxiliary stations.

We are mindful that the issue of scale can be a troubling element when arriving at conclusions through laboratory foraging studies. Actual foraging territories of *C. formosanus* can extend 100 m and

involve millions of foragers (Su and Scheffrahn 1988). In this study we dealt with an area of 0.5625 m² and only a small fraction of a functional colony's foraging population. The cryptic habits of termite populations make field observations of behavior quite challenging. However, to further substantiate our laboratory findings, we are currently exploring methods to direct termite foragers from field colonies into arenas that will permit observation.

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