

Nesting Biology of the Drywood Termite, *Incisitermes minor*
(Hagen)

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CHAPTER

1

GENERAL INTRODUCTION

This first chapter gives an overview of the background of the study and outlines the thesis.

1.1. Introduction

The western drywood termite, *Incisitermes minor* (Hagen) (Kalotermitidae) is considered to be the most destructive drywood termite in the western United States (USA) and is one of the five most economically important termites in the USA (Su and Scheffrahn 1990). The colonies live entirely within sound and dry wood (Light 1934), and derive their food and water from a single piece of wood (Evans *et al.* 2010). Because of this hidden ecology, *I. minor* has been easily transported within an infested wood around the world by human activities. In the modern and mobile society, the introductions of such wood-inhabiting termites are very difficult to deal.

Although *I. minor* is an economically important pest, there is very few scientific literature investigated the nest-gallery systems of *I. minor*. The most detailed description of *I. minor*'s biology was provided by Harvey (1934b), eight decades ago. The cryptic behavior of *I. minor* hinders studies of its nest-gallery development and colony establishment; thus, the early detection of *I. minor* infestation is difficult.

There have been many published investigations pertaining to intermediate and separate termite nesters; however, we still lack a detailed understanding of the creation and maintenance of the gallery systems produced by single-piece nesters due to the difficulty of visualizing the galleries inside the wood. Most studies of nesting and ecology in termites have focused on higher termites (Clarke and Garraway 1994; Lys and Leuthold 1991; Thorne *et al.* 1996; Wilson 1971), with a few studies that include a group of drywood termites (Calleri *et al.* 2010; Harvey 1934a). Mapping the entire gallery system of drywood termites has been technically difficult, with

wood dissection being the most common method used (Grace *et al.* 2009; Harvey 1934b; Lewis and Forschler 2014). However, wood dissection destroys the fine details of the main chambers, minor chambers, foraging tunnels, and the three-dimensional (3D) connectivity among them.

In recent years, X-ray computed tomography (CT) has been developed as a reliable indirect method of analyzing termite nests without damaging the fine details of the gallery system. Fuchs *et al.* (2004) was the first to report the use of X-ray CT to describe the 3D nest-gallery of *Cryptotermes secundus*. However, the study only revealed the relatively simple architecture of chambers and tunnel-galleries in a small piece of wood block. It did not present the anatomical profile of the wood as the drywood termite's habitat and environment. More recent investigations of termite nests using X-ray CT were conducted by Perna *et al.* (2008a; 2008b). In these studies, the authors included the more detailed 3D gallery networks of a nest of a soil-feeding termite, *Cubitermes* *sp.* Although the topological efficiency and the accessibility of networks inside the nest were reported (Perna *et al.* 2008b; Viana *et al.* 2013), these findings were limited to soil-feeding rather than single-piece nesting species of termites.

In the present study, X-ray CT will be used to reveal the 3D hidden structure of gallery systems contained within woods that were naturally infested by *I. minor*. By combining CT scan and the volume-rendering technique, a graphics technique which directly renders CT data using a reasonable approximation of the physical properties of timber, will be able to assess the whole systems of nest-galleries and evaluate the important biological information of this drywood termite.

1.2. Biology of the Western drywood termite, *I. minor* (Hagen)

The western drywood termite, *I. minor*, is the most common structure-infesting drywood termite in the southwestern USA (Cabrera and Scheffrahn 2001). Hagen (1858) characterized the species as the common drywood termite, *Calotermes minor*, before Krishna (1961) reclassified it into genus *Incisitermes*. This termite is adapted to the Mediterranean climate, a relatively dry climate with hot summers and little rainfall. Originally from the southwestern USA and northern Mexico (Light 1934), the distribution of this invasive species (Evans *et al.* 2013) has been reported to include Canada (Grace *et al.* 1991), China (Xie *et al.* 2001), Hawaii (Haverty *et al.* 2000) and Japan (Harunari and Tomioka 2004; Indrayani *et al.* 2004; Yasuda *et al.* 2003; Yoshimura 2011).



Fig. 1.1 Soldier of *I. minor*.

Incisitermes minor has distinct characteristics (color, wings, ocellus, arolium, soldier eye, etc.) that distinguish it from most other species in the genus *Incisitermes* (Cabrera and Scheffrahn 2001). Generally, there are three types of termites within the colony: the soldier, the worker, and the alate (swarmer). The soldiers of *I. minor* have a large, sclerotized dark-brown head capsule (Fig. 1.1). The mandibles are large and black with two prominent teeth visible on the inner margin of the left mandible (Cabrera and Scheffrahn 2001). The soldiers of the western drywood termite are identified by their enlarged, club-like third antennal segment (Fig. 1.1) that is as long as or longer than the fourth through sixth antennal segments combined. Soldiers of mature, older colonies are large, ranging from 8- to more than 12-mm long and weighing as much as 20 to 25 mg (Cabrera and Scheffrahn 2001).

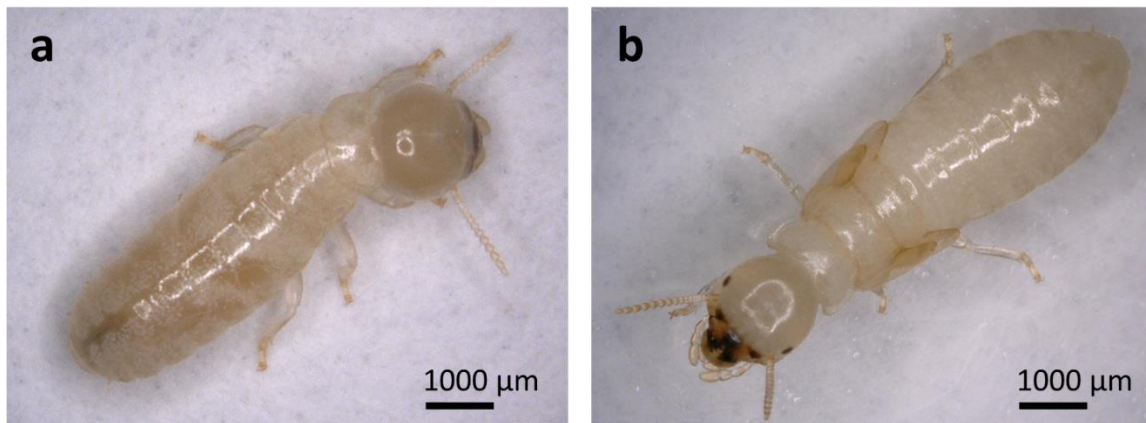


Fig. 1.2 Worker of *I. minor*: **a** Pseudergate; **b** Nymph.

Drywood termites are devoid of a true worker caste (Korb 2007; Roisin 2001) and are often termed as “false” workers (Korb and Hartfelder 2008). Individuals without wing pads are called pseudergates (Fig. 1.2a), while those that possess wing pads are called nymphs (Fig. 1.2b). The workers in drywood

termite are not a sterile worker caste, but totipotent individuals (Roisin 2000) that can moult into secondary reproductives, soldiers, or, most commonly, into nymphs and alates. They may rather be regarded as large immatures with delay reproductive maturity ('hopeful reproductives') (Korb and Hartfelder 2008; Myles 1999).

The alate has an orange-brown head and pronotum, a dark brown abdomen and dark, color-tinted wings (Fig. 1.3a). Alates are estimated to range in size from 11- to 12.5-mm long, including the wings. The alates (winged male and female individuals) leave the colony during the nuptial flight. The swarming season is reported to vary depend on geographical location. In the earlier report, the alates of *I. minor* was reported to be night-fliers (Banks and Snyder 1920); however, they are now known to be day-fliers (Cabrera and Scheffrahn 2001; Harvey 1934b). In southern California, alates fly during the day from late September through November (Cabrera and Scheffrahn 2001). In Japan, the swarming season has been reported to be in September (Yoshimura 2011). In areas where the average day time temperatures are warmer, alates may swarm as early as May. In Florida, nuptial flights have been reported to occur in all months of the year except December, with 50% of the flights in September, October, or November (Cabrera and Scheffrahn 2001). Upon landing from swarming, the alates get rid of their wings by bracing them against the substrate. Wingless alates – now termed dealates (Fig. 1.3b) – crawl to find a mate. Once they have formed a pair, they start to excavate the first chamber (Fig. 1.3b) to establish a new colony and become primary king (Fig. 1.3c) and queen (Fig. 1.3d).

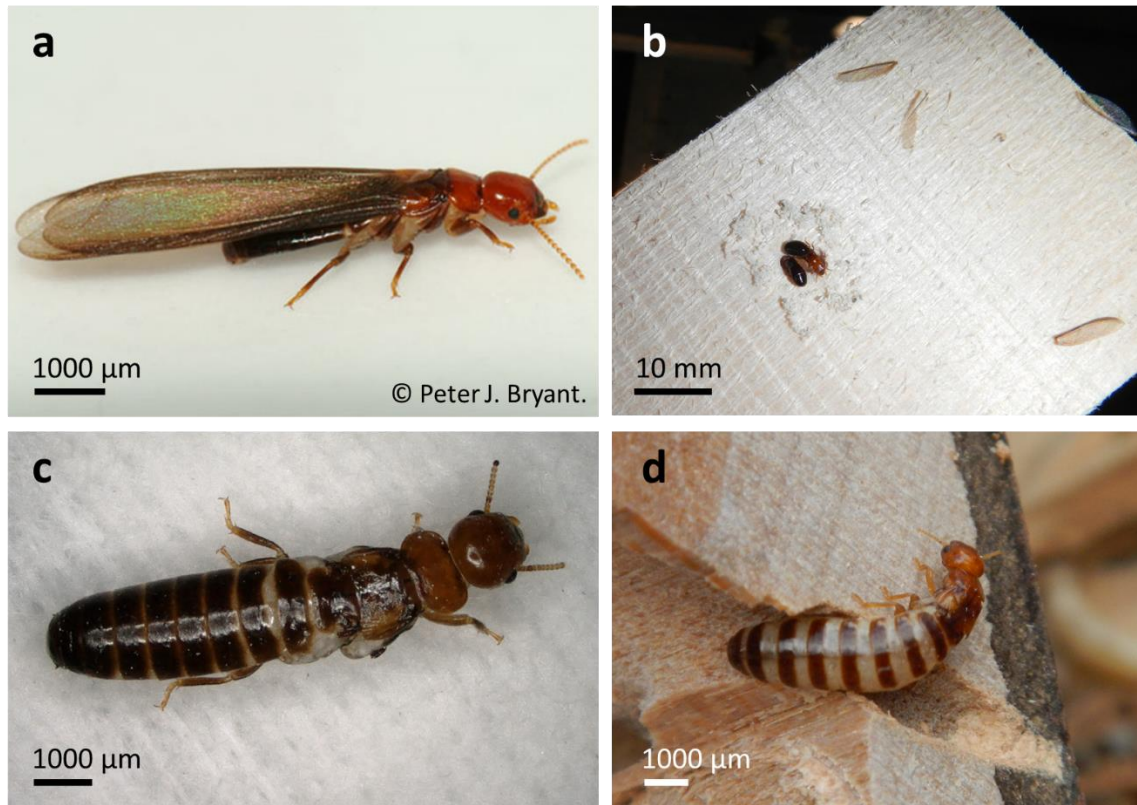


Fig. 1.3 Reproductive caste of *I. minor*: **a** The alate; **b** A pair of dealate reproductive; **c** King; **d** Queen.

1.3. X-Ray CT: Principle and application

Colony development in drywood termites is difficult to assess as the termites live and forage inside timber. In recent years, X-ray CT has been developed as a reliable indirect method for use in insect studies. Fuchs *et al.* (2004) introduced the CT technique as a non-destructive approach to visualizing the galleries of the drywood termite, *C. secundus*, albeit with low image resolution. Using a more advanced CT technique, Perna *et al.* (2008a) managed to reveal the structure of gallery networks in the nests of the termite *Cubitermes* spp., as well as the topological efficiency of this species (Perna *et al.* 2008b). This technique was also reported to reveal important biological

attributes of the insects that are hidden from view, as reported by Kigawa *et al.* (Kigawa *et al.* 2008) and Torigoe *et al.* (Torigoe *et al.* 2009).

In this section, we will introduce 3D volume visualization of the hidden gallery system of drywood termites using CT. The term 'volume visualization' is commonly used to describe all techniques that can be used to visualize volumetric data-sets (Bosma 2000). An important field in which these volume visualization methods can be applied is the visualization of volumetric data-sets generated by 3D scanners. We use a computerized x-ray imaging technique in which a narrow beam of x-rays is aimed at drywood termite-infested timbers to produce signals that are processed by the computer to generate two-dimensional (2D) tomographic images —or “2D image slices” — of those respective timbers (Fig. 1.4). Once a number of successive 2D image slices are rendered by the volume-graphic application, they can be digitally “stacked” together to form a 3D image (Fig. 1.4). This technique is able to map the whole 3D connectivity of the drywood termite nest-gallery, which until now has been a technically difficult task. The technique is also able to estimate and monitor the extent of the termite’s infestation inside the timber and thus improve our level of understanding of its nesting biology.

Direct volume rendering uses an optical model to map data values to optical properties, such as colour and opacity (Ikits *et al.* 2004). The classification method, as presented in Fig. 1.4, is the process of assigning opacity to volume data. Classification is an essential part of the volume visualization (Praßni *et al.* 2010), as it enables the user to assign optical properties to individual parts of a volumetric data set. Volume data are stored as a stack of 2D image slices or as a single 3D texture object (Fig. 1.4). The

term “voxel” in a 3D image refers to an individual "volume element," similar to the term “pixel,” which refers to a "picture element" in a 2D image. Each voxel corresponds to a location in data space and has one or more data values associated with it. In this study, voxel data are shown in a histogram of pixel intensity value (opacity), which represents the absorptance intensity of the X-ray.

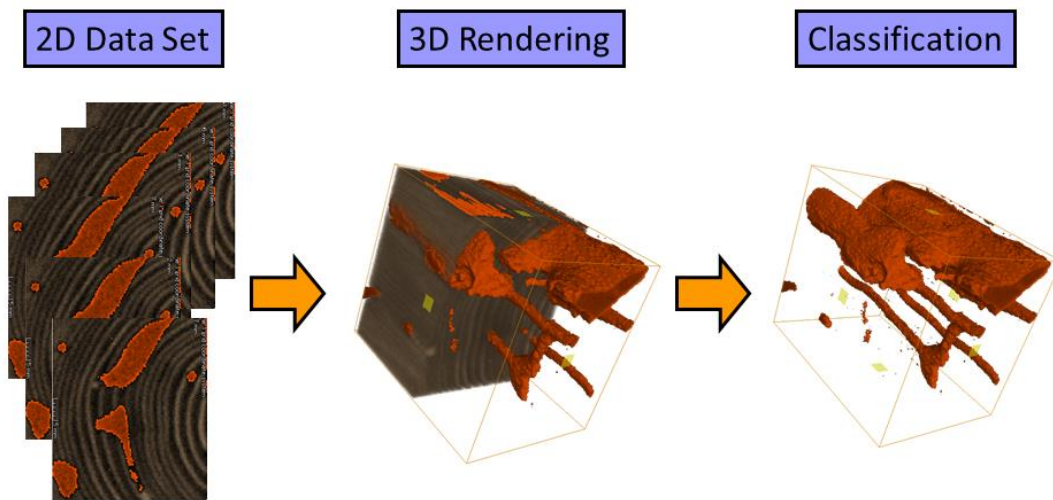


Fig. 1.4 The outline of a 3D rendering of the tomographic data obtained from a CT scan. The volumetric data are stored as a stack of 2D image slices or as a single 3D texture object, and the complete dataset was then rendered using the software’s volume renderer tool.

Figure 1.5 shows the result of direct volume rendering using the volume graphics application. The volume-rendering technique delivered each voxel of the whole volume data with different opacity, as presented on the histogram of the image data (Fig. 1.5a). Different opacities were expressed with different frequencies of the image data value, resulting from the different absorptance intensities of the X-ray signal received by the timber. This technique enabled

precise analysis of the timber properties by specifying the opacity of the voxel data on their gray value using the opacity transfer function (Praßni *et al.* 2010). The opacity transfer function is represented by the gray line plotted over the histogram.

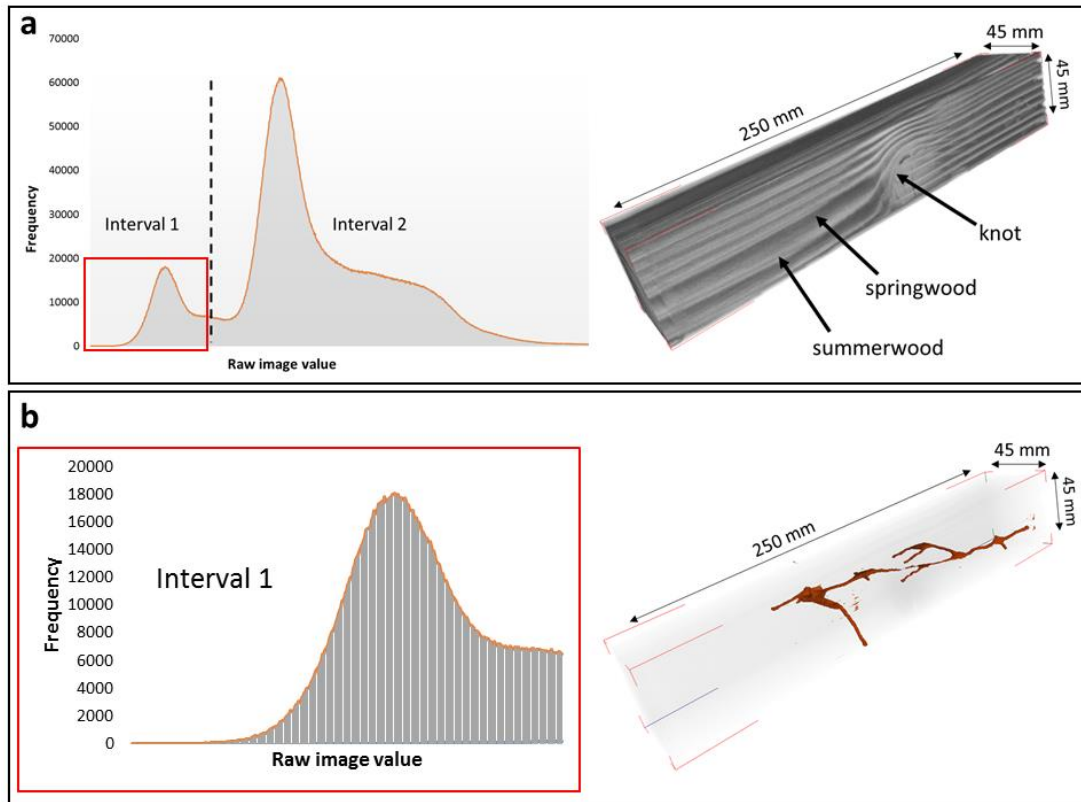


Fig. 1.5. The 3D rendering technique using a volume graphic application. **a** The histogram of CT image data and 3D visualization in volume renderer (pong) mode. **b** Visualization of the nest-gallery inside the timber created by extracting interval 1 of the complete volumetric data set and creating an independent volumetric data set.

In the histogram, an opacity curve specifies the transparency mapping of the gray levels; the lower the line, the more transparent the corresponding gray value is rendered. The shape of the opacity curve can be adjusted by

adding control points. By rendering the complete data set (Fig. 5a), timber properties such as springwood, summerwood and knots can be characterized, and by further specifying the opacity area (Fig. 1.5b), the gallery systems inside the timber can be visualized.

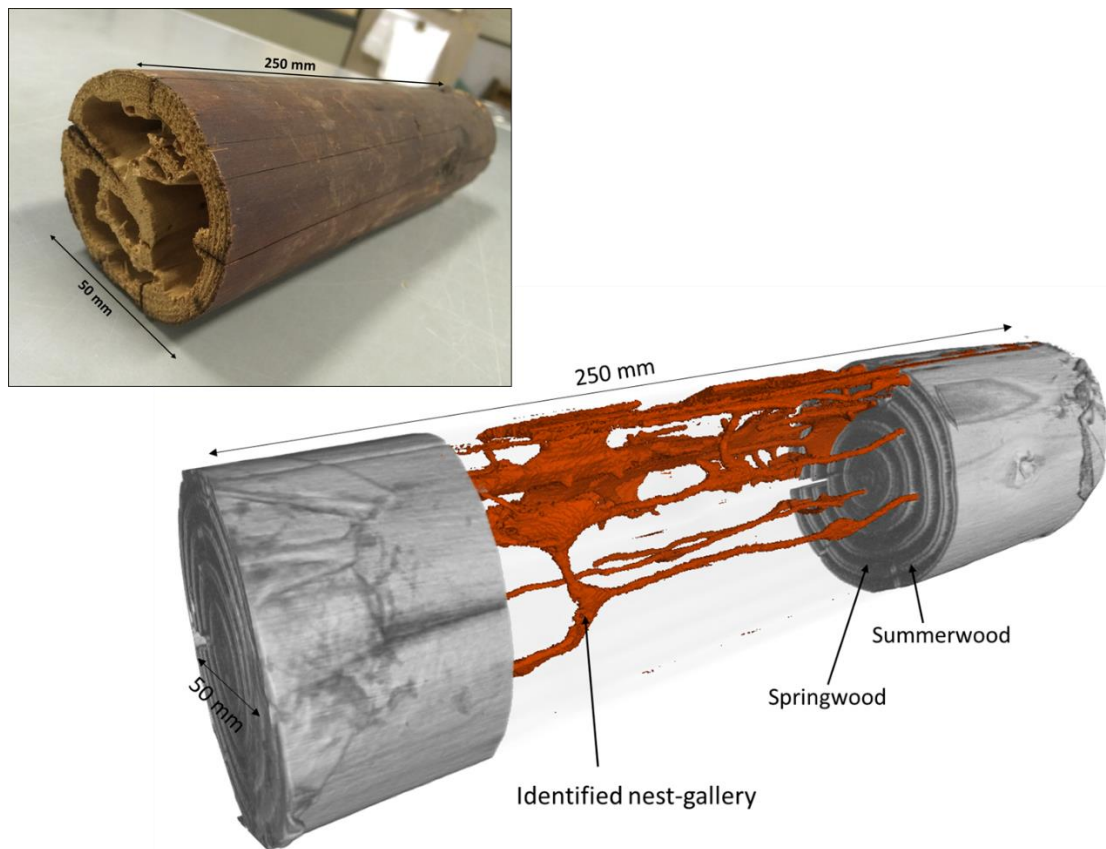


Fig. 1.6 The 3D isosurface volume rendering: By specifying a certain value (isovalue) on image data, the isosurface will render all the voxels with bigger values. Therefore, only the parts of the timber that are correlated with those voxels are visualized.

Figure 1.6 presents the isosurface volume-rendering technique. The isosurface is defined as the surface in a continuous area of the volume data at which the value equals a pre-defined isovalue (Bosma 2000). Direct volume

rendering (in Fig. 1.5) was performed by generating images of a 3D volumetric data set without explicitly extracting geometric surfaces from the data. On the other hand, isosurface rendering was conducted by dragging the isosurface line on the histogram of the data set either to the left or right to respectively decrease or increase the gray level at which the 'isovalue' was rendered. Various densities of timber physical properties generated different data values. By specifying a certain value, the isosurface will render all of the voxels with values bigger than the isovalue. Therefore, we were able to specify the density profile of the timber and to show the respective part, such as the summerwood part (Fig. 7), and to omit the other parts of the timber with lower isovalues.

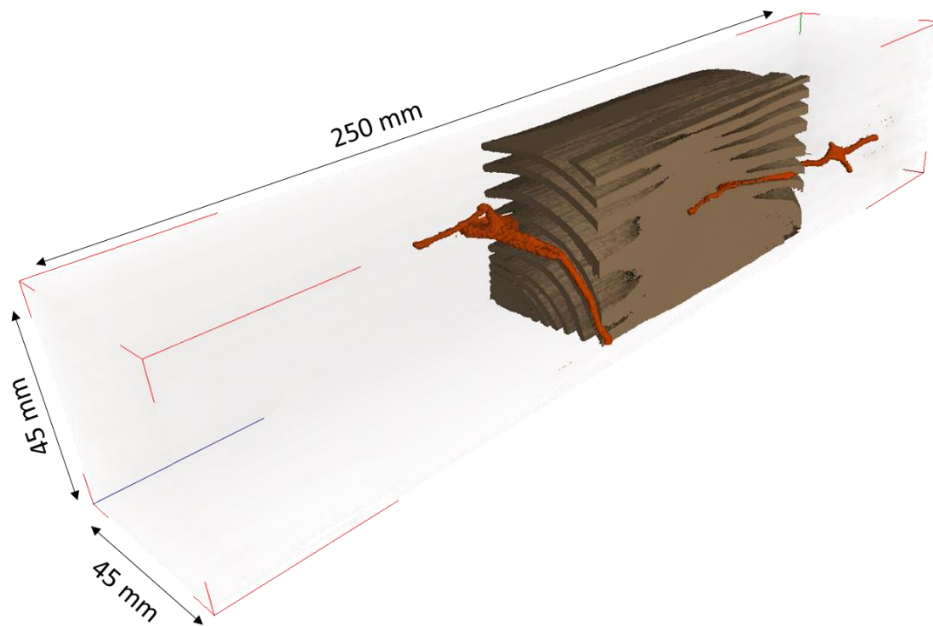


Fig. 1.7 The 3D isosurface volume rendering: The summerwood part of annual growth rings can be visualized, while the springwood parts, which have a lower density value, can be visually omitted.

1.4. Objective and outline of the study

The present study aims to elaborate the nesting biology of the western drywood termite, *I. minor* (Hagen). This study focuses on the observation of nest-founding activities in the natural environment and X-ray tomographic analysis of the nest-gallery systems of *I. minor*. The study is divided into seven chapters.

Chapter 1 introduces the background, objective and outline of the study. The chapter also explains the biology of *I. minor* and gives a short description of the principles and application of X-ray CT, which was used in most parts of the study.

Chapter 2 thoroughly discusses the nest-founding activities of *I. minor* following the nuptial flight on six commercial timbers. The chapter evaluates the timber preference of *I. minor* reproductives in establishing the royal chamber, and nest-site selectivity on various parts of the timber, including the preference of nest-site location in response to the timber arrangement.

Chapter 3 presents X-ray tomographic analysis of the structure of the initial chambers excavated by *I. minor* as part of its nest-founding activities and foraging. The chapter also discusses the new nest-founding activity of *I. minor* either following the nuptial flight or from the foraging activities of the colony to extending the nest-gallery to a new timber.

Chapter 4 presents X-ray tomographic analysis of the development of the royal chamber and an incipient colony of *I. minor* following the nuptial flight, in the first year of the founding of a new colony. The chapter presents important information regarding the nesting biology of *I. minor* in initiating the colony.

Chapter 5 presents X-ray tomographic monitoring of the colonization process of foraging groups of *I. minor* in previously unoccupied timber and discusses how the groups maintain their nest-gallery system. The study also examines the caste composition of isolated groups in search of a better understanding of how one-piece nester types sustain their colonies.

Chapter 6 provides important insights into how drywood termites establish and maintain their nest-gallery systems in response to the internal structure of fibers, growth rings and other anatomical properties of timbers. The chapter reveals the selectivity of *I. minor* in its foraging pattern and its adaptability with regard to the timber environment.

Chapter 7 provides the conclusion of the study.

CHAPTER

2

TIMBER PREFERENCE OF THE DRYWOOD TERMITE, *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae) IN FOUNDING A COLONY FOLLOWING THE NUPTIAL FLIGHT

In this chapter, the nest-founding activities of I. minor reproductives on six species of commercial timbers are evaluated. The chapter discusses the timber preference, nest-site selectivity on various parts of the timber and nest-site preference in response to the timber arrangement.

2.1 Introduction

The information of nutritional ecology of *I. minor* and its feeding preference among various timbers is very important in order to examine the nest-founding activities as well as nest-gallery development. *Incisitermes minor* has been reported to attack most of the common USA timbers, including sycamore, oak, alder, Monterey cypress, redwood, California laurel, buckeye, eucalyptus, willow, peach, pear, almond, cherry *etc.* (Light 1934). Rust *et al.* (1979) suggested that Douglas-fir was the most preferred wood of *I. minor*, while Indrayani *et al.* (2006) found that spruce (*Picea abies* Karst.) was the wood species most susceptible to *I. minor* after evaluating its feeding responses to 10 commercial timbers from Japan, the USA and Malaysia.

As one-piece nester, drywood termites live and forage in a single piece of timber. Therefore, information on their feeding ecology may provide important feedback regarding their nest-founding preferences on timber. In this chapter, the nest-founding activities of *I. minor* from nuptial flight in a natural situation against six commercial timbers were evaluated. Until now, there has been no information on the timber preference of this species for nest-founding. Thus, this study will be very important in elucidating which timber is least preferred by *I. minor* reproductives. This information will be valuable for determining which structural timbers should be used in infested areas as part of a sustainable pest management strategy.

2.2 Materials and Method

2.2.1 Wood specimens

Six species of commercial timbers were used in this study to evaluate the nest-founding activities of *I. minor* in a choice preference test in a natural environment. The timbers included three Japanese timbers, hinoki (*Chamaecyparis obtusa* Endl.), karamatsu (*Larix leptolepis* Gord.), and sugi (*Cryptomeria japonica* D. Don), and three USA timbers, Douglas-fir (*Pseudotsuga menziessi* Mirbel), western red cedar (*Thuja plicata* Donn ex D. Don) and spruce (*Picea sitchensis* Bong. Carriere). Eighty pieces were prepared for each species.

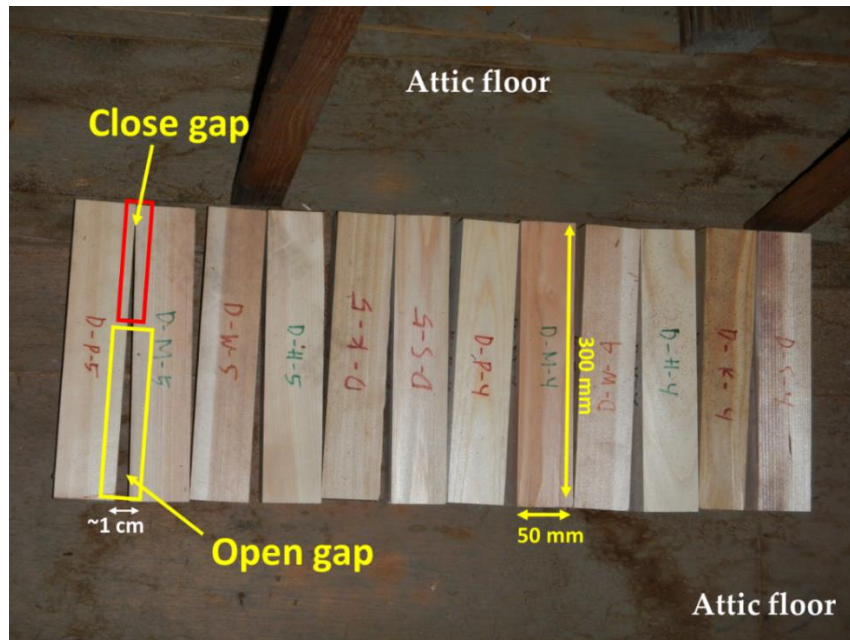


Fig. 2.1 Timber arrangement used to record nest-founding activity of *I. minor* from nuptial flight against six commercial timber.

All timbers were made up of a combination of sapwood and heartwood with dimensions of 50 (R) x 50 (T) x 300 mm (L). In total, 480 pieces of timber

were laid at random positions in highly infested attics in four houses in Wakayama Prefecture, Japan (Fig. 2.1). The timbers were arranged with a closed gap (CG) between two timbers at one end and an open gap (OG) between the two timbers at the other end. The CG is the area (red rectangle on Fig. 2.1) where the edges of the two timbers are touching each other, while the OG is the area (yellow rectangle on Fig. 2.1) where edges of the two timbers are separated by a ~1-cm gap.

3.2.2 Monitoring and Analysis

Annual monitoring was conducted in November, two months after the *I. minor* swarming season, from 2012 - 2015. The infested timbers were recorded in detail by identifying the positions of excavated holes in the timbers (sapwood, heartwood and border line between sapwood and heartwood), and the locations of excavated holes with respect of the timber arrangement (CG, OG, and the cross-sectional surface (CS), bottom surface (BS) and upper surface (US) of the timbers). In July 2016, all of the infested timbers were collected from the field.

One-way analysis of variance (ANOVA) following Tukey's honest significant difference (HSD) test ($p < 0.05$) using IBM SPSS 23 (IBM Inc., USA) was used to analyze the preferences of *I. minor* for the type of timber, nest-site position on the timbers (sapwood, heartwood and border line between sapwood and heartwood) and nest-site location (CG, OG, CS, BS and US of the timbers) in nest-colony founding.

2.3 Results

2.3.1 Nest-founding activities on six species of commercial timbers

Field experiments were set up in four houses, to be referred to as Houses A, B, C and D. The infestation by royal pairs on timbers following the nuptial flight can be identified from the presence of an entrance hole on the timber surface. The infestations were classified into two groups: (1) successful nest-founding activity, as indicated by sealed entrance holes surrounded by chewed wood powders, and (2) attempted infestations resulting from the timber assessment activities of *I. minor* reproductives in search of preferred nest-site locations (Himmi *et al.* 2014), as evidenced by feeding traces, chewed spots and unsealed holes (Fig. 2.2). A sealed entrance hole indicated that the royal pair completed the excavation of the royal chamber, entered the chamber and sealed it using cement pellets (Harvey 1934b; Himmi *et al.* 2014).

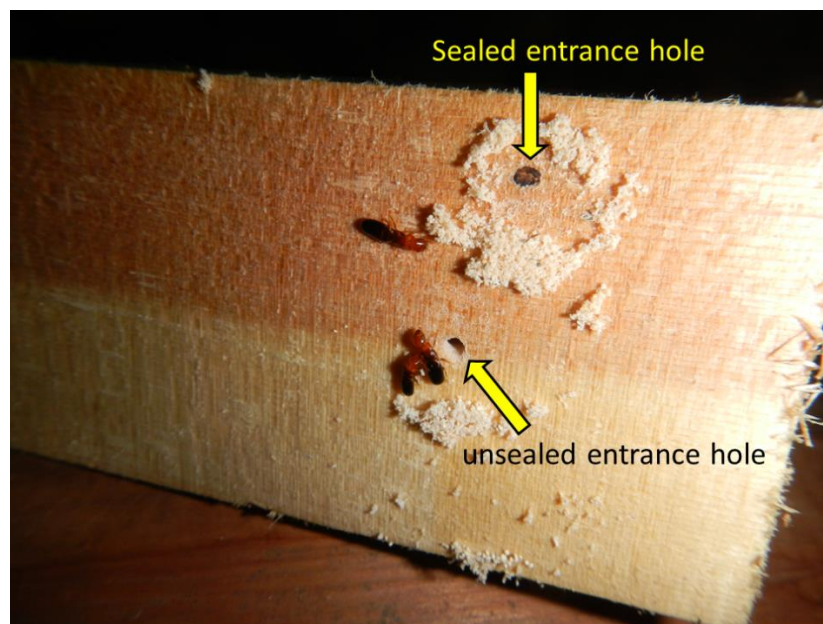


Fig. 2.2 Nest-founding activities of royal pairs on a spruce timber.

Figure 2.3 and Fig. 2.4 present the results of successful nest-founding activities and attempted infestations by *I. minor* reproductives in six species of commercial timbers in four houses. In total, the successful nest-founding activities resulted in 218 royal chambers, with 0, 100, 65 and 53 royal chambers recorded in Houses A, B, C, and D, respectively. The attempted infestations generated a total of 124 attacks, with 1, 33, 33, and 57 attacks on timbers in Houses A, B, C and D, respectively.

Among the six commercial timber species, hinoki showed the highest total number of royal chambers with 69 sealed entrance holes, followed by spruce, western red cedar, sugi, Douglas-fir and karamatsu, with 61, 50, 24, 12 and 2 sealed entrance holes, respectively (Fig. 2.3). On the other hand, data on attempted infestations showed that the highest number of attacks, at 33, occurred in western red cedar, followed by spruce, hinoki, Douglas-fir, sugi and karamatsu with 26, 23, 23, 15 and 4, respectively. (Fig. 2.4).

There was no record of successful nest-founding in House A (Fig. 2.3) and only one attempted infestation on sugi timber (Fig. 2.4) during 4 years of monitoring. Therefore, the data from House A are omitted from further statistical analysis, and we relied on the results from the other three houses (B, C and D).

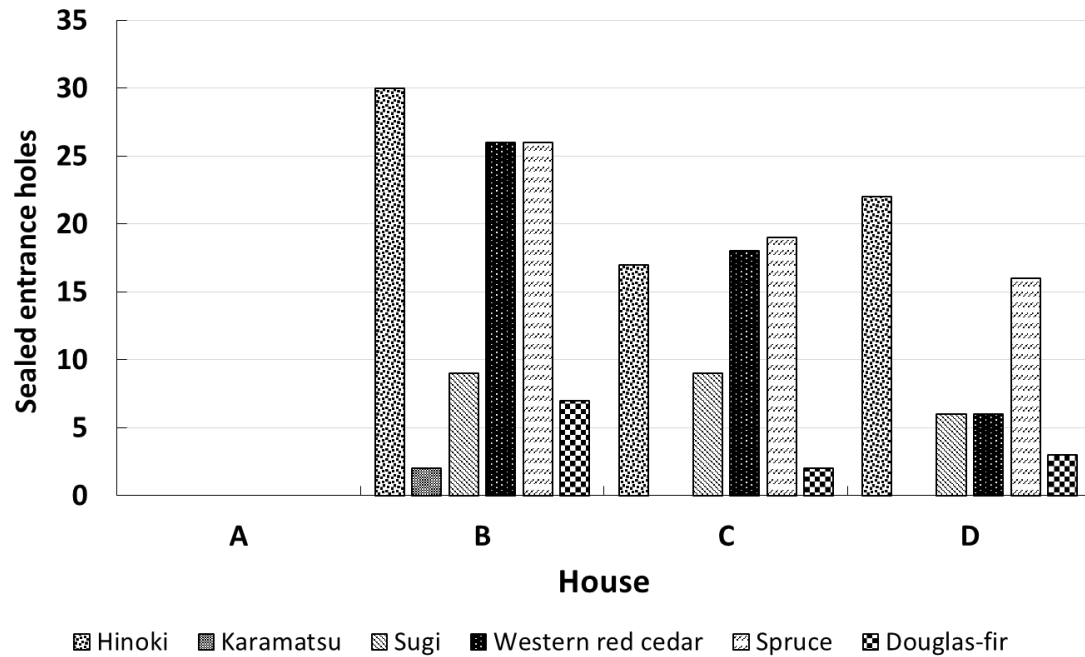


Fig. 2.3 Successful nest-founding activities of *I. minor* reproductives on six species of commercial timbers.

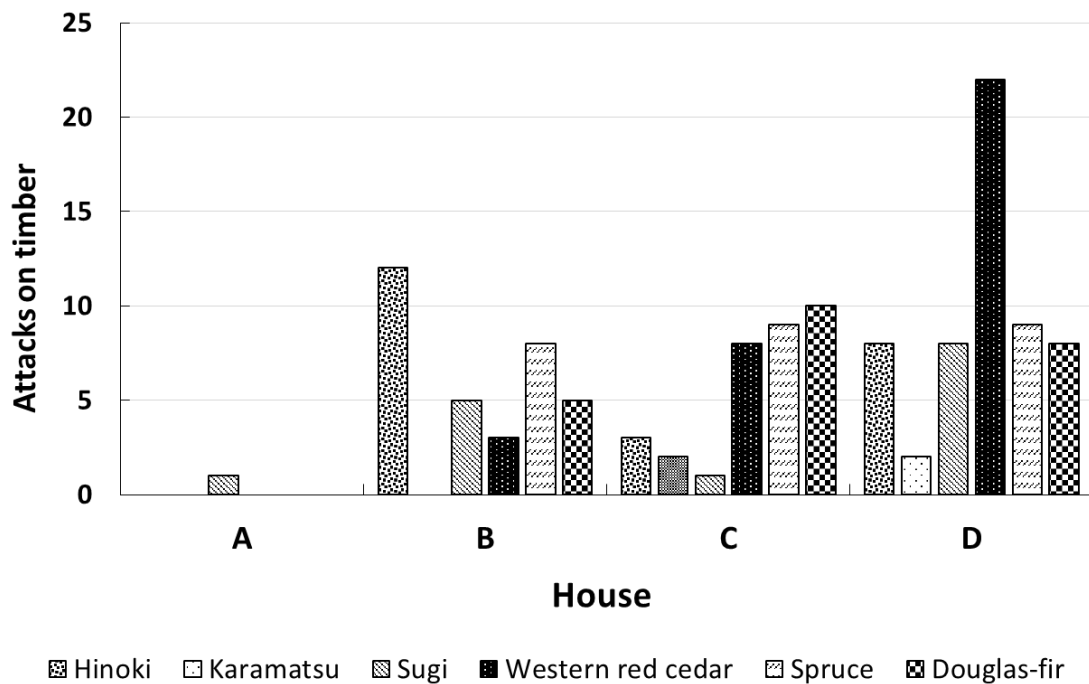


Fig. 2.4 Attempted infestations by *I. minor* reproductives on six species of commercial timbers.

2.3.2 Timber preference

Figure 2.5 shows the mean numbers of successful nest-founding activities on the six commercial timber species. The data showed a statistically significant difference among the mean values of the timber specimens ($F = 8.319$, $df = 5$, $p = 0.001$).

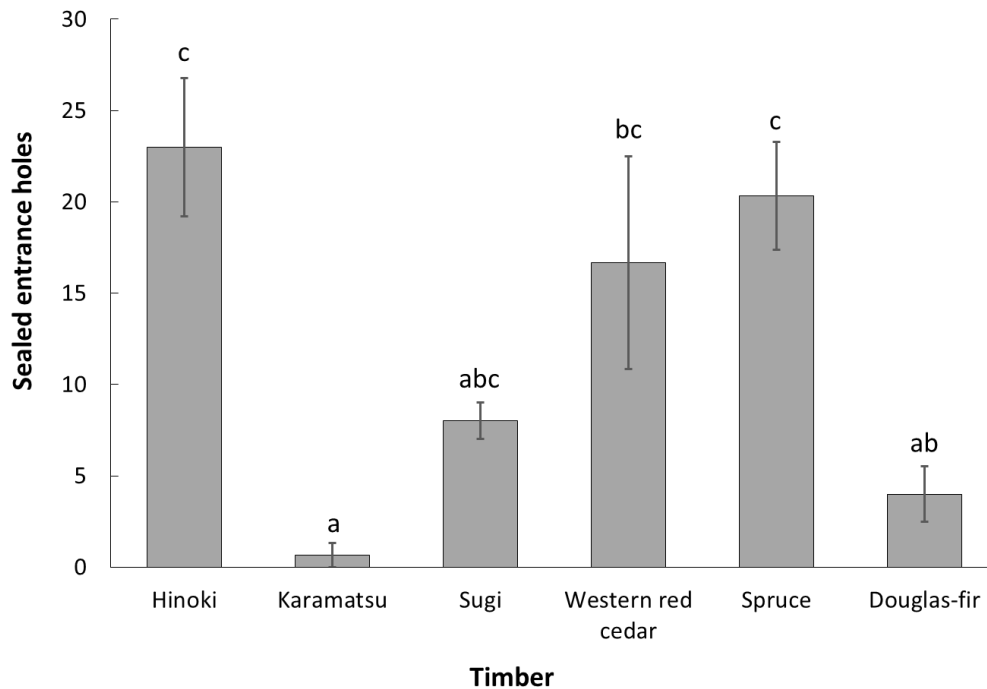


Fig. 2.5 Mean numbers of successful nest-founding activities by *I. minor* reproductives on six species of commercial timber as observed in three houses (B, C and D). Common letters across timbers show that no statistically significant difference was found by Tukey's HSD test ($p < 0.05$). Error bars show standard errors.

Hinoki (23.00 ± 3.79) showed statistically significant differences with karamatsu (0.67 ± 0.67 , $p = 0.003$) and Douglas-fir (4.00 ± 1.53 , $p = 0.012$), but no statistically significant differences with spruce (20.33 ± 2.96 , $p = 0.990$), western red cedar (16.67 ± 5.81 , $p = 0.722$), or sugi (8.00 ± 1.00 , $p = 0.052$)

(Fig. 2.5). Spruce showed a statistically significant difference with karamatsu ($p = 0.009$) but statistically significantly lower to Douglas-fir ($p = 0.32$). It showed no statistically significant differences with hinoki ($p = 0.990$), western red cedar ($p = 0.959$) or sugi ($p = 0.137$). The results suggested that hinoki and spruce were significantly preferred over karamatsu and Douglas-fir by royal pairs of *I. minor* for the purpose of establishing royal chambers.

Figure 2.6 shows the mean numbers of attempted infestations by royal pairs during nest-founding activities. The data suggest that there were no statistically significant differences among the six species of commercial timbers ($F = 1.492$, $df = 5$, $p = 0.263$).

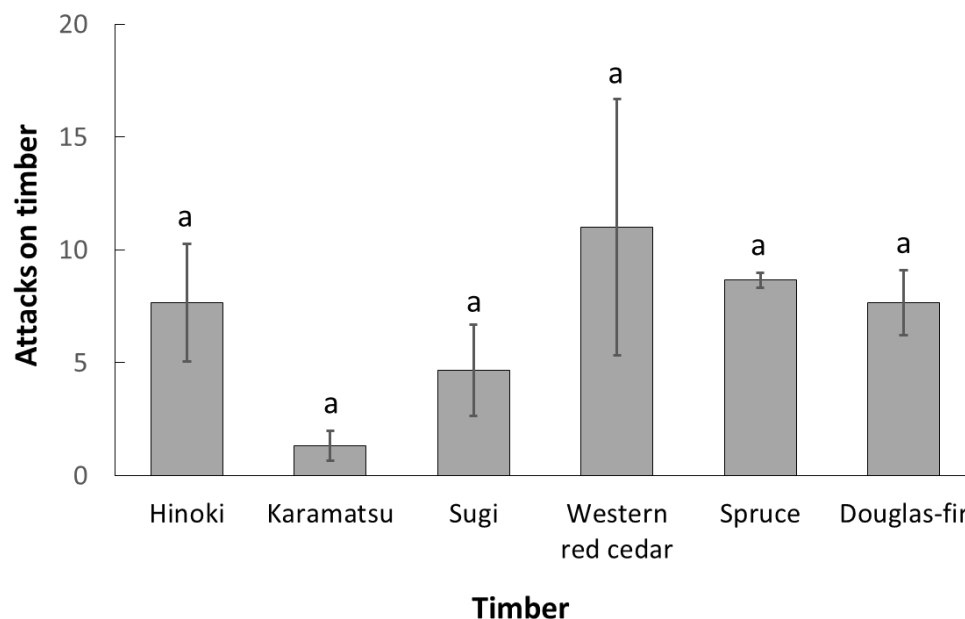


Fig. 2.6 Mean numbers of attempted infestations by *I. minor* reproductives on six commercial timber species as observed in three houses (B, C and D). Common letters across timbers show that no statistically significant difference was found by Tukey's HSD test ($p < 0.05$). Error bars show standard errors.

2.3.3 Nest-site position on the timber

The nest-site position on the timber is determined by the nest-site selectivity of *I. minor* reproductives in excavating the royal chambers in the sapwood part, the heartwood part, or on the border line between the sapwood and heartwood of the timber. In total, 88.53% of royal chambers were excavated in the sapwood, while 9.17% and 2.29% of total excavations were found in the heartwood and on the border line between the sapwood and heartwood, respectively (Fig. 2.7a).

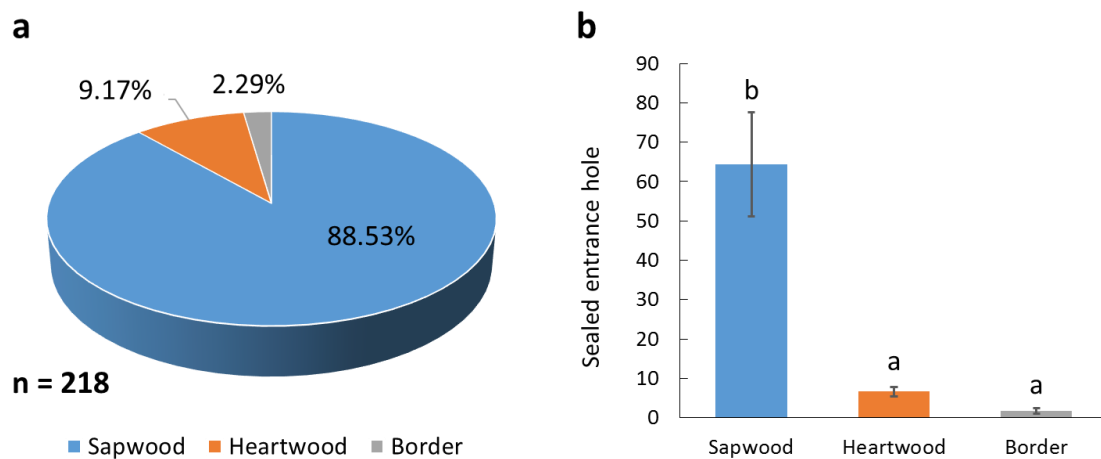


Fig. 2.7 Nest-site positions on the timbers as observed in three houses (B, C, and D). **a** Percentage of total nest-sites located on each part of the timbers. **b** Mean numbers of nest-sites located on each part of the timbers. Common letters show that no statistically significant difference was found by Tukey's HSD test ($p < 0.05$). Error bars show standard errors.

Figure 2.7b suggests that there is a statistically significant difference between timber parts (sapwood, heartwood and border line between sapwood and heartwood) ($F = 20.597$, $df = 2$, $p = 0.002$) in the overall establishment of royal chambers. The data suggested that sapwood ($64.33 \pm$

13.22) was statistically significantly preferred over heartwood (6.67 ± 1.20 , $p = 0.004$) and the border line between sapwood and heartwood (1.67 ± 0.67 , $p = 0.003$) by *I. minor* reproductives as the location at which to establish royal chambers.

Table 2.1 shows the nest-site selectivity of *I. minor* reproductives on each timber specimen. The data suggested that the royal pairs preferred the sapwood part of hinoki, sugi, spruce, and western red cedar for establishing royal chambers over the heartwood part and the border line between the sapwood and heartwood (Tukey's HSD test, $p < 0.05$). In western red cedar, 100% royal chambers were found on the sapwood part. Even though 100% of royal chambers on karamatsu were also found on the sapwood part, the record of infestations was very low (only 2 royal chambers in 4 years). The results from karamatsu and Douglas-fir, the two timbers which were less preferred by *I. minor* reproductives (Fig. 2.5), showed no statistically significant differences among the sapwood part, the heartwood part, and the border line between the sapwood and heartwood (Tukey's HSD test, $p > 0.05$).

Table 2.1 Nest-site position on each timber species as observed in three houses (B, C, and D)

Timber specimens	Timber part	Royal chambers*	<i>p</i> value**
Hinoki	Sapwood	21.33 $\pm$ 4.10 b	0.001
	Heartwood	1.00 $\pm$ 0.58 a	
	Border	0.67 $\pm$ 0.67 a	
Karamatsu	Sapwood	0.67 $\pm$ 0.67 a	0.422
	Heartwood	0.00 $\pm$ 0.00 a	
	Border	0.00 $\pm$ 0.00 a	
Sugi	Sapwood	7.67 $\pm$ 1.33 b	0.001
	Heartwood	0.33 $\pm$ 0.33 a	
	Border	0.00 $\pm$ 0.00 a	
Douglas fir	Sapwood	2.33 $\pm$ 0.33 a	0.296
	Heartwood	1.33 $\pm$ 1.33 a	
	Border	0.33 $\pm$ 0.33 a	
Spruce	Sapwood	15.67 $\pm$ 2.67 b	0.001
	Heartwood	4.00 $\pm$ 0.58 a	
	Border	0.67 $\pm$ 0.33 a	
Western red cedar	Sapwood	16.67 $\pm$ 5.81 b	0.019
	Heartwood	0.00 $\pm$ 0.00 a	
	Border	0.00 $\pm$ 0.00 a	

*Values are means $\pm$ standard errors from three replications. Means followed by the common letters within the row for the same timber specimen show no statistically significant difference (Tukey's HSD test: $p < 0.05$).

**Values are the significance values (p) in ANOVA analysis. A significance value below 0.05 ($p < 0.05$) indicates that there is a statistically significant difference between the nest-site positions within a timber specimen.

2.3.4 Nest-site location in response to timber arrangement

The timbers were randomly arranged to observe the preference of royal pairs in selecting nest-site locations in response to the timber arrangement. Each sealed entrance hole on the timber specimens was ascribed to one of 5 different nest-site locations (Fig. 2.1): the CG area, OG area, CS area, US area and BS area. In total, 83.49% of royal chambers were established in the CG area, followed by the BS area, OG area, US area, and CS area with 11.01%, 3.21%, 1.38%, and 0.92%, respectively (Fig. 2.8a).

Figure 2.8b suggests that there were statistically significant differences among nest-site locations ($F = 20.315$, $df = 4$, $p = 0.000$). The CG area (60.67 ± 12.78) showed statistically significantly higher numbers of nest-sites than the BS area (7.67 ± 0.67 , $p = 0.001$), OG area (2.33 ± 1.45 , $p = 0.000$), CS area (1.00 ± 0.00 , $p = 0.000$), and US area (0.67 ± 0.33 , $p = 0.000$). The results indicated that the CG area of the timber arrangement was significantly preferred by *I. minor* reproductives over other areas as a location in which to establish royal chambers.

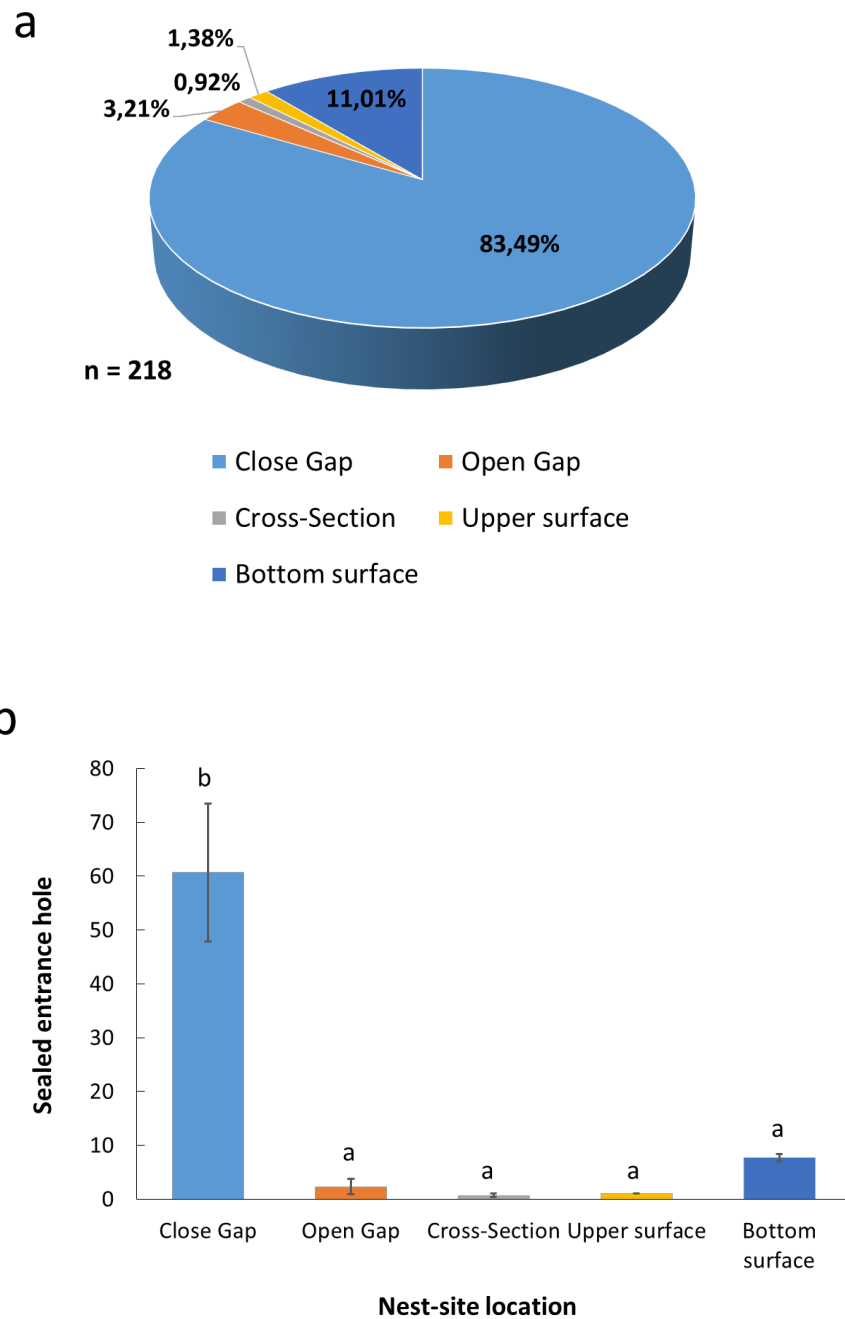


Fig. 2.8 Nest-site location in response to timber arrangement (Fig. 2.1) as observed in three houses (B, C and D). **a** Percentages of total numbers of nest-sites in various locations. **b** Mean numbers of nest-sites in various locations. Common letters show that no statistically significant difference was found by Tukey's test ($p < 0.05$). Error bars show standard errors.

2.4 Discussion

2.4.1 Timber preference

Based on the number of sealed entrance holes on the six commercial timber species, the ranking of timber species in order of the *I. minor* reproductives' preference for establishing a new colony was as follows: hinoki, spruce, western red cedar, sugi, Douglas-fir, and karamatsu (Fig. 2.3). Hinoki and spruce were the most preferred by dealate reproductives of *I. minor* for excavating royal chambers, but no statistically significant difference in preference was shown between these two species. Karamatsu was the least preferred timber among the specimens tested.

Indrayani *et al.* (2006) ranked the feeding preference of *I. minor* among those six timber species as follows: spruce > hinoki > Douglas-fir > western red cedar > sugi > karamatsu. These results suggested that the preference among timber species was almost similar between nest-founding and feeding. In our study, Douglas-fir was the second-least preferred by *I. minor* for establishing nests, while Indrayani (2006) suggested that Douglas-fir was preferred over western red cedar and sugi for feeding. However, Indrayani (2006) also reported that there was no statistically significant difference in feeding preference among Douglas-fir, western red cedar and sugi, a finding that was in accordance with our results (Fig. 2.5). Moreover, in a choice feeding test involving combined sapwood and heartwood from all of the timber specimens, Douglas-fir showed resistance against *I. minor* (Indrayani *et al.* 2006), which can explain the low number of successful nest-founding activities on this timber. Interestingly, in comparison with redwood, sugar

pine, sugar bush and jojoba timbers, Douglas-fir was reported to be the timber most preferred by *I. minor* (Rust *et al.* 1979).

Table 2.2 Earlier studies on physiochemical properties of six commercial timbers and its natural durability of against *I. minor*

Timber species	Density ^a (g/cm ³)	Extractive, % (EtOH/Benzene)	Feeding resistance against <i>I. minor</i> ^e	
			Sapwood	Heartwood
Hinoki	0.44	3.2 ^a	NR	MR
Karamatsu	0.50	2.6 ^a	R	R
Sugi	0.38	5.1 ^a	MR	R
Douglas-fir	0.55	4.4 ^d	R	R
Spruce	0.44	1.2 ^c	NR	NR
Western red cedar	0.37	14.1 ^b	MR	R

R = Resistance; MR = Moderate resistance; NR = Nonresistance.

^aThe handbook of wood industry (2004); ^bGardner and Hillis (1962); ^cAcetone-soluble extractives (gravimetric) (Caron *et al.* 2013); ^dScalbert (1992);

^eCombined-choice feeding test (Indrayani *et al.* 2006)

As presented in Table 2.2, both karamatsu and Douglas-fir showed feeding resistance against *I. minor* in both their sapwood and heartwood. The number of sealed entrance holes was very low in both karamatsu and Douglas-fir, with only 2 and 12 chambers were established in 4 years (Fig. 2.3), respectively. Douglas-fir has the highest density among the tested timbers at 0.55 g/cm³ and a high extractive content of 4.4% (Table 2.2). Karamatsu has a density of 0.50 g/cm³ and an extractive content of 2.6% (Table 2.2). By conforming the data to our results, we can conclude that physical factors

might be more important than the chemical contents of the timber in influencing the nest-founding preference of *I. minor* reproductives.

Hinoki and spruce have a similar density, i.e. 0.44 g/cm³, while western red cedar and sugi have densities of 0.37 g/cm³ and 0.38 g/cm³, respectively (Table 2.2). Those four timber species showed no statistically significant differences in terms of the nest-founding preference of *I. minor* reproductives (Fig. 2.5). The results suggested that *I. minor* may prefer timbers with a certain density profile (<0.50 g/cm³), as drywood termites have the ability to differentiate between food sources (Evans *et al.* 2007; Evans *et al.* 2005; Inta *et al.* 2007).

2.4.2 Nest-site selectivity

Along with timber preference, *I. minor* also showed nest-site selectivity, which was indicated by nest-site excavation in preferred positions on the timbers and in preferred locations with respect to the timber arrangement. The overall data suggested that *I. minor* reproductives prefer sapwood over heartwood (Fig. 2.7). Himmi *et al.* (2014) reported the nest-site selection behavior of dealate reproductives. The royal pairs assessed the timbers when attempting to establish the royal chamber as indicated by chewed spots, feeding traces, and unsealed holes in the timbers (Fig. 2.6). The assessment activities on the six timber species showed no statistically significant difference in timber preferences, indicating that these activities were an ongoing process that were part of the effort to establish the royal chamber in a preferred part of the timber.

In karamatsu and Douglas-fir, the two timber species least preferred by *I. minor* reproductives, no statistically significant preference for sapwood or heartwood was shown. The results are consistent with those of a previous study, which reported that both the sapwood and heartwood of these timbers showed resistance against *I. minor* (Table 2.2). Selectivity data regarding nest-site position on hinoki, spruce, sugi and western red cedar suggested that *I. minor* significantly preferred sapwood over heartwood (Table 2.1). In western red cedar, zero infestations were recorded in the heartwood. The heartwood of western red cedar was reported to show the only high resistance against *I. minor* in a no-choice feeding test (Indrayani *et al.* 2006). The high extractive content (14.1%, Table 2.2) and chemical factors may have deterrent effects against *I. minor* (Alfaro *et al.* 1981; Gardner and Hillis 1962; Scheffrahn 1991).

Infestation of the heartwood part of sugi and hinoki was rare (Table 2.1), with only one royal chamber established in the heartwood part in both timber species. The heartwood of sugi has been reported to have resistance against termites (Kano *et al.* 2004), and its extractives have a termicidal effect (Arihara *et al.* 2004; Sogabe *et al.* 2000) and show anti-termite activities (Shibutani *et al.* 2007). A colony of *I. minor* was also reported to avoid the heartwood part of sugi during the expansion of the nest-gallery system (Himmi *et al.* 2016c). The heartwood of hinoki was also reported to have termicidal activity (Kinjo *et al.* 1988; Matsushima *et al.* 1990).

The heartwood of spruce has been reported to have 70% extractives than the sapwood (Caron *et al.* 2013). However, the heartwood of spruce was classified as nonresistant to *I. minor* (Table 2.2). Our previous study also suggested that spruce heartwood shows no deterrence against *I. minor*, as

indicated by the extension of the nest-gallery by the colony throughout the sapwood and heartwood parts of spruce timbers (Himmi *et al.* 2016c). Even though the feeding response of *I. minor* showed that both the sapwood and heartwood of spruce were nonresistant to feeding, *I. minor* reproductives significantly preferred sapwood to heartwood when establishing the first royal chamber.

Incisitermes minor reproductives were also reported to show selectivity for the springwood part of the annual growth ring of the sapwood when establishing royal chambers (Cabrera and Scheffrahn 2001; Harvey 1934a; Himmi *et al.* 2014). Yoshimura (*personal communication*) assumed that the density ratio of springwood to summerwood in the annual growth rings in karamatsu and Douglas-fir was a factor in these timbers being the least preferred by *I. minor* reproductives. The springwood in the growth rings of karamatsu has a density of 0.35 g/cm³, while the summerwood has a density of 0.77 g/cm³, resulting in a density ratio of 2.2 (FPPRI 2004). Douglas-fir has a density ratio of 2.6, as the density values of springwood and summerwood are 0.30 g/cm³ and 0.79 g/cm³, respectively (FPPRI 2004). The higher the density ratio, the bigger the difference in density between springwood and heartwood, and, therefore, the more difficult it is for *I. minor* reproductives to determine a preferable spot at which to establish the royal chamber.

With respect to the timber arrangement, *I. minor* reproductives significantly preferred the CG area (Fig. 2.7). Previous studies have reported that *I. minor* reproductives love wood cracks, crevices, and holes as locations in which to establish the royal chamber (Cabrera and Scheffrahn 2001; Harvey 1934a). When designing the timber lay out in the present study, the CG area

was situated to resemble wood cracks and crevices, and the results confirmed that royal pairs do exhibit selectivity in determining their nest location.

2.5 Chapter summary

Incisitermes minor reproductives showed a timber preference in establishing their royal chambers to initiate new colonies. Among the six timber species studied, the order of timber preference was as follows: hinoki, spruce, western red cedar, sugi, Douglas-fir, karamatsu. *Incisitermes minor* reproductives also showed nest-site selectivity for their preferred part of the timbers, *i.e.* the sapwood; and for their preferred location within the timber arrangement, *i.e.* in the CG area.

CHAPTER

3

THE STRUCTURE OF INITIAL CHAMBER AND NEST-FOUNDING ACTIVITY OF THE DRYWOOD TERMITE, *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae)

*In this chapter, X-ray tomographic analysis of initial structure of the royal chamber; nest-founding activity and nest-site selection of the drywood termite *Incisitermes minor* will be discussed. The chapter has been published on *Journal of Wood Science* (2014), 60(6): 453-460.*

3.1 Introduction

The structure of initial chamber in drywood termite has been examined by dissection (Harvey 1934a; Harvey 1934b; Morimoto 2000), however it is difficult to assess important properties of the structure, such as volume and diameter. The introduction of X-ray computed tomography (CT) as a non-destructive approach to analyzing termite nests will bring us a better understanding of the nesting biology of this cryptic insect.

This chapter presents X-ray tomographic analysis of the structure of initial chambers excavated by *Incisitermes minor* as part of nest-founding activities and foraging. Important qualitative and quantitative properties of the initial chamber such as volume, diameter, depth and length, will be able to be determined. Nest-site selection mechanism in the target timber, nest-founding and foraging activities of *I. minor* will also be discussed.

3.2 Material and Method

3.2.1 Wood specimens and nest-founding activity

The wood specimens were selected based on the feeding responses of *I. minor* to 10 commercial timbers as reported by Indrayani *et al.* (Indrayani *et al.* 2006). Sitka spruce (*Picea sitchensis* Bong. Carriere) timbers, with dimensions of 50 (R) x 50 (T) x 1000 mm (L) and made up of a combination of sapwood and heartwood portions, were used in this study. Evaluation of the initial nest-founding activity of *I. minor* in a natural context was carried out at four infested houses in Wakayama Prefecture, Japan. Fifteen pieces of Spruce timbers were laid with gaps of ~10 mm between them in the highly infested attic area at each house. The timbers were laid in random positions, without

considering whether tangential sections, radial sections, sapwood parts, and heartwood parts were oriented in any particular direction. The experimental set-up was conducted on August 3, 2012, one month before *I. minor* swarming season in the Wakayama area, which was reported to be in September (Yoshimura 2011).

3.2.2 Analysis of initial structure of royal chamber

The monitoring was conducted in the following fall season, on November 15, 2012. All of the infested timbers were brought back to the laboratory and prepared for CT scan analysis. Each infested timber was imaged using a large-scale X-ray CT (Y.CT Modular, YXLON International GmbH, Germany) at Kyushu National Museum. The digital data thickness was 1 mm, 1024 x 1024 pixels, with 3.17 two-dimensional (2D) image slices per mm.

CT data were reconstructed into 3D images, 2D section images, and series of virtual cuts using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH Germany). The CT images revealed important properties of the chambers: depth, length, diameter and volume. Volume properties were measured by calculating the voxel properties in 3D-image reconstructions. The 3D images of *I. minor* nests were reconstructed from 2D digital image data and displayed as discrete values of a density function $F(x, y, z)$, which represented physical properties. The other important properties were obtained by 2D image analysis.

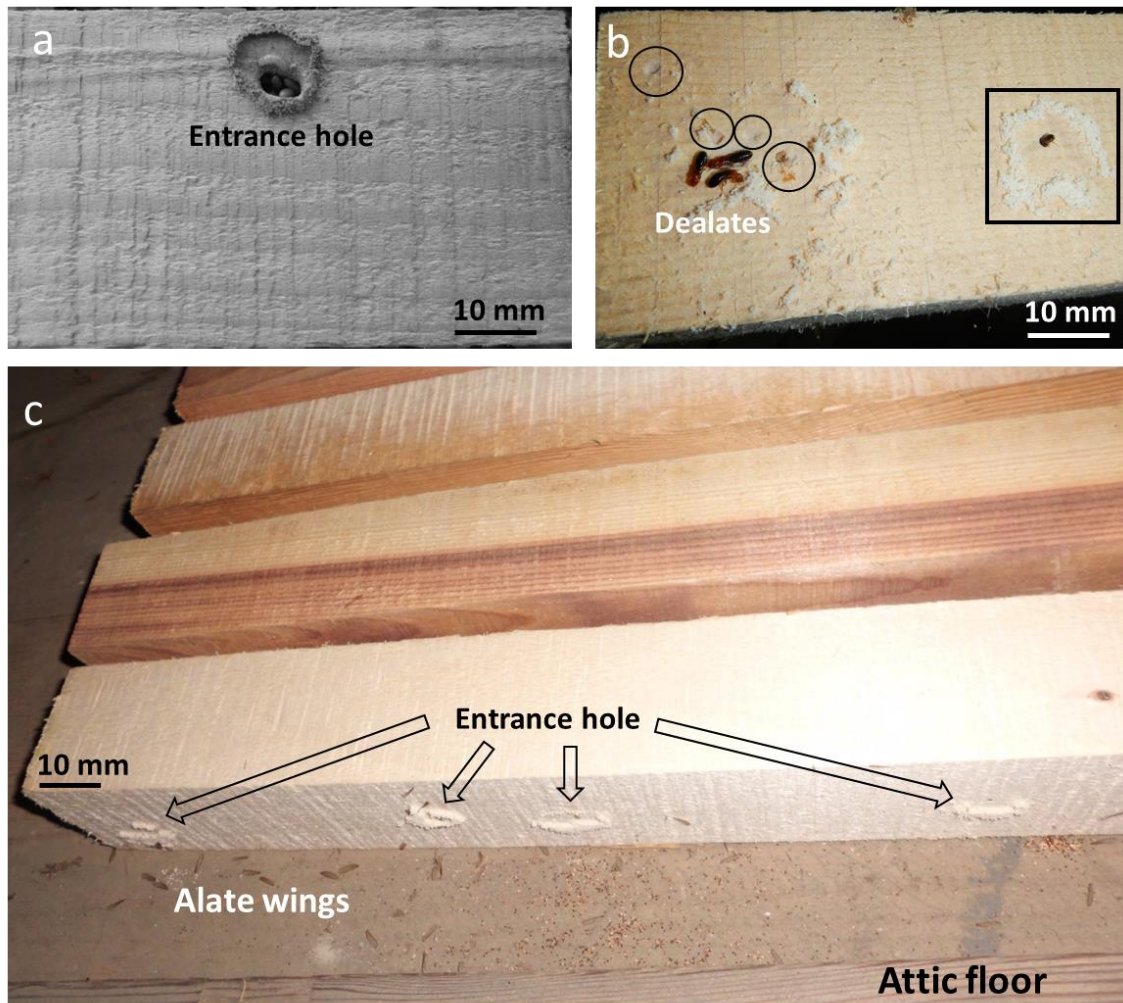


Fig. 3.1 Nest-founding activity of *I. minor*. **a** New infestation hole created due to colony expansion found on the Spruce A timber. **b** Nest-site selection behavior: dealate reproductives assessed the timber to establish the royal chamber. Chewed spots are marked with circles, while an under-excavated entrance hole is marked with a square. **c** Nest-founding activity following the nuptial flight in the Spruce B timber: four sealed royal chambers. Alate wings were found on the attic floor near the excavated holes.

3.3 Results

The monitoring was conducted two months after the *I. minor* swarming season in Wakayama. Three infested spruce timbers, namely the Spruce A, Spruce B, and Spruce C timbers, were analyzed by X-ray CT scan. Two kinds of infestation were found on those three timbers. Spruce A was infested by a group of termites that emerged from their natal nest. The other two timbers were infested by dealate reproductives following the nuptial flight, with four and one entrance holes on the Spruce B and C timbers, respectively. The details of the infestations of each timber are presented in Table 3.1, while the nest-founding activities in spruce timbers are displayed in Fig. 3.1.

Table 3.1 Detail of infestations in spruce timbers

Timber specimen	Infested by	Number of entrance hole	Nest-site location					
			Sa	He	Bo	TS	RS	CS
Spruce A	A group of foragers	1	○			○		
Spruce B	Royal pair	4	○				○	
Spruce C	Royal pair	1	○				○	

Sa = Sapwood, He = Heartwood, Bo = Border line of sapwood-heartwood, TS = Tangential section, RS = Radial section, CS = Cross section

The Spruce A timber was infested by a group of termites that emerged from the attic floor and attacked the adjacent bottom surface of the timber. The term pseudergates (false workers) is used to refer to a group of individuals that infested the Spruce A timber, since the profile of the caste pattern of the group members was not fully characterized. This definition of pseudergates follows Roisin's description (2000) of pseudergates as

totipotent individuals that can give rise to any of the three terminal castes (alate, replacement reproductive, soldier). The group excavated an entrance hole in a fairly wide springwood (Fig. 3.1a) of a tangential section of the sapwood. The entrance hole was excavated as an ample passageway (~8 mm) and was located adjacent to the emergence hole of the natal colony. Termites surrounded the entrance hole with cement pellets to connect it with the emergence hole of the natal colony. A group of pseudergates was highly engaged in excavation activities when the timber was inspected.

Figure 3.1b shows the nest-site selection behavior of dealate reproductives. During the monitoring, we observed two pairs of dealate reproductives were assessing a spruce timber apart from the three infested Spruce timbers (Table 3.1). Several chewed spots, indicated by circles (Fig. 3.1b), were encountered on the heartwood and sapwood of the radial section. The pairs were assessing the timber in order to establish an entrance hole. One entrance hole on the sapwood, marked by a square (Fig. 3.1b), was under excavation by a pair of dealate reproductives when the timber was inspected. The entrance hole was not yet sealed as the pair were digging the spot and placing the chewed wood in the surrounding area.

Figure 3.1c shows four royal chambers found on the Spruce B timber. The royal chambers were completely excavated, as all the entrance holes were sealed. Alate wings were found in the nearby excavated holes. According to Harvey (Harvey 1934b), the alates divest of their wings upon landing from their dispersal by lowering and bracing the wing tips against the timber. Then the dealates crawl to find a mate, and the new pair of reproductives work

together to establish the entrance hole (Harvey 1934b) in accordance with the case shown in Fig. 3.1b.

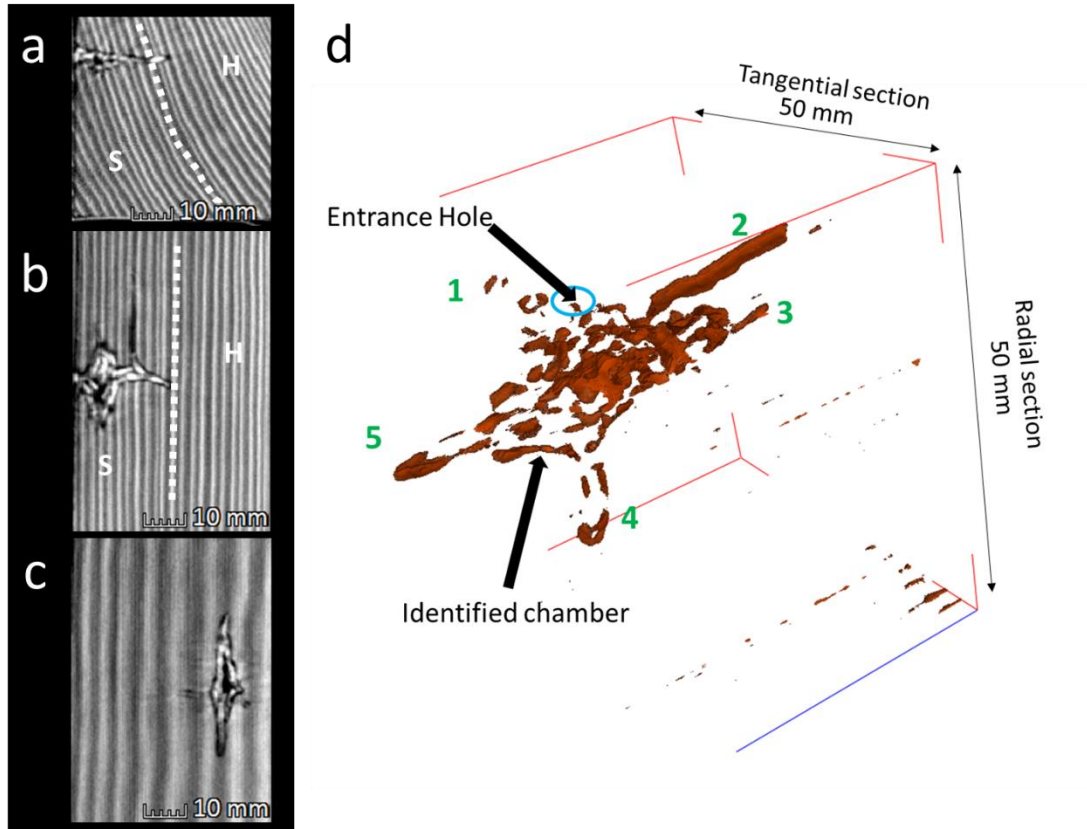


Fig. 3.2 CT images of the newly excavated chamber in the Spruce A timber. **a** A tunnel gallery was transversely excavated to several layers of annual growth rings. **b** A 2D image of the chamber as viewed along from the radial section, showing an aggregation of termites inside the chamber. **c** A 2D image of the chamber as viewed along the tangential section (sapwood). **d** A 3D image of the chamber: exploratory tunnels in five directions are indicated by number (1 – 5). The red color on the 3D image indicates the empty space inside the chamber, and termite presence is indicated by the uncolored area inside the chamber.

S: sapwood; H: heartwood. Springwood and summerwood are indicated by the darker and lighter colors in 2D CT images, respectively.

Figure 3.2 shows 2D and 3D CT images of the newly excavated chamber in the Spruce A timber, while the measurements of relevant properties are shown in Table 3.2. Termites excavated the big entrance hole (diameter 8.2 mm) on the wider springwood of the tangential section (Fig. 3.1a) at the edge of the timber (Fig. 3.2d). The first chamber was transversely excavated 26.2 mm from the surface (Table 3.2) across several annual growth rings and elongated parallel to the axial system in the sapwood part of the timber (Fig. 3.2a – c). The chamber (11.9 mm max. diameter and 114.8 mm³ volume) was spacious enough to accommodate the aggregations of dozens to twenties of termites (Fig. 3.2b, d). This chamber was evaluated as an extension for infesting a new timber given that the entrance hole was adjacent to the emergence hole of the natal nest.

As shown in Fig. 3.2d, five exploratory tunnels were excavated by the invasive colony. One tunnel gallery (No. 1 in Fig. 3.2d) was excavated perpendicularly to the axial system to follow the springwood of a particular growth ring of the sapwood. Three tunnel galleries were excavated parallel to the longitudinal axis of the springwood of the sapwood (Nos. 2, 3 and 5 in Fig. 3.2d). Another tunnel gallery (No. 4 in Fig. 3.2d) was transversely excavated across the grain from the sapwood to the heartwood (Fig. 3.2a). The average diameter of the tunnel galleries was so narrow (2.2 mm, Table 3.2) that only a single termite could pass through it at one time (Fig. 3.2a – b, d).

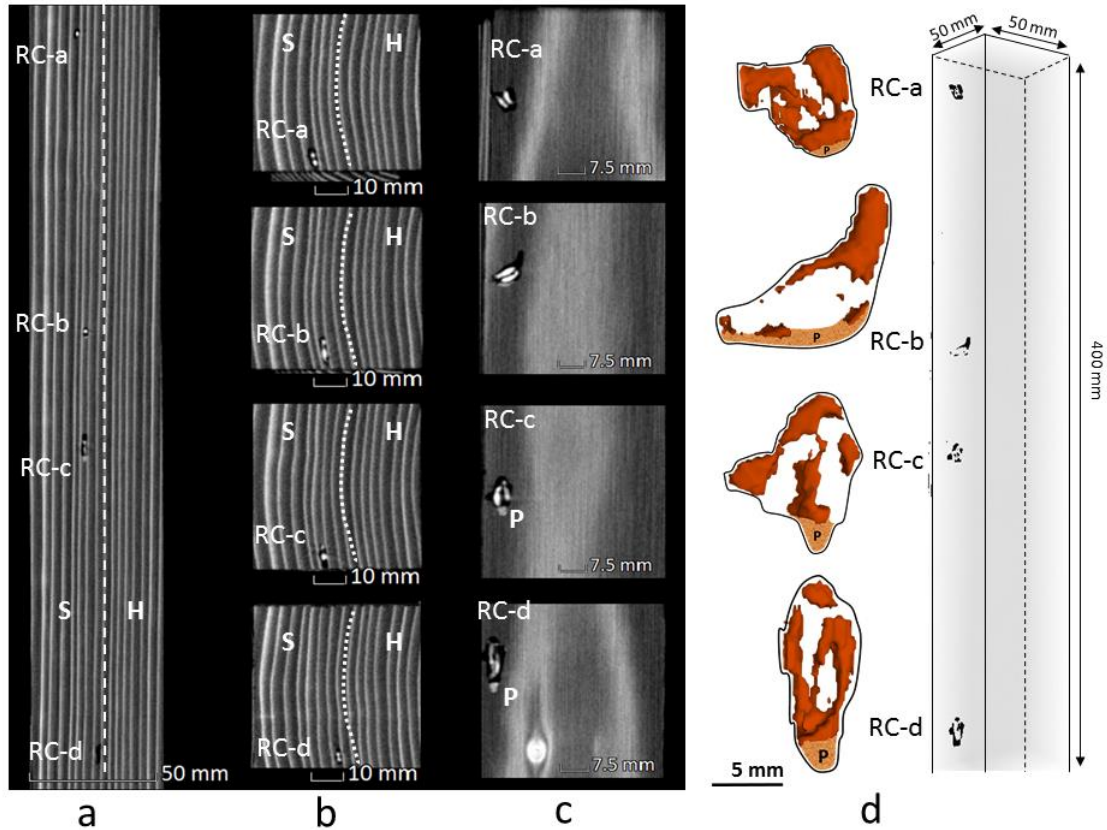


Fig. 3.3 CT images of four royal chambers in Spruce B timber. **a** A 2D CT image of a radial section, showing the sites of the entrance holes on the timber. **b** 2D CT images of cross sections, indicating that the royal chambers were excavated following the grain of the annual growth ring. **c** 2D CT images of a tangential section showing the presence of royal pairs and the shape and depth of the chambers. **d** 3D CT images of four royal chambers; the red color represents the empty space inside the chamber; the presence of royal pairs is indicated by the uncolored area inside the chamber.

RC: royal chamber; S: sapwood; H: heartwood, P: fecal pellets. Springwood and summerwood are indicated by the darker and lighter colors in the 2D CT images, respectively.

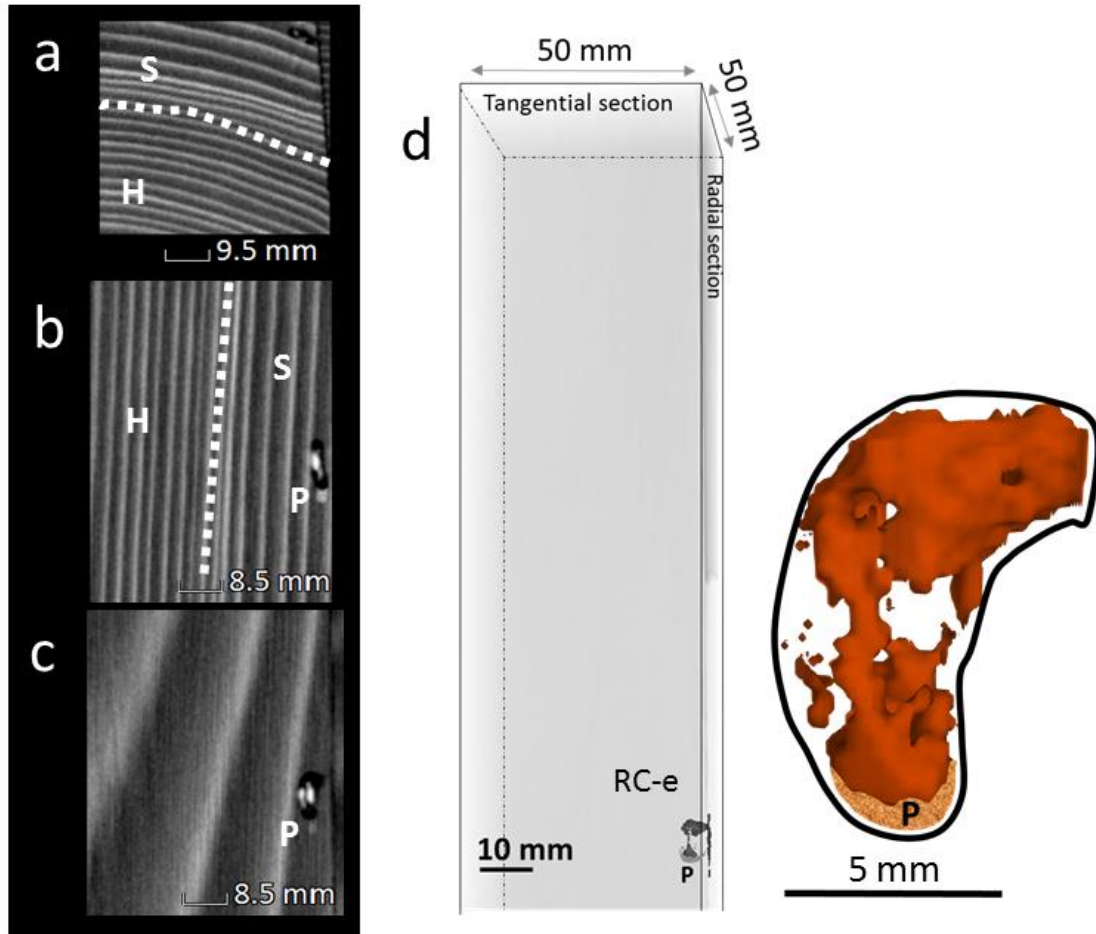


Fig. 3.4 CT images of a royal chamber in Spruce C timber: **a** A 2D image of a cross section; **b** A 2D image of a radial section; **c** A 2D image of a tangential section; (d) A 3D image of the royal chamber.

RC: royal chamber; S: sapwood; H: heartwood, P: fecal pellets. Springwood and summerwood are indicated by darker and lighter colors in the 2D CT images, respectively.

Figure 3.3 presents a CT image reconstruction of four royal chambers found in the Spruce B timber in 3D and 2D CT images of a radial section, cross sections and tangential sections. The measurements of important properties of the chambers are presented in Table 3.2. The royal chambers in Spruce B is referred to as RC-a, RC-b, RC-c, and RC-d (Fig. 3.3). All newly established

chambers with sealed entrance holes were found on the sapwood (Fig. 3.3a – b) with an average diameter of 1.8 mm (Table 3.2). The excavation followed the springwood grain of an annual growth ring, resulting in a sloping entrance (Fig. 3.3b). The chambers were excavated on the plane of the springwood (indicated by the darker color in the CT image) (Fig. 3.3c). The pair excavated a narrow oval-shaped chamber sufficient for them and to store some fecal pellets (Fig. 3.3c – d).

Table 3.2 Measurement of relevant initial nest-gallery properties

Properties	Spruce A	Spruce B				Spruce C
		RC-a	RC-b	RC-c	RC-d	RC-e
Entrance hole diameter (mm)	8.2	1.9	1.9	1.9	1.9	1.7
Volume* (mm ³)	114.8	33.5	17.9	25.6	41.0	41.4
Depth** (mm)	26.2	7.4	11.9	9.0	6.4	6.7
Length (mm)	49.4	8.7	14.9	12.1	15.6	13.0
Max. chamber diameter (mm)	11.9	6.4	6.3	7.0	6.3	4.6
Average gallery diameter	2.3	-	-	-	-	-

*Volume refers to the empty space in the chamber.

**Depth is measured from the particular surface where the entrance hole was excavated.

The chambers were located 6.4 – 11.9 mm beneath the surface to the bottom edge of the chamber (Fig. 3.3c, Table 3.2). The initial structures of the royal chambers were varied to follow the anatomical texture of the particular excavation spot. Two chambers (RC-a and RC-c) resembled the shape of a European pear, supporting the previous findings (Harvey 1934b; Morimoto 2012), while the other two chambers (RC-b and RC-d) resembled a cashew-nut shape. The cashew-nut-shaped chambers were longer but narrower than those with the European pear shape (Table 3.2). Fecal pellets were found

inside of all the royal chambers (Fig. 3.3c – d). RC-d was the chamber had the highest volume, 41.0 mm³, followed by RC-a, RC-c and RC-b with volumes of 33.5 mm³, 25.6 mm³, and 17.9 mm³, respectively.

Figure 3.4 shows CT images of a royal chamber in the Spruce C timber, referred to as RC-e, and the chamber properties are presented in Table 3.2. A pairing reproductive excavated a sloping entrance to follow the springwood of the sapwood (Fig. 3.4a – c) on the bottom side of the timber (radial section). The royal chamber was excavated in an elongated cashew-nut shape (Fig. 3.4d). The chamber had a 4.6-mm diameter and a 13.0-mm length, and was established 6.7 mm beneath the surface (Table 3.2). The pair selected a particular spot with a somewhat wider growth ring for establishing the royal chamber (Fig. 3.4d).

3.4 Discussion

3.4.1 Nest-site selection

Two types of infestations found in the three Spruce timbers reflected nest-site selection mechanisms. Even though the three spruce timbers were infested from different planes, all of the entrance holes were excavated on the springwood of the sapwood (Table 3.1). The selective excavations may be related to the feeding biology of drywood termite, which is suggested to be influenced by both the physical and chemical properties of the wood (Kasseney *et al.* 2011; Sajap and Sahri 1983).

The drywood termite has the ability to assess physical properties of the wood such as mass, density, and internal damping (Inta *et al.* 2007) through the reception of vibratory signals (Evans *et al.* 2005). Indrayani *et al.*

(Indrayani *et al.* 2007) also observed that *I. minor* touches the surface of the timber with its antennae before penetrating the timber by engaging in cutting, pulling and collecting activities. This behavior is apparently suggested as “selection” mechanism, as indicated by the concentration of excavation and foraging activities on the softer part of the timber, i.e. the springwood. Both the foraging activities of individuals in the Spruce A timber in excavating tunnel-galleries and the nest-founding activities of the royal pair in the other two timbers were observed to occur on the springwood, and the termites were seen to avoid the summerwood (Figs. 3.2 – 3.4).

The wood selection mechanism is also known to be affected by wood chemistry (Abe and Higashi 1991) and the wood’s nutritional value (Shellman-Reeve 1994). Wood is primarily composed of three major components: cellulose, hemicelluloses, and lignin, with various other components such as organic extractives and minerals. Extractives are responsible for the color, smell, and durability of the wood (Rowell *et al.* 2005). In Sitka Spruce, the heartwood contained about 70% more extractives than the sapwood (Caron *et al.* 2013). Therefore, nest-site selection in the sapwood may be determined by the extractive content.

The existence of nest-site selection mechanisms was confirmed by the fact that dealate reproductives chewed several areas on the timber before selecting a favorable spot for establishing the royal chamber (Fig. 3.1b). This result suggests that drywood termites effectively search for nesting sites and start their colonies in favorable environments. This kind of behavior may be highly related to foraging efficiency (Lee and Su 2010), i.e. it may serve to

reduce the energy spent on excavation, and at the same time maximize the termites' access to an important nutritional diet (Shellman-Reeve 1994).

3.4.2 Nest-founding and foraging behavior

One-piece nesters spend their entire colony life in a single piece of wood that functions both as a nest and as food (Abe 1987). Individuals of one-piece termites with flexible linear caste development (Roisin 2000) have only two options, staying at the natal nest as helpers/workers or developing into winged reproductives that disperse to establish new colonies (Korb and Schmidinger 2004). The decision as to whether to remain or disperse is influenced by ecological constraints, such as food availability and habitat saturation (Korb and Lenz 2004; Roisin 1999). Korb estimated that the decision to remain in the natal colony had two philopatric benefits: a long-term direct benefit through nest inheritance and an indirect benefit from raising young individuals. However, as the availability of food decreases, both benefits of philopatry decline, thus leading the reproductives to favor choosing dispersal to found new nests (Korb and Lenz 2004; Korb and Schmidinger 2004).

An interesting result was observed on the Spruce A timber, as new chamber-gallery establishment was mediated by the foraging activities of individuals that had emerged from their natal nest. The result implied that “one-piece” terminology could not explain this kind of foraging activity outside the natal nest. The designation of drywood termites as “one-piece” termites is only useful as an ecological distinction to distinguish them from “intermediate” (nesting in wood but foraging outside the nest wood) and “separate” (nesting in and foraging through the soil) termites (2009). This

finding is in accordance with a previous report (2009), suggesting that drywood termites foraged outside the natal nest throughout several adjacent timbers and had extensive gallery systems. The fact that some pseudergates were encountered and collected using a ground sticky trap also supported the outside foraging activity of *I. minor*.

The chamber-gallery excavation in the Spruce A timber showed a different pattern of development compared to the nest-founding activities from nuptial flight in the other two timbers. Entrance holes were excavated in springwood (Fig. 3.2c), consistent with the wood selection mechanism. The chamber, which was still connected with the natal nest when it was found, functioned as a starting chamber for establishing extended nest-gallery systems on the newly infested timber. Five exploratory tunnel galleries were excavated in five different directions, which may have been part of an “environmental assessment” by the colony before deciding on further nest-gallery excavation. The colony development in the Spruce A timber was in a vulnerable stage, since the presence of a royal pair in the incipient colony was unconfirmed. However, with highly flexible linear caste development, individuals of an *I. minor* colony can develop into replacement reproductives in order to sustain the colony (Evans *et al.* 2010; Roisin 2000).

Nest-founding activity by pairing reproductives in the Spruce B and Spruce C timbers indicated that the royal pair carefully “selected” the springwood of the sapwood (Figs. 3.3 and 3.4) as the site for excavating the entrance hole (~1.8 mm in diameter) as well as the royal chamber. The pairs excavated the royal chambers beneath the surface, parallel to the annual growth rings, and avoided the summerwood. The royal chambers were pear-

shaped, consistent with the previous descriptions given by Harvey (1934b) and Morimoto(2012), and oval cashew-nut shaped with 6 ~ 7 mm elongated narrow diameters, 8~16 mm lengths, and range volumes of 17 ~ 41 mm³ (Table 3.2).

Volume properties can be used to estimate in order in which the royal chambers were excavated. The other properties (depth, length, and diameter) are discrete values, as they depend on the anatomical texture of the particular excavated area in the timber. The volume of the chamber represents how much timber was consumed by the royal pair, which is related to how much time has passed since the pair started the excavation. Based on the volume measurements of the royal chambers, RC-e in the Spruce C timber was apparently the first excavated chamber, followed by RC-d, RC-a, RC-c and RC-b in the Spruce B timber.

3.5 Chapter summary

The structure of royal chambers resemble European pear-shape and cashew-net shape, in response toward timber environment. The reproductives of *I. minor* showed selectivity in nest-founding activities, i.e., on the springwood part of annual growth-ring of the sapwood. The study also encountered outside foraging activity by *I. minor* colony, suggested that drywood termite has greater foraging flexibility in response of environmental conditions.

CHAPTER

4

THE FIRST YEAR DEVELOPMENT OF AN INCIPIENT COLONY FOLLOWING THE NUPTIAL FLIGHT OF THE DRYWOOD TERMITE, *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae)

In this chapter, X-ray tomographic analysis of the development of a royal chamber and incipient colony of I. minor in the first year of the new colony founding is discussed. The chapter reveals important information regarding the nesting biology of I. minor.

4.1 Introduction

The establishment of a new colony following the nuptial flight has been described as “vulnerable solitary founding” (Cronin *et al.* 2013). The level of failure is high, as the queen must brood and forage at the same time. Harvey (Harvey 1934b) observed that the royal pair of *I. minor* had a period of inactivity after spending tremendous energy in swarming, shedding their wings, mating and excavating the chamber. The hibernation period was estimated to last for nine months, and many pairs do not survive through it. Successful colony-founding depends on the royal pair’s ability to maintain their energy while producing a workforce and to maintain their foraging efficiency. The colony has to distribute the time and energy spent in excavating the nest-gallery and foraging in such a way as to maintain its fitness (Lee and Su 2010).

This chapter reports the first-year development of royal chambers and incipient colonies following the nuptial flight of *I. minor* reproductive as monitored by X-ray CT. The chapter provides important information regarding the nesting biology of the royal pairs in the very early stage of colony founding.

4.2 Materials and Method

4.2.1 Wood specimens

As described in Chapter 3, two spruce (*Picea sitchensis* Bong. Carriere) timbers were infested by five royal pairs of *I. minor*. The timbers had dimensions of 50 (R) x 50 (T) x 1000 mm (L), with sapwood and heartwood portions. The timbers were collected from the field in November 2012, and

kept in a laboratory under controlled temperature and humidity ($28 \pm 20^\circ\text{C}$; RHs $80 \pm 10\%$).

3.2.2 Monitoring of first year chamber development

The first-year development of incipient colonies following the nuptial flight in five royal chambers, referred to as RC-a, RC-b, RC-c, RC-d, and RC-e, in those two spruce timbers using X-ray CT scan (YXLON International GmbH, Germany). Biannual scanning analysis was conducted in Kyushu National Museum.

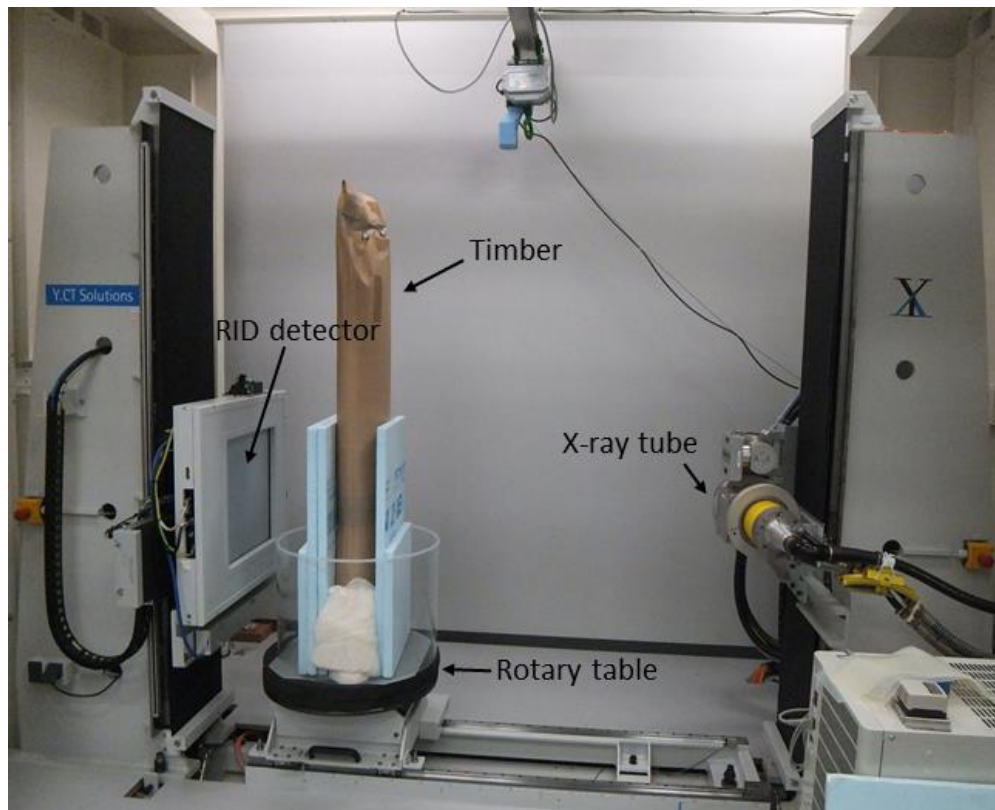


Fig. 4.1 X-ray CT machine used to investigate wood at Kyushu National Museum, Japan.

The scanning was performed in vertical measurements using a 320 kV X-ray source (2.0 mA) with a 400 mm x 400 mm digital flat-panel refractive index detector (YXLON International GmbH, Germany) in the dynamic range of 16-bit, with a pixel pitch of 20 μm , and 1024 x 1024 pixel resolution. The timbers were placed on a rotary table that continuously rotated during the scanning process, while the X-ray tube and the detector were simultaneously moved vertically and downward along the entire length of the timbers (Fig. 4.1). The X-ray data obtained during the scanning process were stored in files containing 2D image stacks (*.raw image file), with each file representing a single image-slice of the timbers.

3.2.3 Data and statistical analysis

The 2D CT-image stacks for each timber were rendered into 3D images (each 2D image slice was 0.3 mm in thickness) using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH, Germany). The relevant properties of the development of the chambers such as the volume, depth, length, and maximum diameter of the chambers were measured using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH, Germany).

We relied on the nest volume as the dependent variable with which to measure the development over time (i.e., at the initial stage, the half-year stage and the one-year stage), and generated the differences across those three stages. The statistical analysis was carried out by repeated measures ANOVA ($p = 0.05$) using IBM SPSS 23 (IBM Inc., U.S.A).

4.3 Results

All five royal chambers were established on the radial surface of the timber, on the springwood part of an annual growth ring in the sapwood (refer to Chapter 3 for details).

4.3.1 *The development of RC-a*

Figure 4.2 shows the development of RC-a in 3D and 2D CT images. The figure indicates that the royal pair survived the first year of the establishment of the colony. The chamber was extended perpendicular to the longitudinal axis of the timber to follow the direction of the annual growth rings (Fig. 4.2a – c, 2D cross-section CT images), in the springwood part (Fig. 4.2a – c, 2D tangential-section CT images) of the particular annual growth ring where the entrance hole was established.

Inside the chamber, we observed some fecal pellets. The position of the fecal pellets changed over time. The position could have been affected by external disturbances, as the timbers were periodically transported for X-ray scanning analysis. During the X-ray CT scanning analysis, the timbers were positioned vertically, and interestingly, some fecal pellets did not move or were attached to the top of the chamber (Figs. 4.2b – c). The fecal pellets that remained at the top of the chamber might have been newly excreted and hydrated sticky pellets, while the fecal pellets found at the bottom of the chamber may have been dehydrated pellets.

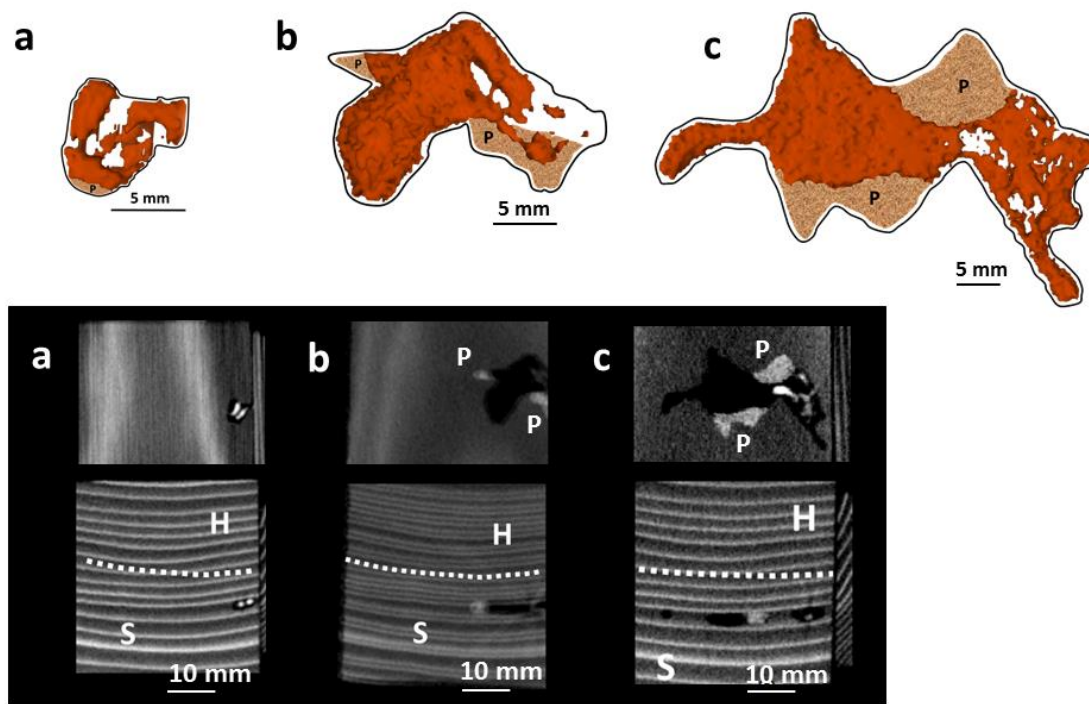


Fig. 4.2 3D and 2D CT images of the development of RC-a: (a) initial scan; (b) half-year later; and (c) one year later. The 2D CT images include tangential-section images (*top*) and cross-section images (*bottom*).

In the 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively.

S, sapwood; H, heartwood; P, fecal pellets.

In the first six months, no new colony members were observed, but an image taken after one year indicates that one or two new individuals were present. The pear-shaped initial structure of RC-a (Fig. 4.2a) had developed into a single large chamber six months later (Fig. 4.2b), and two exploratory tunnels (Fig. 4.2c) were further developed after one year, along with the breeding of new colony members.

4.3.2 The development of RC-b

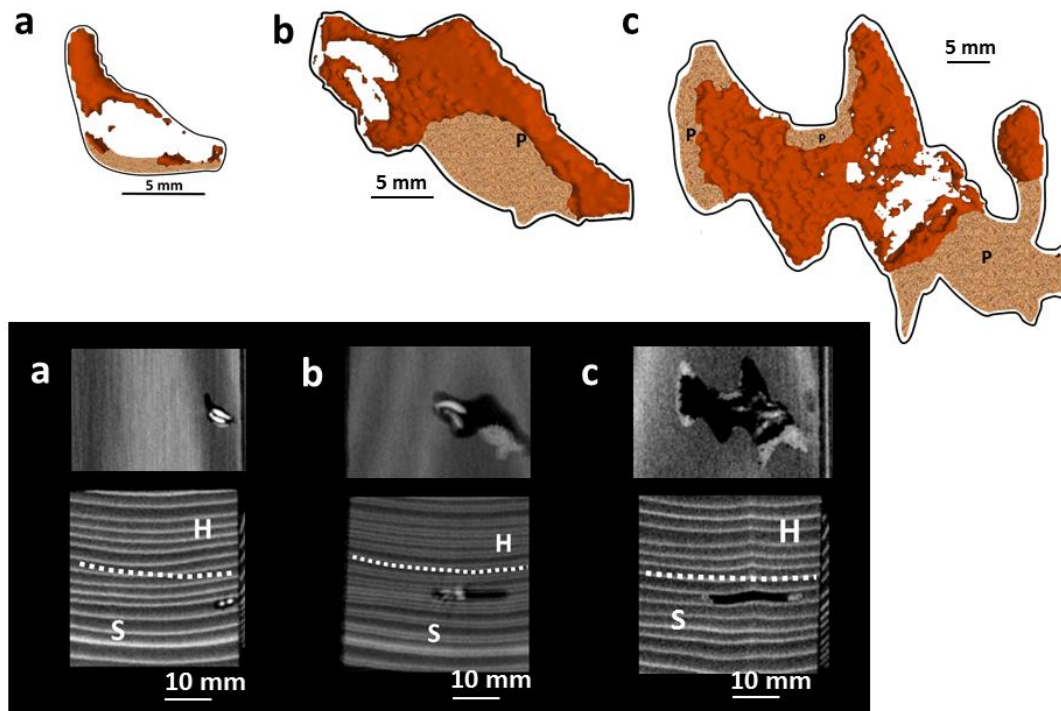


Fig. 4.3 3D and 2D CT images of the development of RC-b: (a) Initial scan; (b) half-year later; and (c) one year later. The 2D CT images include tangential-section images (*top*) and cross-section images (*bottom*).

In the 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively.

S, sapwood; H, heartwood; P, fecal pellets.

The development of RC-b over time is presented in Fig. 4.3. The royal pair survived the process of colony-founding. The cashew-nut-shaped initial-chamber was extended along the springwood part of the particular annual growth ring where the entrance hole was established, perpendicular to the longitudinal axis of the timber. The royal pair hatched a young colony member

in one year. Some hydrated sticky fecal pellets remained in their position on the top and side walls of the chamber (Fig. 4.3c), separated from the dehydrated fecal pellets. An exploratory tunnel was excavated near the sealed entrance hole, parallel to the longitudinal axis of the timber (Fig. 4.3c).

4.3.3 The development of RC-c

Figure 4.4 presents the development of RC-c over time. The pear-shaped initial-chamber was extended parallel to the longitudinal axis of the timber on the springwood of the particular growth ring where the entrance hole was excavated. Half-year CT images revealed the emergence of new colony members. Four young individuals were observed inside the chamber (Fig. 4.4b) and spotted close to the royal pair. One-year CT images showed that the incipient colony was excavating a lengthwise tunnel gallery (Fig. 4.4c). The tunnel had been excavated parallel to the longitudinal axis of the springwood of the growth ring, with small chambers were established at certain distances along the tunnel.

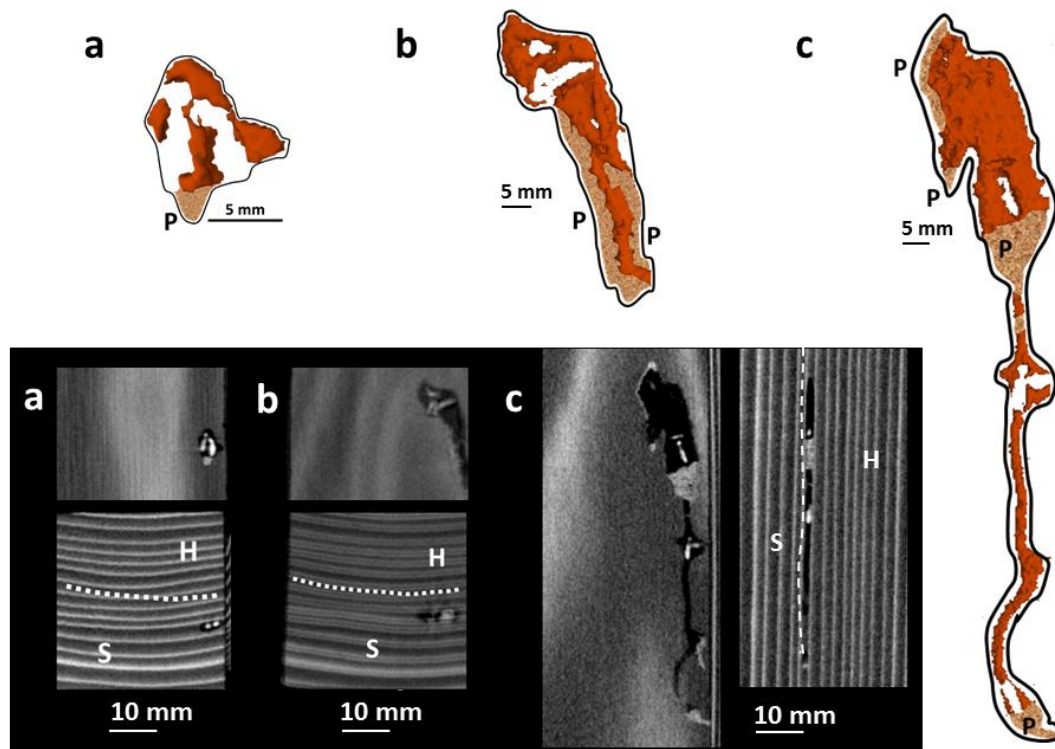


Fig. 4.4 3D and 2D CT images of the development of RC-c: (a) Initial scan; (b) half-year later; and (c) one year later. The 2D CT images in the initial and half-year stages include tangential-section images (*top*) and cross-section images (*bottom*), while the images taken at the one-year stage are tangential section-images (*left*) and radial-section images (*right*).

In the 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively.

S, sapwood; H, heartwood; P, fecal pellets.

4.3.4 The development of RC-d

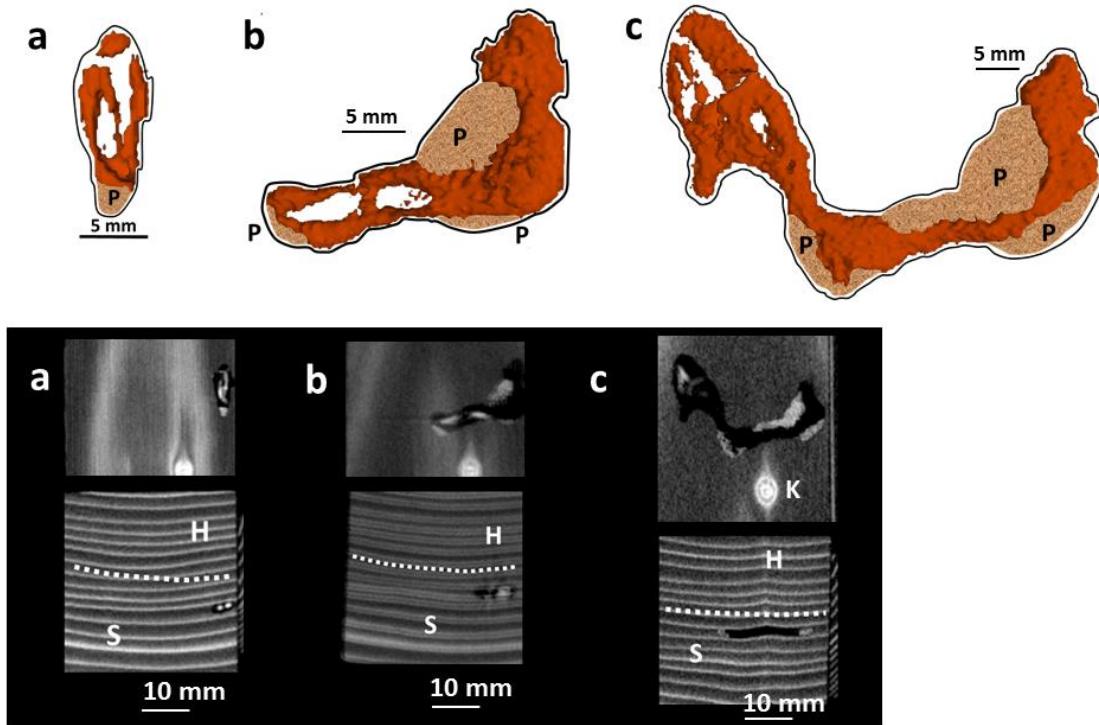


Fig. 4.5 3D and 2D CT images of the development of RC-d: (a) Initial scan; (b) half-year later; and (c) one year later. The 2D CT images include tangential-section images (*top*) and cross-section images (*bottom*).

In the 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively.

S, sapwood; H, heartwood; P, fecal pellets; K, knot.

Figure 4.5 displays the development of RC-d over time. The cashew-nut-shaped initial chamber was extended perpendicular to the longitudinal axis of the timber on the springwood part of the particular annual growth ring where the entrance hole was established. The direction of chamber extension avoided the knot (Fig. 4.5c, 2D tangential-section image), which is consistent

with a previous report (Himmi *et al.* 2016a) that suggested that *I. minor* expresses wood-anatomical selectivity in excavating a nest-gallery. After one year of development, no new individuals were observed, indicating that there was no breeding activity from the royal pair. Hydrated sticky fecal pellets continued to be observed on the top of the chamber (Fig. 4.5b – c), while dehydrated fecal pellets were on the bottom of the chamber.

4.3.5 The development of RC-e

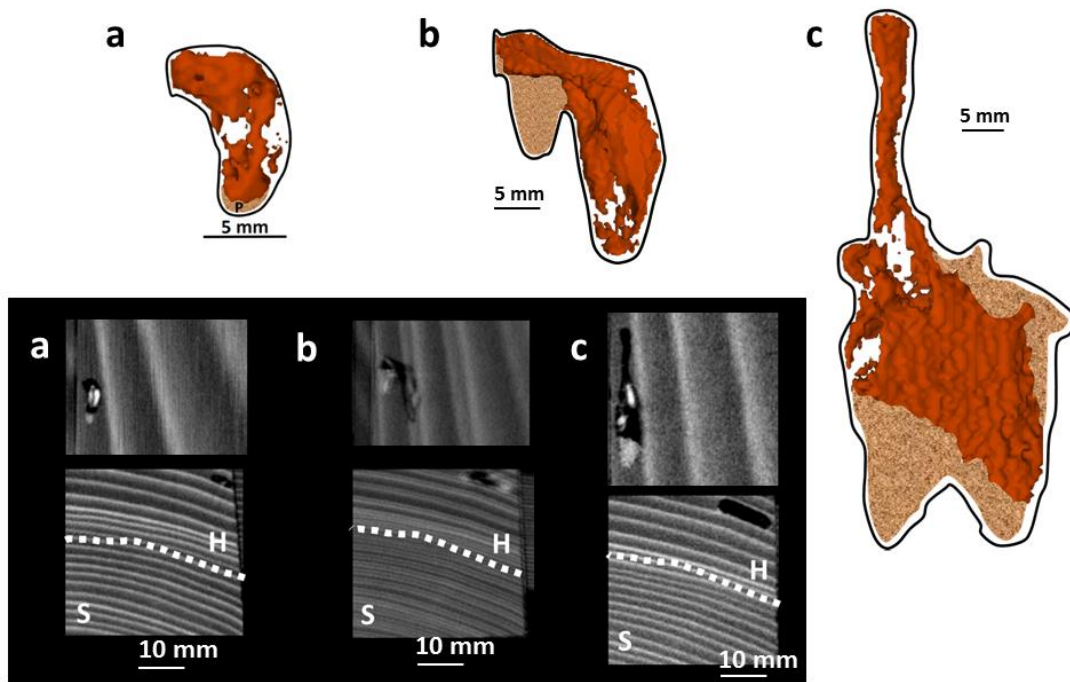


Fig. 4.6 3D and 2D CT images of the development of RC-e: (a) Initial scan; (b) half-year later; and (c) one year later. The 2D CT images include tangential-section images (*top*) and cross-section images (*bottom*).

In the 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively.

S, sapwood; H, heartwood; P, fecal pellets.

Figure 4.6 illustrates the development of RC-e over time. The chamber was excavated perpendicular to the longitudinal axis in the first six months, and parallel to the longitudinal axis of the timber through extension of the tunnel gallery (Fig. 4.6c) in the one-year CT images. Hydrated sticky pellets were located in the one-year chamber (Fig. 4.6c) but not in the half-year chamber (Fig. 4.6b). The first new colony member was observed in the first six months. The colony had a royal pair and two colony members after one year of development.

4.3.6 Relevant properties of nest development

Figure 4.7 shows the relevant properties of five chambers: volume, depth, length and maximum diameter. The properties of the initial chambers was presented in Table 3.2 and extensively discussed in Chapter 3. This section will discuss the further development of the chambers. Volume refers to the empty space in the chamber, which was estimated from 3D image data using volume graphics software. The other three properties (depth, length and maximum diameter) were analyzed using 2D image data. The depth is defined as the deepest point of the chamber, measured from the surface of the timber where the entrance hole was established. The length is defined as the length of the nest-gallery determined by following the direction of development, whether parallel or perpendicular to the longitudinal axis of the timber. The maximum diameter was determined from the biggest diameter value of the chamber, which was measured from 2D tangential-section CT images.

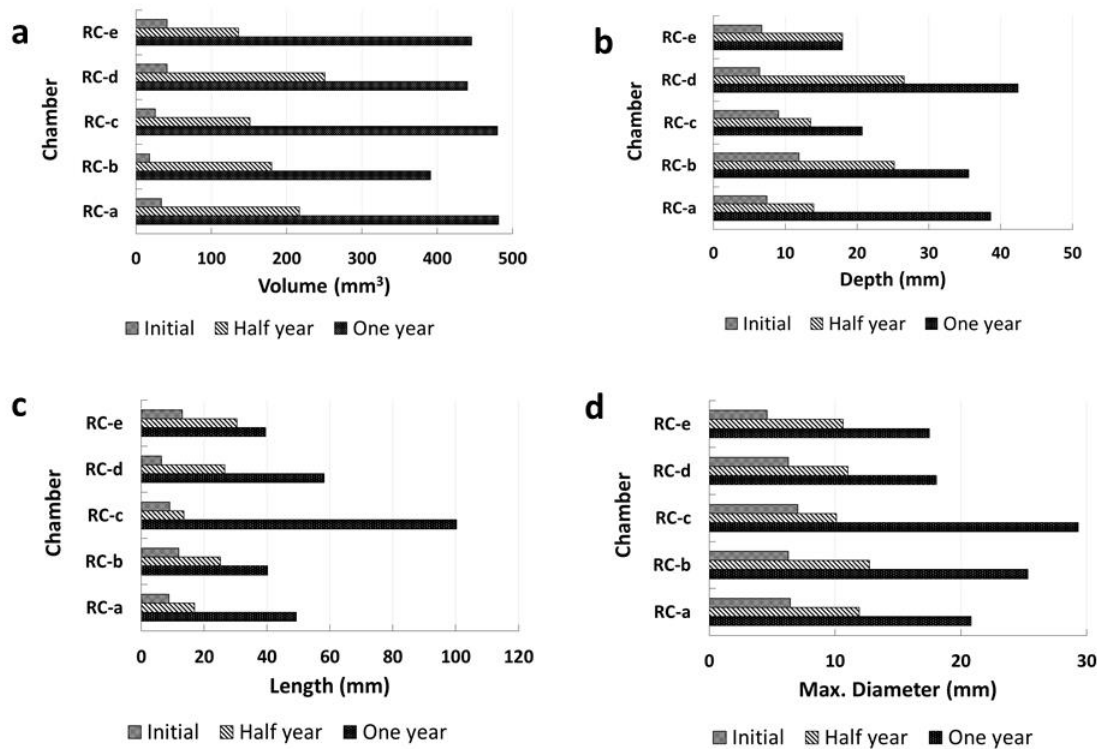


Fig. 4.7 The relevant properties of chambers development; **a** Volume; **b** Depth; **c** Length; **d** Maximum diameter.

In the first six months, RC-e had the lowest volume at 135.9 mm^3 , followed by RC-c at 151.1 mm^3 (Fig. 4.7a). The volume of a chamber is correlated with wood consumption by the royal pair. As the royal pairs of RC-c and RC-e had already started breeding after six months (Fig. 4.4b), the royal pairs in those two chambers may have needed to distribute their energy to forage and breed at the same time, and therefore, the volume of these chambers was less than those of the other three chambers. The royal pairs in RC-a, RC-b and RC-d chambers showed no breeding activity in the first six months, and concentrated their energy on foraging. RC-d had the highest volume in the first six months (250.8 mm^3), followed by RC-a and RC-b at 216.9 mm^3 and 179.8 mm^3 , respectively. The volume in the first six months

may also be related to which chamber was established earlier, as in chambers established earlier, the royal pair had more time for foraging and excavation. After one year of development, RC-a had the highest volume at 481.3 mm³, followed by RC-c, RC-e, RC-d, and RC-b (479.9 mm³, 445.8 mm³, 440.0 mm³, and 391.2 mm³, respectively). The volume of RC-c caught up with the other chambers by acquiring volume due to the presence of four new colony members hatched in the previous six months.

The values of the other relevant properties of the chambers, i.e. depth, length and maximum diameter, were dependent on the timber environment. RC-c had the smallest depth value (Fig. 4.7b) but the greatest length (Fig. 4.7c). This development was related to the timber environment where the royal chamber was established. The royal pair in RC-c encountered summerwood part of the annual growth ring (Fig. 4.4b, 2D tangential-section image) right after the pair penetrated the timber, and then avoided the summerwood by extending the chamber in a sloping excavation parallel to the longitudinal axis of the timber. This adaptation resulted in a lengthwise chamber (100.2 mm in length, Fig. 4.7c) with a small depth value (20.7 mm, Fig. 4.7b).

The three chambers (RC-a, RC-b and RC-d) were established in relatively similar environments, namely wide planar springwood areas, and the chambers were further excavated perpendicular to the longitudinal axis of the timber. This kind of development resulted in a greater chamber depth value and a relatively smaller development in terms of length. RC-a, RC-b and RC-d had depths of 38.6 mm, 35.5 mm and 42.4 mm after one year (Fig. 4.7b), respectively, and lengths of 49.3 mm, 40.1 mm, and 58.2 mm (Fig. 4.7c), respectively. The maximum diameters of these three chambers were

relatively similar (between 11 ~ 12 mm, Fig. 4.7d). The adaptability of *I. minor* in response to the timber environment was expressed in RC-d. A knot located close to the chamber (Fig. 4.5c, 2D tangential-section image) affected the direction of chamber development.

RC-e was established close to the edge of the timber on an inclined growth ring (Fig. 4.6). In the first six months, the chamber had been extended to include a new spacious chamber, its depth increased from 6.7 mm to 17.9 mm, its length increased from 13.0 mm to 30.5 mm, and its maximum diameter increased from 4.6 mm to 10.6 mm. After six months, no further development occurred in the direction perpendicular to the longitudinal axis of the timber, as the chamber had reached the edge of the timber (Fig. 4.6b – c, 2D cross-section images). Therefore, the depth value remained the same at 17.9 mm, even after one year of development (Fig. 4.7b). The chamber was further extended parallel to the longitudinal axis of the timber, resulting in an increase in length to 39.5 mm after one year of development (Fig. 4.7c).

Qualitative generalization regarding the development over time of the breeding chambers was carried out using repeated measures ANOVA ($p < 0.05$) by relying on the volume properties of the five chambers as a dependent variable. Repeated measures ANOVA with a Greenhouse-Geisser correction determined that the volume differed significantly ($p < 0.0005$) among the time stages (initial, half-year and one year). The estimated marginal means of nest volume at three time points, i.e. initially, after a half year and after one year, were 31.9 mm³, 201.7 mm³, and 447.6 mm³, respectively (Fig. 4.8). Post hoc tests using the Bonferroni correction also revealed that the initial volume of the chambers was significantly different from the half-year volume and one-

year volume ($p = 0.001$ and $p < 0.0005$, respectively). Moreover, the volume of the chambers after one year also showed a statistically significant difference from the volume after six months ($p = 0.002$).

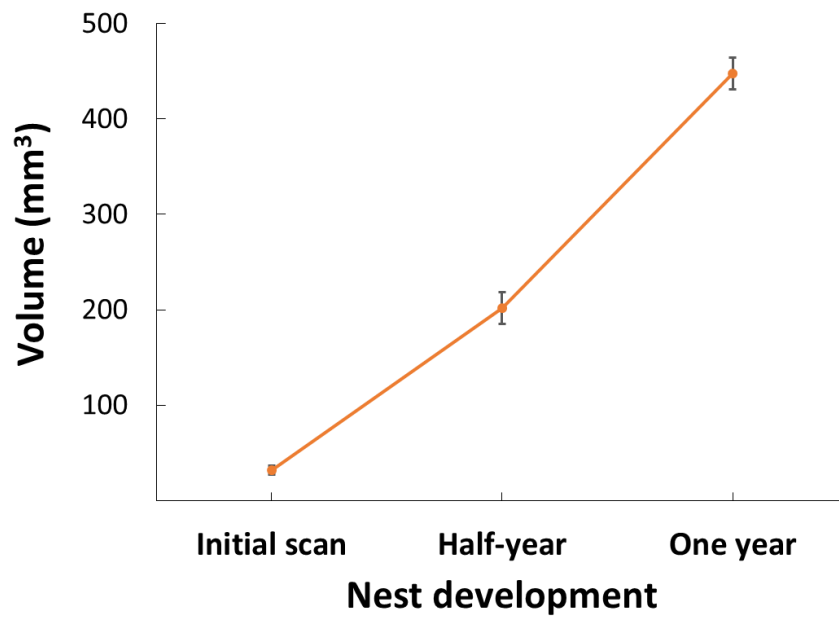


Fig. 4.8 Estimated marginal means of the volume of the nest at three time points: Initial scan, one half-year later and one year later. Error bars show standard errors.

4.4 Discussion

4.4.1 Nest development

The monitoring of the development of royal chambers using X-ray CT obtained important biological feedback. Harvey (1934b) argued that royal pairs enter nine-month periods of inactivity in the early stage of colony establishment following the nuptial flight, due to the spending of a tremendous fund of energy by the royal pair in swarming, divesting their wings, mating and excavating the royal chamber. In the present study, the primary reproductives in all of the observed chambers showed no inactivity

period in the first six months and continuously excavated the galleries or had already started breeding to produce new colony members, in contrast with Harvey's findings. One-year CT analyses also confirmed that the reproductives continued foraging, as indicated by the significant accretion in the volume and structure of the chambers.

The first-year development of the royal chambers followed a certain pattern. The royal pair showed selectivity in excavating the chambers in the springwood part of the particular annual growth ring where the entrance hole had been established. The springwood part is softer than the summerwood part of the annual growth ring. CT data indicated that *I. minor* foraged selectively on springwood and avoided summerwood (Figs. 4.2 – 4.6) and knots (Fig. 4.5c) in a direction either perpendicular or parallel to the longitudinal axis of the timber. Foraging selectivity in response to timber properties has been previously reported in *I. minor* (Himmi *et al.* 2016a; Himmi *et al.* 2016c).

It is premature to draw definitive conclusions regarding any period of inactivity from the results of the present study. All the naturally infested timbers were kept in climate-controlled rooms (28 ± 2°C; RHs 80 ± 10%), and thus the primary reproductives in the observed chambers never experienced any seasonal changes. Even though temperature and humidity have been reported to be essential factors influencing the feeding activities of *I. minor* (Cabrera & Rust, 1994; Cabrera & Rust, 1996; Indrayani *et al.*, 2006), the inactivity period of primary reproductives in the early stage of colony establishment may not last as long as nine months.

In the second field monitoring in November 2013, we encountered a spruce timber infested by a royal pair. When we analyzed the timber using X-ray CT, we found that the chamber had volume properties within the range observed in half-year chambers (Himmi, unpublished data). The volume data of this chamber indicated that the timber might have been infested earlier in the summer, much earlier than the common nuptial flight in the Wakayama area which was reported to occur in September (Yoshimura, 2011). The primary reproductives in this chamber were experiencing natural seasonal change; however the volume properties of the chamber were similar to those of the chambers in the climate-controlled room.

Inside the five chambers, we observed hydrated sticky pellets that remained at the tops of the chambers. The function of these pellets is not yet clearly understood, as there has been no specific report discussing their role in drywood termite nests. Indrayani *et al.* (2008) reported that *I. minor* built a barrier using fecal pellets and wet feces near the bait-gel chemicals to block contact with the colony. Himmi *et al.* (2016c) also reported that an *I. minor* colony sealed a tunnel which led to outside of timber environment using cement pellets as part of a defense mechanism. These previous findings and our observation that hydrated sticky pellets were always present in the developing chambers suggest that drywood termites may store these sticky pellet as a preemptive strategy in case the chamber is exposed to the outer environment. The drywood termites can close the hole immediately once the situation happened.

4.4.2 *The establishment of a new colony*

Nest development cannot be separated from new colony establishment. The first six months of CT data from the five chambers showed two different outcomes of initiating a new colony following the nuptial flight, as follows. The royal pairs in two chambers (RC-c and RC-e) spent their energy for both breeding and foraging simultaneously, but in the other three chambers (RC-a, RC-b, and RC-d) the royal pairs only spent energy for foraging. After one year, the royal pair in only one chamber (RC-d) showed no breeding activity, while the other four chambers had 2 - 5 new colony members.

The decision to start breeding may be influenced by the fitness level of the royal pair after having spent a tremendous amount of energy in the nuptial flight and the first chamber excavation (Harvey, 1934b). Foraging is one key strategy for regaining fitness by obtaining essential nutrition (Shellman-Reeve, 1994). If their fitness is regained, the pairing reproductives start breeding. At the same time, their foraging activities slow down, as indicated in the present study by the smaller accretion in volume in the chambers of breeding pairs compared to those of non-breeding pairs (Fig. 4.7a).

There is no adequate literature on the actual time interval that passes after the royal chamber is established until the primary reproductives start laying eggs, either under natural seasonal changes or in the laboratory. The only comparative report was provided by Atkinson (1994), who recorded seasonal changes in the caste composition of 38 *I. minor* colonies dissected from their natal nest. However, he did not provide information as to whether the observations were carried out under actual seasonal changes or under a

controlled temperature and humidity level. He estimated that eggs were not encountered in the colonies from October through May, with the peak production range from August to September. However, our data suggested a different life cycle. The first half-year CT evaluation conducted in May, 2013, showed breeding activities in RC-c and RC-e, indicating that the oviposition occurred between November 2012 and April 2013.

Atkinson (1994) also reported that seasonal trends in colony composition were relatively weak in *I. minor*. He emphasized that the major factor driven by seasonal changes seemed to be the reduction of oviposition and the slower development rate of immatures during the cooler months of the year. Himmi *et al.* (2016b) evaluated 22 incipient colonies following the nuptial flight in different years of infestation in the natural environment. The caste composition and colony size were examined by dissecting the timbers. The colony size in the first and second years varied from 0 – 5 new colony members. The new colony members were mostly in larval instars, sometimes with the presence of one small soldier. After three years, the number varied from 4 – 16 new members with various caste compositions. The results suggested that there is no difference in colony size in the first year of development (0 – 5 new members), either in a controlled or natural environment.

4.5 Chapter summary

Royal pairs of *I. minor* can start breeding new colony members in the first six months, and by the end of the first year, an incipient colony can have 0 – 5 new members. The development of royal chambers in the first year

showed a preference for the springwood part of the particular growth rings where the entrance holes were excavated.

CHAPTER

5

NEST-GALLERY DEVELOPMENT AND CASTE COMPOSITION OF ISOLATED FORAGING GROUPS OF *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae)

In this chapter, the colonization process of foraging groups of I. minor in previously unoccupied timber and how the groups maintain their nest-gallery system will be monitored using X-ray tomography. The study also examines the caste composition of isolated groups in search of a better understanding of how one-piece nester types sustain their colonies. The chapter has been published on Insect (2016), 7(3).

5.1 Introduction

In chapter 3, two kinds of nest-founding activities have been identified in drywood termites (Himmi *et al.* 2014): (1) from nuptial flight by pairing dealate reproductives; or (2) from nest-gallery extension through colony foraging to a new piece of adjacent timber (Grace *et al.* 2009). Chapter 4 has thoroughly discussed the development of initial chamber from nuptial flight. In this chapter, the development of nest-gallery in timbers which were naturally infested by an *I. minor* colony through foraging activity will be presented.

The study intends to capture the colonization process of foraging groups of *I. minor* in previously unoccupied timber and how the groups maintain their nest-gallery system. The study also examines the caste composition of isolated groups in search of a better understanding of how one-piece nester types sustain their colonies.

5.2 Material and Method

5.2.1 Wood Specimen and Experimental Setup

We set up a field experiment on the nest-founding activity of *I. minor* in Wakayama prefecture, Japan, in August 2012 using two commercial timbers, Sitka spruce (*Picea sitchensis* Bong. Carriere) and sugi (*Cryptomeria japonica* D. Don). The timbers had dimensions of 50 (R) × 50 (T) × 1000 mm (L) and were made up of a combination of sapwood and heartwood portions. The timbers were laid in the attic areas of four highly-infested houses. A total of 10 sugi (S) timbers and 15 spruce (P) timbers were placed in each house, arranged at five different test areas. Each test area consisted of five pieces of

timbers, with a “P-S-P-S-P” arrangement and narrow gap (~1 cm) between the timbers. The timbers were arranged at random orientations, without considering whether tangential sections, radial sections, sapwood parts, and heartwood parts faced any particular direction.

Table 5.1 The record of new nest-founding activity by *I. minor*

Timber specimens	Nest-founding following the nuptial flight		Nest-founding through foraging activity	Total
	Successful*	Attempt**		
Spruce (n = 60)	29	21	2	52
Sugi (n = 40)	10	8	1	19

*Successful nest-founding is a sealed entrance holed, indicated that the pair of dealate reproductive successfully established the royal chamber.

**Attempt is an unsealed entrance-hole or an abandoned entrance-hole without the pair of dealate reproductives.

5.2.2 Monitoring

Annual monitoring was conducted in November, around two months after the swarming season of *I. minor* in Wakayama. All of the infested timbers were brought back to the laboratory and were kept at controlled temperature and humidity (28 ± 20 °C; RHs $80\% \pm 10\%$). Two nest-founding strategies were observed (Table 5.1): first, nest-founding by a pair of dealate reproductives following the nuptial flight; and second, through foraging activity by extending the nest-gallery to the adjacent bottom surface of the timber. Three timbers infested by colony through foraging activity (Table 5.2) are presented in the present study. Those timbers were collected from the same test area, but at different times. Details of the infestation can be found in Table 5.2.

Table 5.2 Nest-founding activity from colony infestation

Timber			Detail location of infestation on timber				
Specimens	Collected	Opened	Sapwood	Heartwood	Tangential section	Radial section	Cross section
Spruce A	November 2012	March 2016	○		○		
Spruce B	November 2014	March 2016		○	○		
Sugi	November 2014	March 2016	○			○	

5.2.3 X-Ray CT Scanning and Data Analysis

The timbers were analyzed by an X-ray CT Scanning Machine (Y.CT Modular320 FPD, YXLON International GmbH, Hamburg, Germany) at Kyushu National Museum to visualize the nest-gallery system. Spruce A was subjected to biannual analysis (from 2012 to 2014) to observe the in situ gallery development, while the other two timbers were scanned only once, in November 2014. When it was collected, Spruce A had only one chamber and was at the very beginning of new nest-gallery extension (Himmi *et al.* 2014); therefore, we were able to record the development of the nest-gallery by the *I. minor* colony by continuously scanning the timber. Spruce B and Sugi already had an extensive nest-gallery system when it was found; therefore, it was very difficult to follow the development of a nest-gallery system in those timbers.

The scanning analysis was conducted in vertical measurement by 320-kV X-ray source (2.0 mA) with a 400 mm × 400 mm digital flat-panel refractive index detector (RID) (YXLON International GmbH, Hamburg, Germany) in the dynamic range of 16-bit, pixel pitch of 20 μm, and 1024 × 1024 pixel resolution. The timbers were put on a rotary table that continuously rotates during the

scanning analysis while, at the same time, the X-ray tube and the detector were simultaneously moved vertically and downward along the entire length of the timbers. The X-ray CT data were stored in files containing two-dimensional (2D) image stacks (*.raw image file), with a digital thickness of 0.3 mm. Each file of 2D image stacks corresponds to a single 2D image-slice of the timbers. CT data analysis was performed by rendering 2D CT image data into 3D images using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH, Hamburg, Germany).

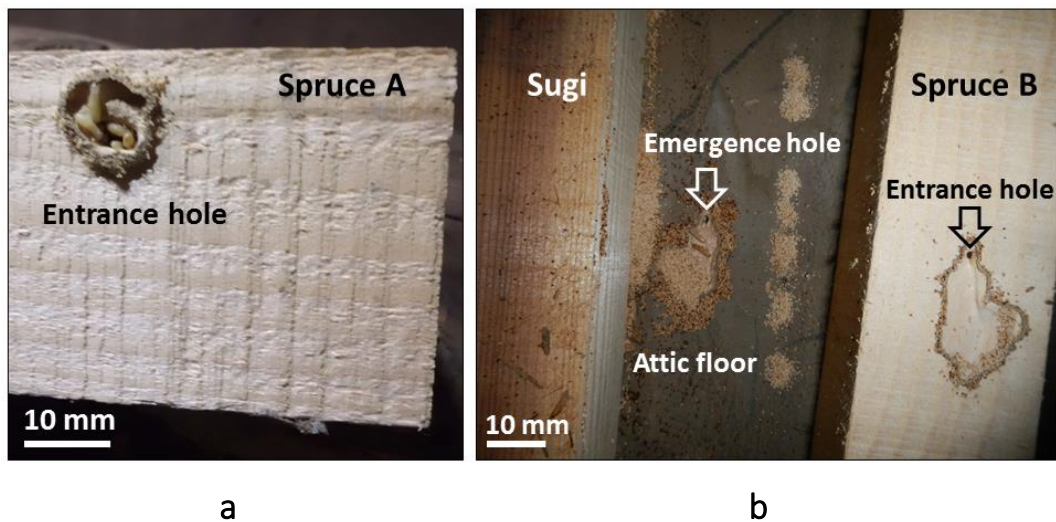


Fig. 5.1 Nest-gallery extension from natal colony to adjacent timbers activity by *I. minor* colony. **a** Entrance hole in Spruce A timber; **b** *I. minor* colony emerged from the attic floor to attack the adjacent bottom surface of Spruce B and Sugi timbers.

5.2.4 Colony examination

The timbers were kept in the laboratory to allow termite colonies to develop and extend the nest gallery system. The timbers were opened in March 2016 (Table 5.2), and all termites were carefully extracted from each

timber. The timbers were cut into pieces no longer than 100 mm, and termites that fell from the timber were collected. The pieces of timber were carefully chopped and all the termites were carefully collected and held. The composition of colony caste was examined with the digital microscope KEYENCE VHX-5000 (Keyence Corp., Japan).

5.3 Results

The three timbers—Spruce A, Spruce B and Sugi—were infested by an *I. minor* colony in a similar process: the infestation was mediated by foraging activities of individuals that had emerged from their natal nest. In both spruce timbers, the colony emerged from the attic floor to attack the adjacent bottom-surface of the timbers (Fig. 5.1). An ample entrance hole was excavated by the colony, surrounded by cement pellets to connect it with the emergence hole of the natal colony. In the Spruce A timber, the entrance hole was excavated on a tangential section of the sapwood, while in the Spruce B timber, it was excavated on a tangential section of the heartwood (Table 5.2). A group of termites was engaged in excavation activities when the timbers were collected (Fig. 5.1). The entrance hole in the Sugi timber could not be located. However, many holes sealed with fecal pellets were identified all over the sapwood surface.

5.3.1 Nest-gallery development in Spruce A timber

The Figure 5.2 presents 3D and 2D CT images of biannual development of the nest-gallery system in Spruce A timber, while the length and volume properties are shown in Fig. 5.3. The initial scan (Fig. 5.3a) showed that the timber had a newly excavated chamber. This chamber was an extension for

infesting a new timber, by the fact that the entrance hole was adjacent to the emergence hole of the natal nest from attic floor. A detailed description of the initial structure of the chamber was reported by Himmi *et al.* (2014). Five exploratory tunnels were extended from the chamber in five different directions (Fig. 5.2a, *3D image*), which may constitute an environmental assessment performed by the isolated group before it further extended the nest-gallery.

A half-year later, the group had extended the nest gallery by excavating another chamber through one of the exploratory tunnels that had been excavated perpendicularly to the axial system (Fig. 5.2a – b, *3D images*). Both chambers were still in the sapwood part of the Spruce A timber. One year later, the nest-gallery system comprised numerous chambers and interconnected tunnel-galleries (Fig. 5.2c, *3D image*) and had expanded throughout both the sapwood and heartwood parts of the timber. Chambers were constructed as spacious cells around an annual growth ring, or involving several growth rings when the width of the growth ring was narrow (< 2 mm). The tunnels had circular cross-sections, and were constructed very narrowly and primarily along the springwood. The diameters of the tunnels were restricted in size (~ 2 mm), a gap that would only allow a single termite passage through.

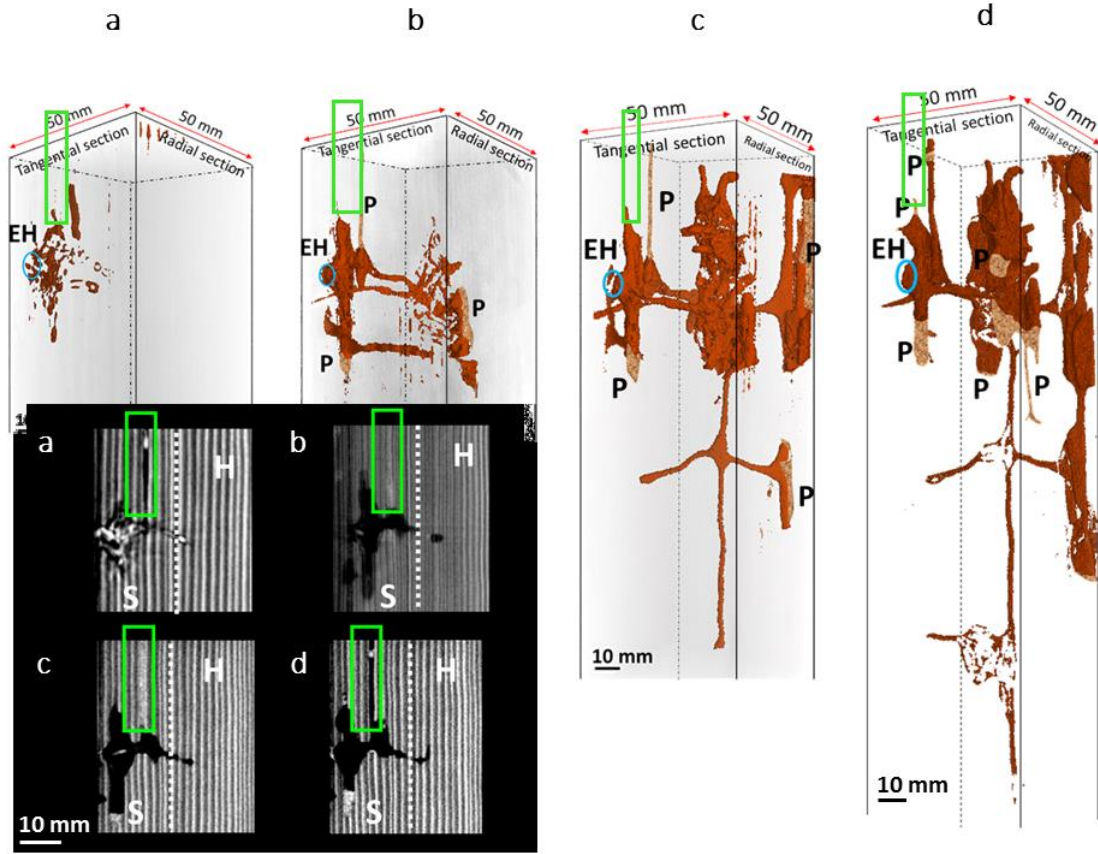


Fig. 5.2 The 3D and 2D CT images of nest-gallery development of Spruce A timber: **a** Initial scan; **b** Half-year later; **c** One year later; **d** One and a half years later. Two-dimensional radial-section images show dynamic change of the nest-gallery system, indicated by the sealing and re-opening of a tunnel gallery (green rectangles).

In 3D images, the presence of termites is indicated by the uncolored area inside the nest-gallery. In the 2D CT images, the gray value of a pixel corresponds to an index of density: the lighter the color, the denser the area (Himmi *et al.* 2016a). Springwood and summerwood are indicated by the darker and lighter colors in the images, respectively.

EH, entrance hole; *S*, sapwood; *H*, heartwood; *P*, fecal pellets.

One and a half years later, the nest-gallery had more extensive chambers and tunnel galleries (Fig. 5.2d, *3D image*). The presence of termites is indicated by an uncolored area inside the nest-gallery, and the pellets are located in certain chambers just beneath the timber surface. The CT images indicated that termites aggregated in particular chambers (Fig. 5.2a – d). The group seemed to keep moving together to new chambers (Fig. 5.2b – d) and to leave the previous chambers empty. Some individuals were observed at the edge of the tunnel galleries (Fig. 5.2d), which may be related with tunneling activity.

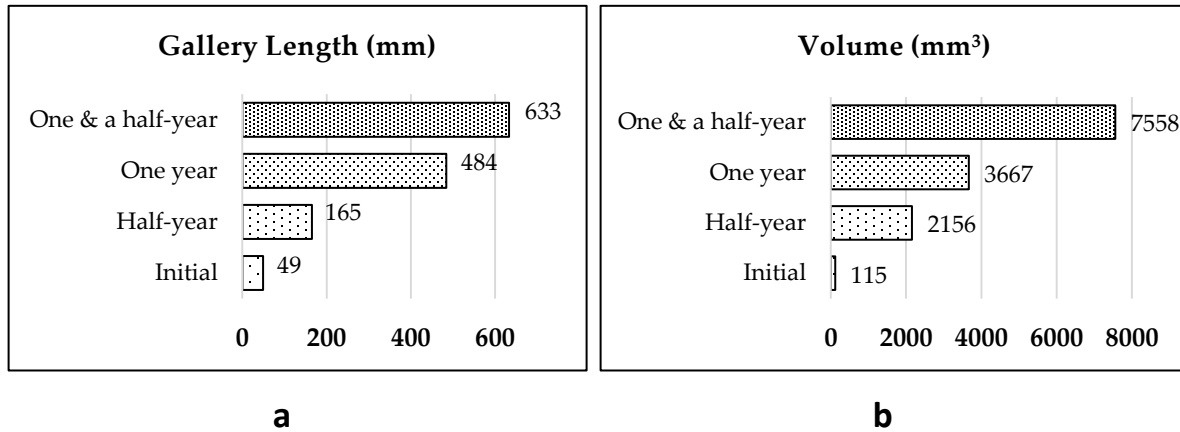


Fig. 5.3 The properties of nest-gallery development in Spruce A timber: **a** Gallery length (mm); and **b** Volume (mm³).

In 2D radial section images, we observed the dynamic change of the nest-gallery system. One of the exploratory tunnels from the first chamber was extended parallel to the longitudinal axis, and reached the outer edge of the timber (Fig. 5.2a, *green rectangle*). A half-year later, the tunnel was sealed by cement pellets and the tunnel remained sealed at the one-year scan (Fig. 5.2b – c, *green rectangle*). Interestingly, the sealed tunnel was re-accessable,

as we found that termites had re-opened the tunnel after one and a half years (Fig. 5.2d, *green rectangle*), leaving tiny cement pellets to close the tunnels to the outside environment.

The development of the length and volume (Fig. 5.3) had a pattern that indicated the basic strategy of the isolated group to extend the nest-gallery system in the Spruce A timber: namely, transverse expansion, followed by longitudinal expansion. Transverse expansion is chamber extension, while longitudinal expansion is tunnel extension. Transverse expansion is the extension of the nest-gallery by the colony to expand the chamber or explore the surrounding space in the cross-sectional direction perpendicular to the longitudinal axis of the timber, which resulted in a notable increase in volume but not in length. Longitudinal expansion is the extension of tunnel-galleries parallel with the longitudinal axis of the timber, which increased the gallery length.

Half-year data indicated that the volume increased importantly, by 2041 mm³ compared to the initial volume (Fig. 5.3b); meanwhile the length only increased 116 mm (Fig. 5.3a). These half-year data correspond with the CT image (Fig. 5.2b), indicating that the group was expanding the nest-gallery to the axial system of the timber, rather than exploring a new area parallel to the longitudinal axis of the timber. The converse pattern was observed for one-year data, where the length notably increased by 319 mm from the previous point (Fig. 5.3a) while the volume increased relatively less, by 1,511 mm³ (Fig. 5.3b). When the group extended the gallery in the axial direction, the volume highly increased by 3,891 mm³ from the previous point (Fig. 5.3b) while the length was extended only 149 mm (Fig. 5.3a). In short, the higher

the rate of axial expansion, the lower the rate of longitudinal expansion and vice versa.

5.3.2 Nest-gallery system in Spruce B timber

The nest-gallery system in Spruce B timber is presented in Fig. 5.4. A wide and large cavity (Fig. 5.4d) was excavated by a foraging group of termites on the bottom surface of the timber on the tangential surface of heartwood (Fig. 5.4c). The cavity was surrounded by cement pellets (Fig. 5.4) and was connected to the emergence hole of the natal nest on the attic floor (Fig. 5.2b) when the timber was collected in November 2014. After being separated from its natal colony, the separated group sealed the entrance hole using cement pellets (Fig. 5.4a, b, & d). The chambers close to entrance hole had been widely enlarged from edge to edge of the tangential plane (Fig. 5.4.a & c), to accommodate an aggregation of large numbers of termites (Fig. 5.4c). The 2D cross-section and radial CT images suggested that the nest-gallery had been extensively excavated throughout the heartwood and sapwood (Fig. 5.4a & b). The nest-gallery consisted of 32 chambers interconnected by tunnel galleries, and measured 56,613 mm³ in volume and 982 mm in length.

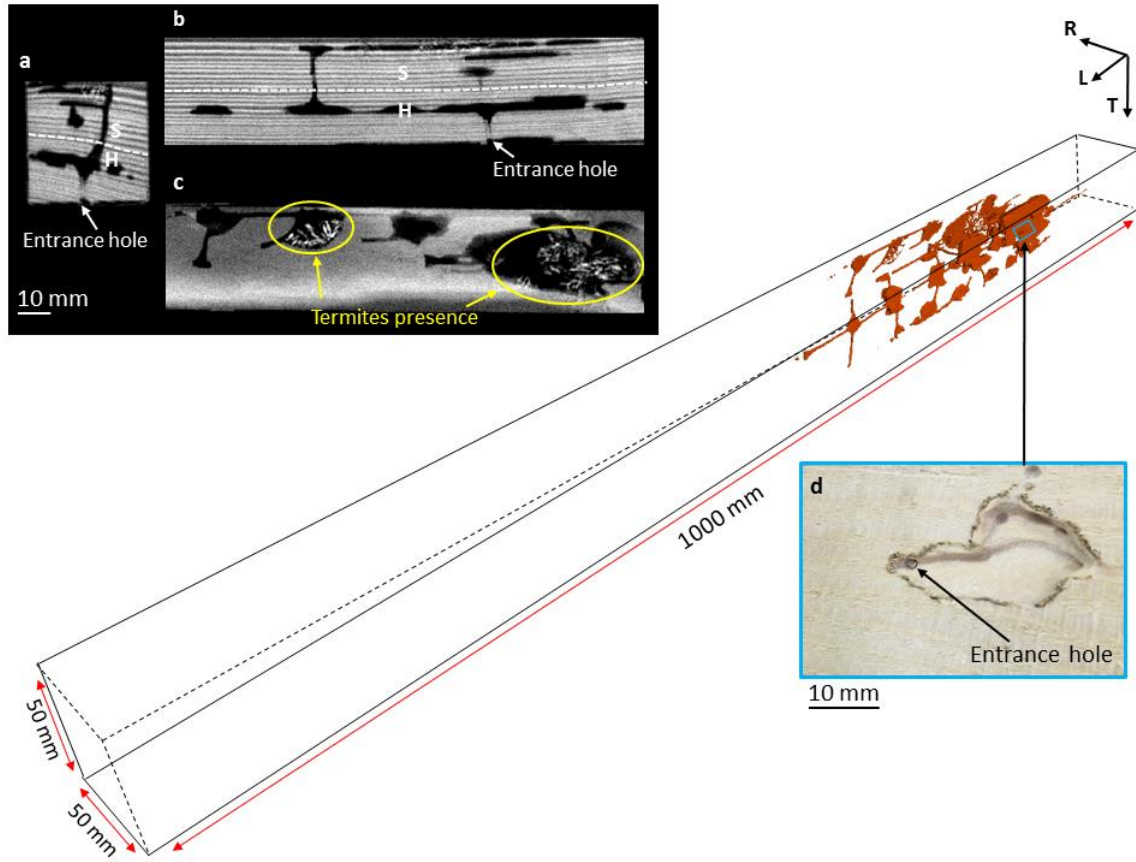


Fig. 5.4 CT images of nest-gallery system in Spruce B timber. (a) A 2D cross-sectional image; (b) a 2D radial image, indicated that the nest-gallery has been extensively excavated throughout the sapwood (*S*) and heartwood (*H*); (c) a 2D tangential image, taken from the sapwood part on the opposite surface of the entrance hole; and (d) a close-up view of the entrance hole.

Termite presence is indicated by an uncolored area inside the nest-gallery in the 3D image or a yellow circular shape in the 2D tangential image. In 2D CT images, the gray value of a pixel corresponds to an index of density: the lighter the color, the denser the area. Springwood and summerwood are indicated by the darker and lighter colors in the images, respectively.

Arrow axis represent longitudinal (*L*), radial (*R*), and tangential (*T*) section of the timber. *S* Sapwood, *H* Heartwood.

5.3.3 Nest-gallery system in Sugi timber

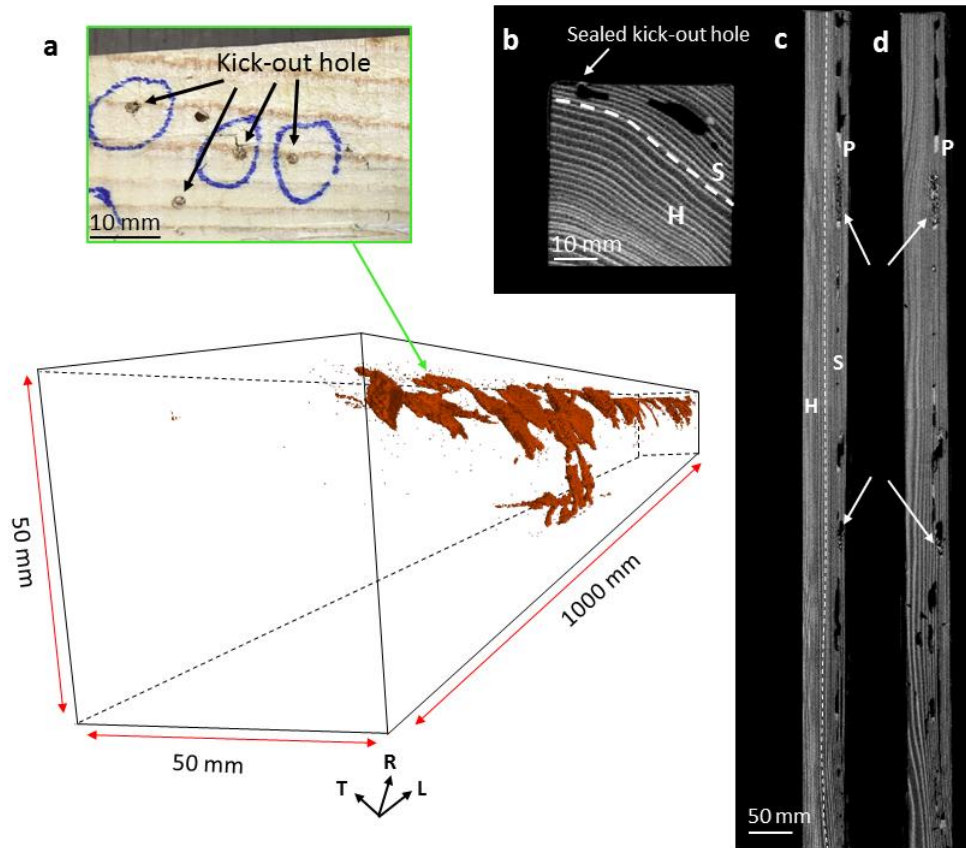


Fig. 5.5 CT images of a nest-gallery system in Sugi timber. **a** A close up view of “kick-out holes”, i.e., holes to dispose pellets from the nest-gallery. **b** A 2D cross-sectional image. **c** A 2D radial image, indicating that the nest-gallery was extensively excavated all over the timber along the sapwood part, parallel to the longitudinal axis of the timber. **d** A 2D tangential image of the sapwood.

In 2D CT images, the gray value of a pixel corresponds to an index of density: the lighter the color, the denser the area. Springwood and summerwood are indicated by darker and lighter colors in the images, respectively. The arrow axis represents the longitudinal (*L*), radial (*R*), and tangential (*T*) directions of the timber. *S*, Sapwood; *H*, Heartwood; *P*, Fecal pellets.

Figure 5.5 displays the nest-gallery system inside a Sugi timber, which shows that the nest-gallery had been excavated all along the timber. The timber was collected in November 2014 and had been located side by side with the Spruce B timber. It is highly possible that the foraging group that attacked the Sugi timber is from the same colony that infested the Spruce B timber, since only one emergence hole was observed from the attic floor (Fig. 5.1b). However, the exact position of the first entrance hole in the Sugi timber could not be located. There were neither spacious entrance holes as found in Spruce A timber (Fig. 5.1a) nor a large cavity as found in Spruce B timber, but rather, numerous holes sealed by cement pellets (Fig. 5.5a). Most of those holes were connected to chambers (Fig. 5.5b), and might have functioned as kick-out holes to dispose pellets out of the nest-gallery. The 2D radial image indicated that the nest-gallery was established parallel to the axial system along the sapwood (Fig. 5.5c), and never extended to the heartwood. The nest gallery measured 114,030 mm³ and 1,368 mm in volume and length, respectively, with 28 interconnected chambers.

5.3.4 Colony composition

The timbers were dissected into 10 pieces (~100 mm/piece) to extract termites and to examine the caste composition. The caste composition of termite colony from each timber is presented in Table 5.2, while the caste profile is displayed in Fig. 5.6. The total number of termite colonies of Spruce A, Spruce B, and Sugi timbers was 50, 117 and 150, respectively (Table 5.2). The colony was assessed into castes differentiation as follow:

- *Primary reproductive* (Fig. 5.6e), defined as a reproductive that found a new colony after a nuptial flight (Roisin 2000). It is characterized by stark sclerotization, the presence of compound eyes and wing marks (Korb and Hartfelder 2008).
- *Pseudergates* (Fig. 5.6g). The term pseudergate follows Luykx (1986), and defines a majority of individuals without wingpads in one-piece nesters (Abe 1987). They are not doing tasks and work (Korb 2007) to the same extent as that of the (true) worker in intermediate and separate nesters (Abe 1987), *i.e.*, they are less involved in truly altruistic working tasks, such as foraging, brood care, or nest duties. Korb *et al.* (Korb and Hartfelder 2008) also defined this caste as false worker.
- *Soldier* (Fig. 5.6h), a permanently sterile caste, defined as an individual with a strongly sclerotized head with defensive attributes (enlarged mandibles and phragmotic shape) (Roisin 2000).
- *Nymph* (Fig. 5.6f), defined as individual with visible wing buds, which indicates progressive moulting into an imago (Korb and Hartfelder 2008; Roisin 2000).
- *Neotenic reproductive* (Fig. 5.6a & c), defined as a secondary reproductive with juvenile morphological characteristics (Roisin 2000), characterized by the absence of wings, the lack of compound eyes, and a cuticle color that is less sclerotized than that of primary reproductives (Korb and Hartfelder 2008).

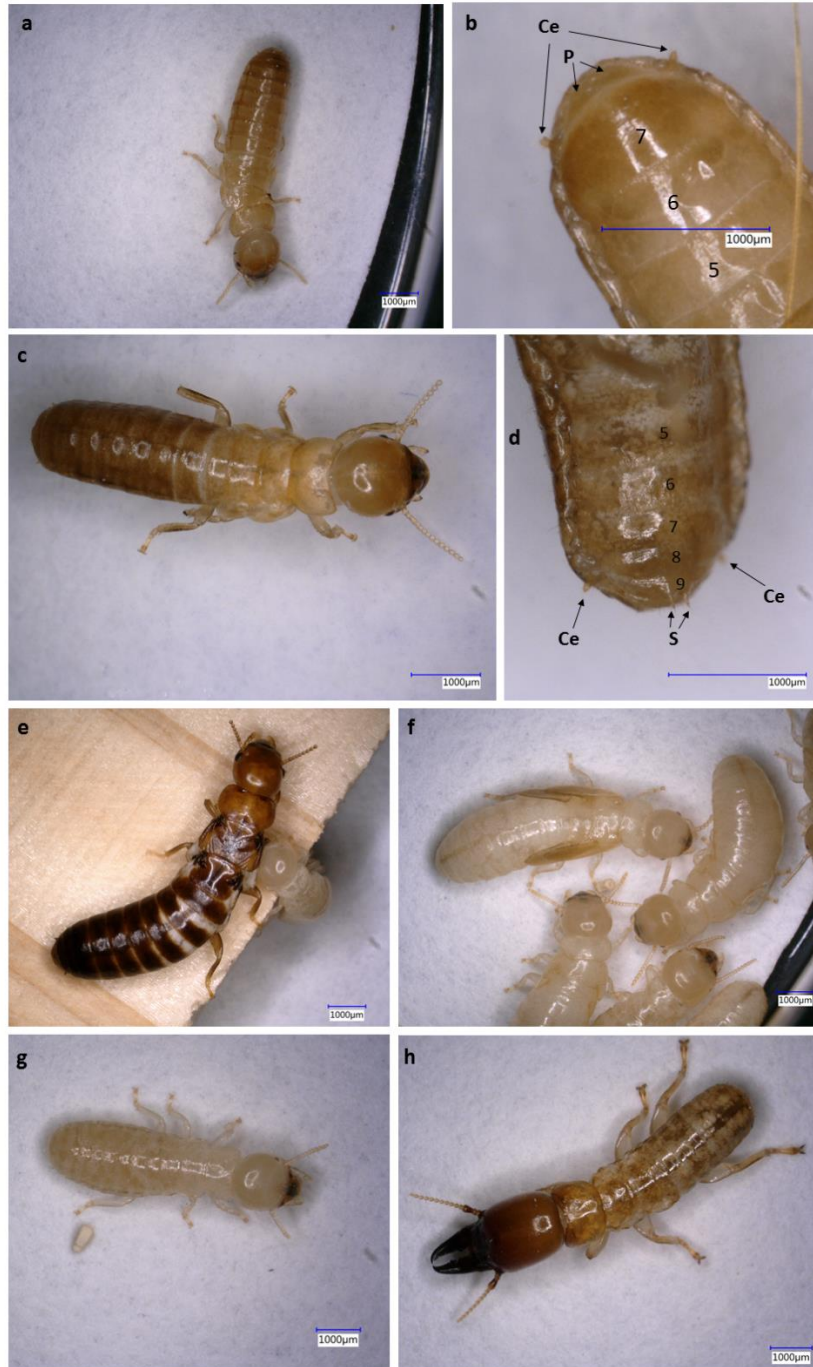


Fig. 5.6 Caste profile of *I. minor* colony. (a) A neotenic reproductive emerged from the colony in Spruce A timber and was identified as a (b) female neotenic. (c) A neotenic reproductive emerged from the colony in Spruce B timber and was identified as (d) male neotenic. (e) A queen (primary reproductive) from the colony in the Sugi timber. (f) Nymphs. (g) A “false” worker. (h) A soldier. *Ce*, Cerci; *P*, Paraprocts; *S*, Styli. The numbers in Fig. 6b & d indicate sternite segment.

Table 5.3 Caste composition of drywood termite colony from each timber

Timber	Caste composition					
	Primary reproductive	Pseudergate	Soldier	Nymph	Neotenic reproductive	Total
Spruce A	-	38	1	10	1*	50
Spruce B	-	89	6	21	1**	117
Sugi	1***	102	2	48	-	153

*female neotenic reproductive (Fig. 5.6a–b), **male neotenic reproductive (Fig. 5.6c–d), ***queen (Fig. 5.6e)

The majority of individuals within the colony in Spruce A, Spruce B, and Sugi timbers were “false” workers, at 76.0%, 76.1% and 66.7%, respectively; the next most prevalent were nymphs, at 20%, 17.9%, and 31.4%, respectively (Table 5.3). Only the colony in Sugi timbers was observed to have a primary reproductive, a queen (e), but the presence of male reproductive could not be located. The colony in both spruce timbers was observed without primary reproductives; however, a neotonic emerged from both colonies to replace the absent reproductives. The neotenic reproductive in Spruce A timber was identified as a female neotenic (Fig. 5.6b), while in Spruce B timber it was a male neotenic (Fig. 5.6d). In female neotenic reproductives, the last abdominal sternite (the seventh, which is enlarged and covers the eighth and ninth sternites) is about as long as it is wide (Luykx 1986), with paraprocts (Morimoto 2000; Weesner 1969) observed at the posterior margin (Fig. 5.6b); in males, the last abdominal sternite (the ninth) is only about 1/3 as long as it is wide (Korb 2007), and styli (Morimoto 2000; Weesner 1969) are observed at a posterior margin (Fig. 5.6d). Soldiers were observed in each colony, comprising 2%, 5.1%, and 1.3% of the entire colony in Spruce A, Spruce B and Sugi timbers, respectively.

5.4 Discussion

By evaluating foraging activities in nest-gallery development, two kinds of foraging behavior are observed: individual foraging and collective foraging. Heidecker and Leuthold (1984) suggested that foraging activity is properly called “individual” when it takes place during solitary foraging, while it is “collective” when it is feasible in relation to the group as a colony. Individual foraging was observed in the excavation of tunnel-galleries. This solitary foraging showed wood selectivity; i.e., it was particularly marked in springwood but not summerwood, and was parallel to either the radial or longitudinal axis of the growth ring. This selective foraging may be driven by foraging efficiency (Lee and Su 2010), by which individuals build efficient tunnels to gain the highest nutritional value (Traniello and Leuthold 2000), and at the same time, maintain its optimum fitness and energy spent. While the concept of foraging efficiency has only been explored in subterranean termites (Lee and Su 2010), the tunneling activity in drywood termites also shows some attributes of foraging efficiency, indicated by selectivity for the softer part of the growth ring, thereby reducing energy spent on foraging and excavation. This result supports previous evidence (Himmi *et al.* 2016a) showing that the interactions between *I. minor* colonies and wood generate a distinctive and unique foraging pattern, which leads to selective excavation of the nest-gallery system.

Collective foraging was expressed in the excavation of chambers and the whole system of the nest-gallery. Chambers were excavated and enlarged by aggregate individuals working together as a group; thus, labor resources were sufficient to excavate across several annual growth rings. The nest-

gallery excavation in spruce timbers was different from that in sugi. In spruce timbers, the nest-galleries were extended all over the sapwood and heartwood (Figs. 5.2 & 5.5) while in sugi, the nest-gallery never extended into the heartwood (Fig. 5.5). The heartwood of sugi has been reported to have resistance to termites (Kano *et al.* 2004), and its extractives have termicidal (Arihara *et al.* 2004; Sogabe *et al.* 2000) and anti-termite activities (Shibutani *et al.* 2007). Therefore, termites in sugi may avoid the heartwood. Even though the heartwood of spruce contains about 70% more extractives than the sapwood (Caron *et al.* 2013), the results indicated that the chemicals have low efficacy against *I. minor*. These different results suggested the adaptability of *I. minor* colonies to the timber environment.

Bonabeau (1998) characterized social insect colonies as a complex adaptive system (CAS) (1998) by six characteristics: (1) dispersed interactions among colony members; (2) decentralized control; (3) hierarchical organization; (4) perpetual reproduction; (5) continual adaptation to changing environmental conditions; and (6) self-organization. These six CAS characteristics can be identified as stigmergic behaviors in social insect. Stigmergy defines a colony as an integrated unit that possesses the ability to process a large amount of information in a distributed manner, such as decisions on task allocations by individuals, coordination of activities colony members, undertaking enormous nest construction works, and also flexibility in response to external and internal challenges (Bonabeau 1998). Stigmergic coordinations result from three essential elements: individual autonomy, localized interactions, and autonomous processes selected from individual-colony interaction.

In the present study, stigmergic coordinations were observed in two particular behaviors: sealing a tunnel gallery that ends at the outer edge of the timber (Fig. 5.2a – b); and transporting fecal pellets to particular chambers located beneath timber surface (Fig. 5.2, *3D images*). When a termite individual encounters a tunnel exposing the nest to the outer environment (Fig. 5.2a., *2D radial image*), this individual information is collected and possibly transferred into the “colony decision” to seal that particular tunnel (Fig. 5.2b) as a part of nest defense and protection. The fact that the sealed tunnel was re-accessable (Fig. 5.2c – d) indicates a dynamic change in the nest-gallery system: self-organization and continual adaptation to a changing environment. Similar fortification behaviors were also encountered by Indrayani *et al.* (Indrayani *et al.* 2008), as *I. minor* individuals were reported to build a barrier using cement pellets to block their contact with bait-chemicals. Locating the fecal pellets in certain chambers inside the nest-gallery could be explained from the same stigmergic decisional process to maintain the connectivity and network distribution of the nest-gallery. This argument is supported by a previous finding that *I. minor* individuals collected the wood fragments they foraged and carried them to other locations (Indrayani *et al.* 2007), which may be a behavior necessary to maintain the optimum condition of the network of nest-galleries.

The presence of primary reproductives may have an effect on the way the colony forages and establishes the nest-gallery. In spruce timbers where the primary reproductives are absent, termites were observed to aggregate in certain chambers (Fig 2 & 4) and always move together as a group. During extraction, termites were collected from only 2 or 3 pieces out of 10 pieces of

cut timber. In CT data, they were found to forage and aggregate relatively close to one other; therefore, excavation of the nest-gallery tended to concentrate on certain areas of the timber (Figs. 5.2 & 5.4). On the other hand, the isolated group with the primary reproductive inside the Sugi timber was found to be more distributed over the entire timber (Fig. 5.5). During extraction of the colony, termites were collected from 8 pieces out of the 10 pieces of cut timber.

The presence of a single queen in the *I. minor* colony inside the Sugi timber (Fig. 5.6e) is not an uncommon situation in nature (Harvey 1934a). Since drywood termites' movement is dynamic, it is highly possible that the colony members may forage separately from the pairing reproductives, and be located in different chambers or even different piece of timbers. In our case, foraging groups of termites emerged from the attic-floor and attacked the adjacent timber samples. When the timbers were collected, the foraging groups were isolated from their natal nest, which was previously contiguous. This interference may force the groups to adjust their caste compositions by replacing the absent reproductives, as we observed in the isolated groups inside both spruce timbers (Fig. 5.6a & c).

In kalotermitidae, the tendency of immature stages to become neotenics is called reproductivity (Nagin). Both neotenics were observed without wing buds, which indicated that they had moulted from the pseudergate stage. Our observations support a previous report suggesting that pseudergate reproductivity is generally quite high, at least as high as, or even higher than those of nymphs and larvae (Myles 1999). Interestingly, the sexes of those neotenics were different: female in Spruce A timber (Fig. 5.6b)

and male in Spruce B timber (Fig. 5.6d). Previously, we thought that the secondary reproductive first to emerge would be a female reproductive; however, these results suggested that is not always the case. Further study is necessary to better understand this issue.

Spruce A timber was opened 3 years and 4 months after collection (Table 5.2). Based on the CT data (Fig. 5.2a), the timber was newly infested just before collection. Spruce B and Sugi timbers were opened 1 year and 4 months after collection (Table 5.2). However, the CT data suggested that those timbers might have been infested 3 to 6 months before collection (Figs. 5.4 & 5.5). An earlier report about the emergence of neotenic reproductives in an orphaned *I. minor* colony was reported by Harvey (1934b), who assessed isolated groups consisting of 20 – 30 individuals in laboratory conditions (under 80°F and RHs 70%). The first evidence of morphological change of the individuals of the sixth instars (equal with first nymphal instar in Roisin's (2000) caste developmental scheme) into supplementary reproductives in his study appeared within four days to one week after orphaning. In three weeks, the eyes became conspicuously pigmented, almost black, and showed distinctive color changing from the mesothorax to the abdomen. At the end of a month these color changes reached their maximum, and then ceased. The orphaned colonies showed that the first egg was laid by the end of the second months, and by the end of the first year, 5 – 12 progenies ranging from first to fifth instar had emerged.

By examining the color of neotenic bodies in the isolated groups from both spruce timbers and comparing it with Harvey's description, it seems that the neotenic in those groups had just emerged. The fact that we didn't find

any eggs indicated that the secondary reproductives had not yet reached complete development. Harvey described that secondary reproductives emerged in perfect moult just one month after isolation; however, our case in both spruce timbers indicates a much longer time for the colony to adjust its caste composition. Atkinson (1994) reported that seasonal trends in colony composition were relatively weak in *I. minor*. He observed that all or most of the alates in *I. minor* colonies appeared to emerge whenever suitable environmental conditions were present. This might mean that the emergence of replacement reproductives in the isolated groups of *I. minor* in spruce timbers occurred when the groups met “suitable environmental conditions” (Atkinson 1994).

Then the major question arises: to what extent does “the suitable environmental condition” facilitate the emergence of reproductives in *I. minor*?

Interesting comparative data regarding reproductive decision-making in another kalotermitid, *C. secundus*, have been reported by Korb and Lenz (2004) and Hoffman *et al.* (2012). They argued that “nest value” mediated the decision-making of replacement reproductives in wood-dwelling termites. Individuals in *C. secundus* continually evaluate societal conditions (e.g., the absence of an existing royal pair), change their development and induce competition in an attempt to take over a higher nest value. Nest value greatly determines an individual’s potential fitness, which means that a high-value nest provides more potential fitness benefits than a low-value nest. In drywood termites, nest value may relate to wood resources (food and nest space) relative to colony size. As drywood termites have the ability to assess

the wood size by vibration [26], the remaining value of the current nest, such as nutritional richness, may be a critical factor in an individual's decision about whether to develop into a replacement reproductive and take over the nest.

However, Lenz questioned whether reproductive decision making in *I. minor* had a similar response as that of in *C. secundus* (Lenz and Yoshimura, personal communication). An experiment set up in orphaned *I. minor* colonies using the same ratio model of nest value and colony size as in *C. secundus* drove a much slower response in the neotenic production rate. *Incisitermes minor* did not produce the same response as *C. secundus*, suggesting that the model might not fit (Lenz, Yoshimura, and Lewis, unpublished observations). This inference contradicts Harvey's description (1934b) about the emergence of replacement reproductives in *I. minor* colonies. Further work is necessary to unravel the factors that trigger and regulate neotenic numbers in orphaned *I. minor* groups.

5.5 Chapter summary

The documentation of colonization process of foraging groups of *I. minor* in previously unoccupied timbers using X-ray CT has provided better understanding on how isolated groups of *I. minor* develop and maintain the nest-gallery system; as well as to sustain the colony. In establishing nest-gallery, *I. minor* showed selective foraging activities and adaptability to different timber environments. Stigmergic behaviors were observed in the way of isolated groups of *I. minor* maintain the nest-gallery system, which was expressed in sealing a tunnel gallery that ends at the outer edge of the timber; and transporting fecal pellets to particular chambers located beneath timber

surface. The isolated groups of *I. minor* showed dynamic change in caste composition to sustain the colony. In both groups in which the primary reproductive are absent, a replacement reproductive has emerged from pseudergate stage. However, the sexes of replacement reproductive, time interval and the suitable conditions to facilitate the emergence of replacement reproductive are not been fully understood yet.

CHAPTER

6

WOOD ANATOMICAL SELECTIVITY OF DRYWOOD TERMITE IN THE NEST-GALLERY ESTABLISHMENT

The chapter provides information on how drywood termites establish and maintain their nest-gallery systems in response to internal structure of fibers, growth rings and other anatomical properties of timbers. The chapter has been published on Journal of Wood Science and Technology (2016), 50(3): 631-643.

6.1 Introduction

Termite nests are among the most complex and sophisticated insect-built structures. The construction of nests is related to feeding biology and is the key factor that affects life history and social evolution among termites (Traniello and Leuthold 2000). Nest structure represents the stigmergic work (Ladley and Bullock 2005) and coherent organization (Perna *et al.* 2008a) of a colony. The interactions between the individuals and the environment generate different collective patterns and decision making (Deneubourg and Goss 1989), which represent the distinctive structure of the nest.

During the colonization and foraging within single pieces of wood, the internal structure of fibers, growth rings and other anatomical properties could provide important insights on how drywood termites establish and maintain their nest-gallery systems, and possibly lead to the development of new and novel control technologies.

6.2 Materials and Method

6.2.1 Wood specimen

All wood samples used in our investigations were structural quality sugi (*Cryptomeria japonica*), collected in 2009 from a warehouse in Wakayama Prefecture, Japan. This warehouse had a history of having timbers that were infested by *I. minor*. The infested timbers were brought back to the laboratory for further examination. Twenty-five timbers were preliminarily subjected to X-ray CT scanning analysis, and two timbers, Timber A and Timber B, are presented in this study as typical case; *i.e.*, revealing the anatomical selectivity of *I. minor* in establishing the nest-gallery. Timber A was a timber block with

the dimensional size of 48 (R) x 56 (T) x 780 mm (L), and Timber B was a log timber with a diameter of 50 mm and a length of 250 mm.

6.2.2 X-ray CT Scanning

The selected timbers were imaged using an X-ray CT (Y.CT Modular320 FPD, YXLON International GmbH, Germany) maintained at Kyushu National Museum, Japan. All scanning of timbers were conducted during August 2012. The scanning was performed in vertical measurement by 320 kV X-ray source (2.0 mA) with 400 mm x 400 mm digital flat-panel refractive index detector (RID) (YXLON International GmbH, Germany) in the dynamic range of 16 bit, pixel pitch 20 μm , and 1024 x 1024 pixels resolution. The timbers were placed on a rotary table that continuously rotated during the scanning process, while simultaneously the X-ray tube and the detector were moved vertically and downward along the entire length of the timbers. The X-ray data obtained during the scanning process were stored in files containing two-dimensional (2D) image stacks (*.raw image file), with each file representing a single one image-slice of the timbers.

6.2.3 Data Analysis

The 2D CT-image stacks for each timber were rendered into 3D images (each 2D image slice was 0.3 mm in thickness) by using a volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH, Germany). Volume pixel (voxel) data was shown in grayvalues (signed 16 bit) and a histogram of pixel intensity value (opacity), which represented the absorptance intensity of the X-ray. Various densities of internal structure of the timber generated different absorptance intensities, which resulted in distinctive opacity and

voxel data regions. The volume-rendering technique delivered 3D visualization of the voxel data as a density function of the timber. Therefore, by specifying the opacity of the voxels data, we were able to map lower and higher density areas of the timber and visualize the nest-gallery system within it. The important properties of nest-gallery systems such as volume and length could be estimated.

We randomly cut each of the lower and higher density parts of the timber generated from the 3D visualization into wood block (10 x 10 x 10 mm) with ten replications to determine the density value. All wood blocks were oven-dried for 24 h at 105 °C, and then the density of those respective parts was confirmed by specific weight and volume measurements.

6.3 Results

Figure 6.2 displays the 3D image reconstruction of the extensive nest-gallery system of *I. minor* inside Timber A. Measurements of important properties of the nest-gallery system are presented in Table 6.1. Timber A was 780 mm long, and the total length of the galley system was 639 mm and represented about 81.9% of the length of the entire timber. The total volume of the nest-gallery was about 6.9% of the total wood volume.

The nest-gallery system mainly consisted of numerous chambers and interconnected tunnel-galleries (Fig. 6.1). The tunnels were primarily constructed along the springwood, and had a narrow diameter, so narrow that only a single termite can pass through it. However, the average of tunnel diameters varied considerably depending on the density of wood fibers encountered by foraging termites during construction. In general, tunnels

among the galley system had a circular cross-section and averaged 2 mm in diameter, and often interconnecting with chambers.

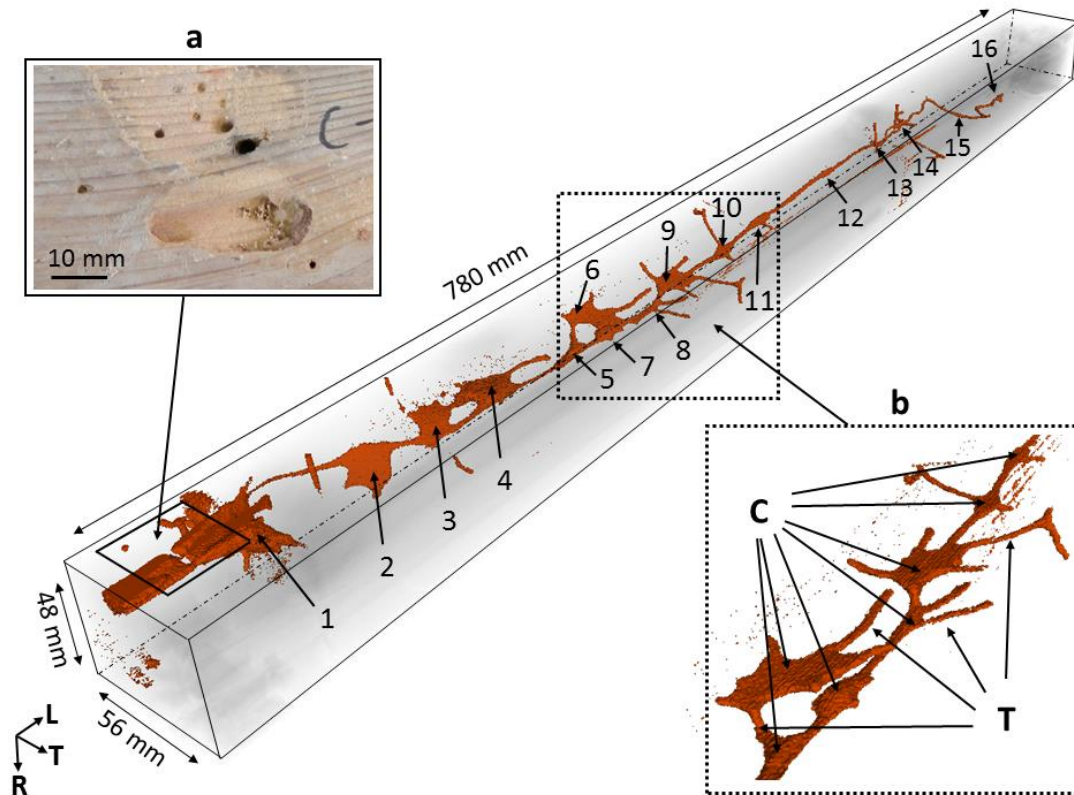


Fig. 6.1 The 3D CT image of an established nest-gallery system of *I. minor* in a naturally infested timber (Timber A). Chambers are numbered (1 – 16) for reference in the main text and in table 6.2.

a The rectangular area represents a close-up view of the entrance hole, which leads to the first chamber, suggesting that the timber was infested by the invasion of *I. minor* colony from another adjacent timber. **b** The dashed-square area represents a close-up of the nest-gallery system, which consisted of interconnected chambers (C) and tunnel-galleries (T).

Table 6.1 The measurements of important properties of the nest-gallery system of *I. minor* in Timber A.

Properties	
- Gallery length (mm)	639
- Nest-gallery volume (mm ³)	144,489
- Total number of chambers	16
- Total wood volume (mm ³)	2,096,640
- Nest-gallery volume to wood volume (%)	6.9

The nest-gallery system observed consisted of sixteen interconnected chambers, which suggested that the timber was occupied by a single colony (Fig. 6.1). The properties of the chambers are presented in Table 6.2. Most of the chambers were constructed as spacious cells to accommodate the aggregation of termite individuals. The width and diameter of the chambers varied from 1.85 mm to 14.69 and from 2.83 mm to 20.04 mm, respectively (Table 6.2). The chambers were cavernously constructed around an annual growth ring (max. width $\leq$ 2.5 mm, Table 6.2), sometimes several growth rings were involved (max. width $>$ 2.5 mm, Table 6.2). The biggest chamber was chamber 1 with estimated volume 133,094 mm³, while the smallest chamber was chamber 16 with estimated volume 64 mm³ (Table 6.2).

Table 6.2 The measurement of chambers properties of the nest-gallery system in Timber A.

Chamber	Volume (mm ³)	Max. width (mm)	Max. diameter (mm)
1	133,094	14.69	20.04
2	526	3.75	17.51
3	284	2.02	15.88
4	1,517	6.94	15.39
5	245	2.97	5.61
6	538	2.72	15.35
7	179	3.04	7.47
8	87	2.15	4.26
9	494	2.81	11.64
10	160	2.07	7.08
11	129	1.95	5.11
12	105	1.85	2.83
13	107	2.01	4.46
14	120	4.16	5.32
15	72	2.12	4.83
16	64	1.92	3.28

Visual examination of the timber revealed the infestation was not originated from a nuptial flight by dealate reproductive, but from an invasion of *I. minor* colony from another timber (Fig. 6.1a). This invasion from another timber was indicated by the wide entrance hole area seen in Fig. 6.1a. Invasions from adjoining timbers by drywood termite had been previously reported (Grace *et al.* 2009; Himmi *et al.* 2014). The typical infestation from

colony invasion was also indicated by a big and spacious chamber (chamber 1 on Fig 2) which was connected with the wide entrance (Fig 2.a), the same indication as reported by Himmi *et al.* (2014).

The intensity of X-rays absorption is highly correlated with the density of wood fibers and growth rings. Due to the different intensity of X-rays absorption, various densities of the timber properties can be classified on the tomographic images. Different varieties of wood density and the annual growth rings generated unique X-ray absorption patterns that can be readily seen with tomographic images in Fig. 6.2. The lower density wood is depicted as white in color, while the higher density wood and knots are dark brown in color and the chambers and gallery system appear in red (Fig. 6.2). The lower and higher density parts of the timber generated $0.386 \text{ g/cm}^3 \pm 0.019 \text{ g/cm}^3$ and $0.454 \text{ g/cm}^3 \pm 0.09 \text{ g/cm}^3$, respectively. These results indicated that foragers did select less dense wood to excavate chambers. The measurements of chambers properties (Table 6.2) suggested that the chambers in the lower density part of the wood (Chamber 1 – 11, Fig. 6.1) had higher volume than those in the higher density part (Chamber 12 – 16, Fig. 6.1). The chambers varied in shapes and structures to adjust the anatomical texture and wood fiber density within the timber (Fig. 6.2a, d – f) and also followed the orientation of growth rings. In 2D CT images (Fig. 6.2a – f), the density variations of wood are highlighted by the grayvalue of a pixel: the denser the wood the lighter the color.

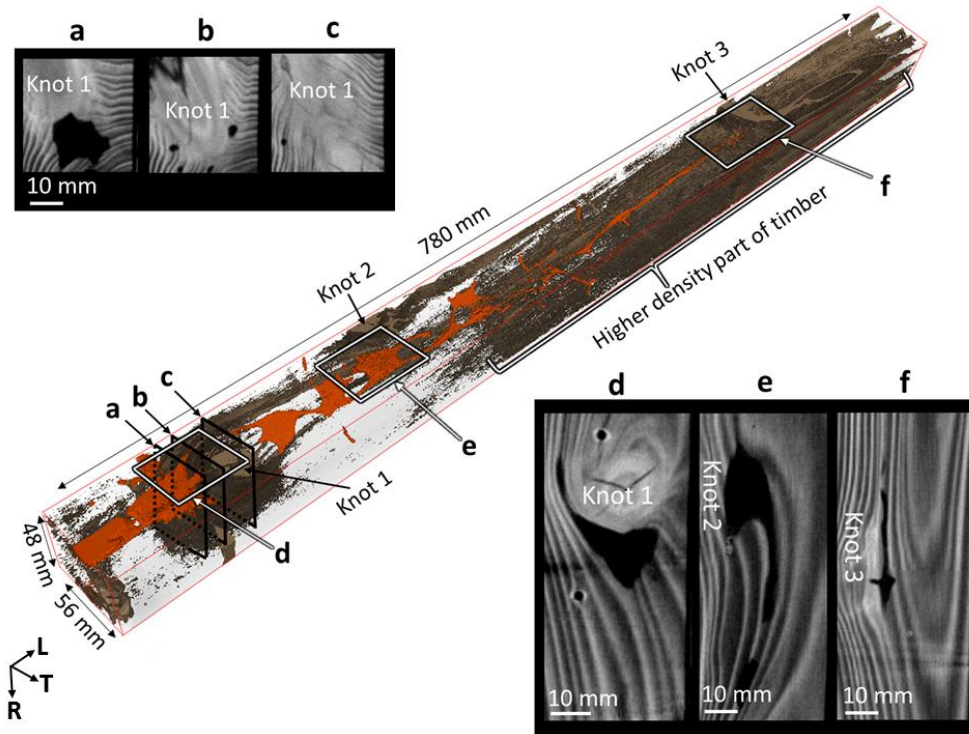


Fig. 6.2 The 3D CT images of the nest-gallery system and anatomical texture of Timber A.

2D cross-section images reveal the tunneling strategy in response to the physical constraints of knot 1: **a** The first chamber, spaciously extended across several annual rings; **b** Four exploratory tunnels were excavated from the first chamber to search for less-dense spots to pass the knot; **c** The selected tunnel, narrowly excavated on a less-dense spot at the periphery of the knot.

2D tangential images present the area around three knots, which show the selectivity on nest-gallery excavation: **d** A narrow passage tunnel from the first chamber to cross knot 1, excavated along a particular annual ring at the periphery of the knot; **e** The shape of the chamber followed the anatomical texture of the timber; **f** tunnel galleries were excavated to avoid the knot.

In the 2D CT images (Fig. 6.2a – f), the grayvalue of a pixel corresponds to an index of density: the lighter the color, the denser the area. Springwood and summerwood are indicated by the darker and lighter colors in the images, respectively.

In the 3D CT images, the grayvalue of voxels corresponds with the density variation of the timber. Timber density is displayed by classifying the opacity value of the tomographic image data. The colored areas inside the timber show the higher density areas of the timber, while uncolored areas represent lower density areas.

To summarize the results from the CT images, a possible explanation for the foraging strategy used by *I. minor* when searching for food and nesting sites within timbers was revealed. First, from the initial chamber (Chamber 1, Fig. 6.1), termites constructed and excavated a spacious chamber that involved several growth rings (Figs. 6.3a and 6.3d). After a period of time in constructing and expanding it, *I. minor* foragers extended out and encountered denser wood or the knot. Four exploratory tunnels were excavated from the first chamber (Fig. 6.2b), which may have been related to wood assessment. The termites then selected a spot in the peripheral knot area to establish a very narrow tunnel-gallery (~1.4 mm) (Fig. 6.2c) for further excavation. Further excavation indicated there was considerable meandering about inside the timber, as the colony navigated in and about between less dense and denser wood, e.g., springwood, summerwood, and knots (Fig. 6.2d – f). The 2D and 3D images also reveal exploratory tunnels that appear to have been built perpendicular to chambers (Figs. 6.2 and 6.3). These narrow perpendicular tunnels radiating out from chambers may indicate the ability of foragers to assess the wood. In other species of drywood termite, *C. domesticus* (Evans *et al.* 2005) and *C. secundus* (Evans *et al.* 2007), researchers have reported its ability to differentiate between food sources using vibrational signals.

We observed a similar pattern of nest-gallery development in a timber which was invaded by an *I. minor* colony that emerged from an attic floor to attack the adjacent bottom surface of the timber (Himmi *et al.* 2014). The timber was subjected to biannual analysis using X-ray CT to observe in situ development of the nest gallery by the colony. Initially, the colony established

the first chamber and enlarged it until it was spacious enough to accommodate an aggregation of dozens to twenties of termites. The colony then assessed the timber by excavating five exploratory tunnels, and selected a particular tunnel for further extension of the nest-gallery. After less than two years, the nest-gallery system consisted of interconnected chambers and tunnel-galleries, in such a way to resemble the established nest-gallery system seen in Fig. 6.1 (Himmi, unpublished data).

Figure 6.4 presents a close detail of the anatomical selectivity of *I. minor* in foraging and excavating the nest-gallery. In at least two spots, we observed that the excavation line had been changed from a narrow growth ring to a fairly wide growth ring (Fig. 6.3a) consisting of springwood. In the second location, the direction of excavation had been changed to avoid the denser wood surrounding a knot (Fig. 6.3b). Previous studies reported that the galleries of drywood termites consist of chambers and galleries that are irregular in their construction (Cabrera and Scheffrahn 2001; Emerson 1938; Harvey 1934a). Our findings, however, suggest that the pattern of construction and connections of chambers to the galley system is quite regular. We found that interactions between the *I. minor* colony and environment, represented by the wood's anatomical properties of fibers and growth rings, generated a distinctive and unique foraging pattern and decision making, which lead to the selective excavation and establishment of the nest-gallery system.

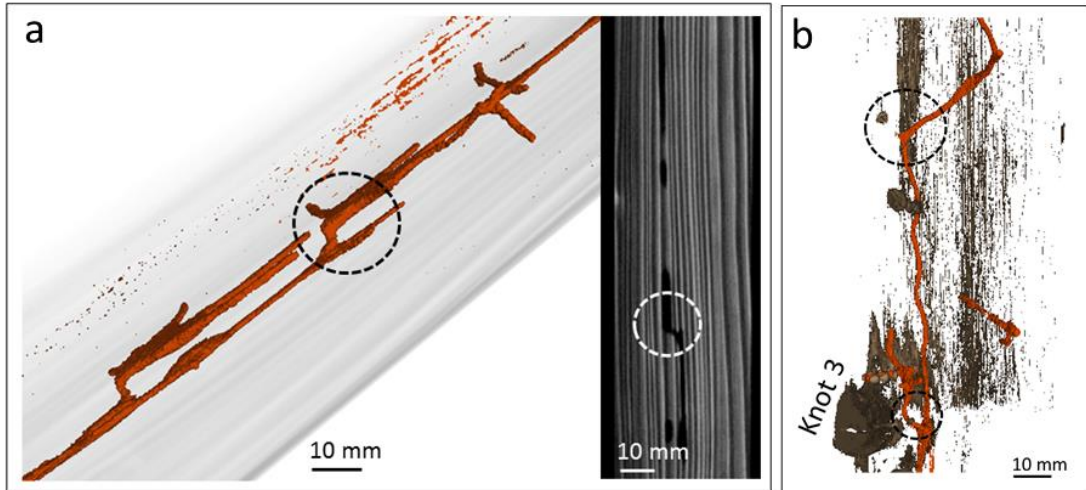


Fig. 6.3 Selective excavation of *I. minor* colony on the anatomical texture of Timber A, marked by dashed-circle.

a The colony changed the line of excavation from a narrow annual ring to a wider annual ring (area marked by dashed-circle in both 3D image and 2D radial section image). The 3D image in this figure is the radial-section view of the image in dashed-square area of Fig. 6.1. In 2D CT image, the gray value of a pixel corresponds to a density index: the lighter the color, the denser the area. Springwood and summerwood are indicated by the darker and lighter colors in the 2D images, respectively.

b The 3D image shows that the colony changed excavation direction to avoid physical constrains. The area is indicated by a dashed-circle. Timber density in 3D CT images is displayed by classifying opacity value of the tomographic image data. Higher density areas of timber are shown by colored area inside the timber, while uncolored area represents the lower density area of the timber. Please refer to Fig. 3 for details regarding the anatomical texture of the timber.

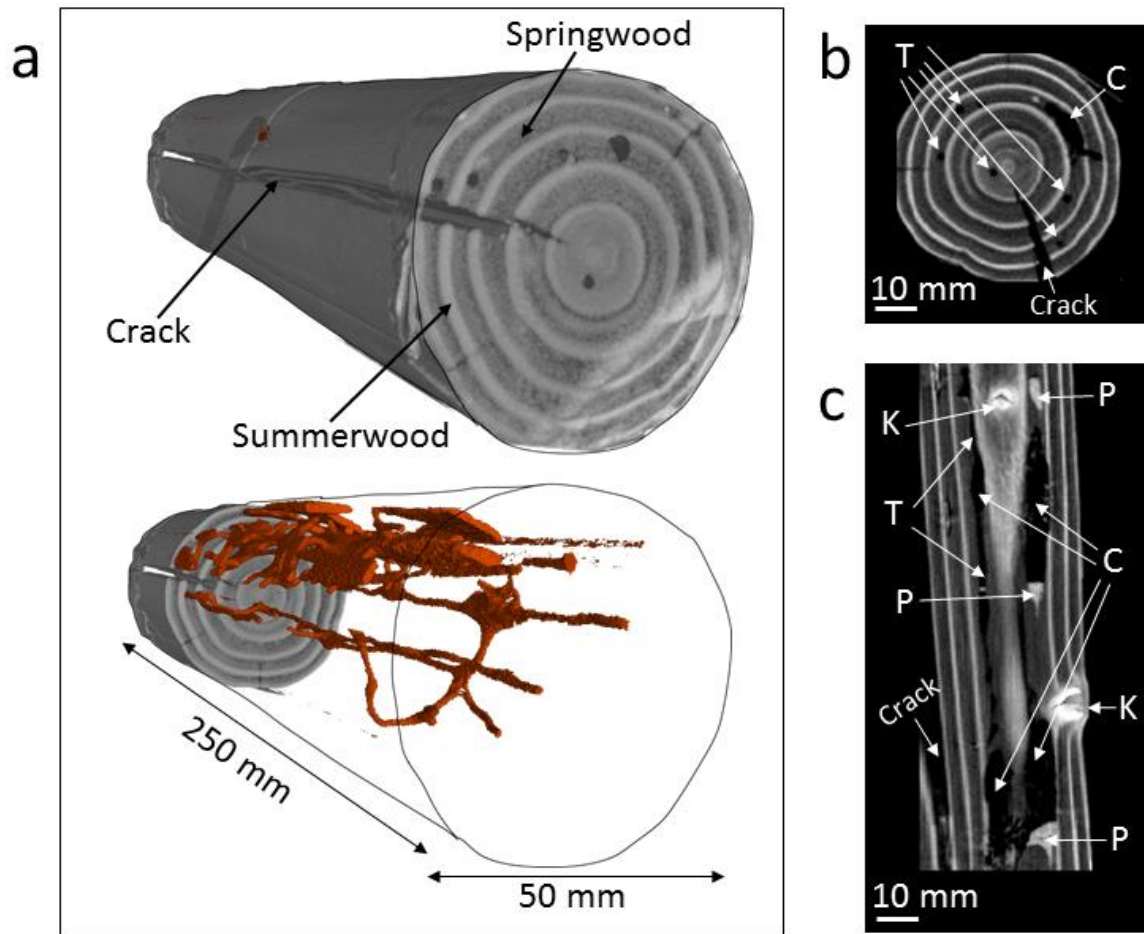


Fig. 6.4 Anatomical selectivity of nest-gallery excavation in Timber B.

a The 3D CT images of a piece of Sugi log timber and the nest-gallery inside the timber demonstrate how the nest-gallery was excavated to avoid the crack and follow the annual growth ring. **b** The 2D cross section image, presents selective excavation of chambers and tunnel-galleries on the springwood. **c** The 2D tangential section image reveals that the excavation of the nest-gallery in the axial direction also followed the anatomical texture of the wood, avoiding knots and summerwood. In the 2D images, the grayvalue of a pixel corresponds to a density index: the lighter the color, the denser the area.

C: Chamber; T: Tunnel-galleries; P: Fecal pellets, K: Knot. Springwood and summerwood are indicated by the darker and lighter colors in the 2D CT images, respectively.

Figure 6.5 shows the nest-gallery system inside a short piece of sugi log timber (Timber B). However, similar to findings from Timber A, foragers demonstrated the same selective excavation pattern in establishing the nest-gallery system. The excavation of chambers and galleries avoided a wood crack (Fig. 6.4a) and several knots (Fig. 6.4c) to follow the springwood portion of the log in either a perpendicular (Fig. 6.4b) or parallel direction along growth rings (Fig. 6.4c). Foragers also excavated some chambers close to the surface, being careful to leave a thin layer of wood between the excavated chambers and the exterior surface of the log that faced the outside environment (Fig 5.a). Unfortunately, volume measurements for chambers and gallery-system could not be estimated due to technical difficulties that included a large wood crack found down the entire length of the log.

6.4 Discussion

The nests built by termites reflect their degree of social organization, diet, biology and environmental factors (Ptáček *et al.* 2013). Based on the information about nest systems and feeding habits, Abe (1987) distinguished evolutionary sequences of life types: one-piece nest, intermediate nest, and separate nest. Bornabeau (1998), by quoting Levin (1998), introduced the concept of social insect colonies as a complex adaptive system (CAS), and defined by six characteristics: (1) dispersed interactions among colony members; (2) decentralized control; (3) hierarchical organization; (4) perpetual reproduction; (5) continual adaptation to changing environmental conditions; and (6) self-organization. Self-organization is dynamic and involves complex collective behaviors and interactions among individuals

exhibiting simple behavior (Bonabeau *et al.* 1997; Deneubourg and Goss 1989). The first four characteristics are common biological attributes and the cornerstones to define eusociality. The latter two characteristics are common among termite colonies and expressed in efficient tunneling (Lee and Su 2009) and in nesting biology: nest-site selection (de Lima *et al.* 2006); topological efficiency (Perna *et al.* 2008b); microclimate regulation and fortification (Noirot and Darlington 2000); and nest expansion (O'Toole D *et al.* 1999) and development (Wilson 1971).

Previous studies that have reported on the important factors and characteristics of self-organization and environmental adaptability have focused on intermediate and separate nesters. However, results from this paper demonstrate that one-piece nesters involving drywood termites also exhibited self-organization and the ability to continually adapt to the changes in food source they encounter in their environment, similar to the foraging and nesting biology found in intermediate and separate nesters, however, at a much limited and smaller ecological scale. For the current study, the continual adaptation of the nest environment was demonstrated by the anatomical selectivity in establishing the nest-gallery system (Figs. 6.3 – 6.5) and in the excavation of less dense and more favorable wood areas inside timbers. Previous studies have also reported that species among drywood termites demonstrated environmental adaptability in nest-site selection (Himmi *et al.* 2014), wood feeding preference (Indrayani *et al.* 2006), and extensive gallery system across multiple adjacent pieces of wood (Grace *et al.* 2009).

Environmental adaptation of the colony is an expression of self-organization, defined as the collective behaviors that emerge from many interactions among individuals. The behavior of individuals of drywood termites can also be driven by environmental cues such as food source (Inta *et al.* 2007; Korb 2006), nutritional ecology (Shellman-Reeve 1994), and local constraints on the food source that include density of wood on growth rings and knots (Figs. 6.3 – 6.5). Self-organization can also be expressed in fortification behavior, such as that demonstrated by the drywood termite foragers when they sealed the gallery system using cement fecal pellets to protect the colony from adverse environmental factors (Indrayani *et al.* 2008). In the present results, self-organization is represented by the modulation of tunnel excavation based on foraging efficiency to compromise with environmental constraints.

A common pattern seen among foraging animals is to maximize and efficiently use food resources as measured by energy and time spent in food gain (Pyke 1978). Foraging efficiency has been reported for subterranean termites (intermediate nesters) (Lee and Su 2010; Reinhard *et al.* 1997), but not for drywood termites (one-piece nesters). For subterranean termites, foraging efficiency was demonstrated in systematic (Reinhard *et al.* 1997), efficient length and distribution of tunnel networks (Lee and Su 2009; Lee *et al.* 2007) in locating food sources away from the nest. Those studies reported that the tunnel networks were constructed from the nest to the food resources in such a way to reduce the energy and time spent. Similar to subterranean termites, in this study we observed that drywood termite colony minimized the energy and time spent in the construction of galleries

and tunnels, albeit within the food source and not in far and distant locations away from the nest. Additionally, our results suggested that drywood termites forage and extend the nest-gallery to avoid anatomical constraints such as dense wood fibers and knots. This finding supports previous investigations showing that drywood termites are able to identify the wood and to modulate their foraging behavior accordingly (Evans *et al.* 2007; Evans *et al.* 2005; Indrayani *et al.* 2007; Inta *et al.* 2007).

At the practical level, infestations of drywood termites are very difficult to detect and control, partly because the colony lives entirely inside the wood and is often hidden from view. The critical challenges faced by inspectors during the inspection process are to identify the existence and extent of the infestation. X-ray tomography can lead us to a better understanding of the nesting biology and architecture of this cryptic pest. Hopefully, the results of our investigation will trigger future studies that involve unreported drywood termite foraging and tunneling behaviors, as well as novel methods and techniques that will lead to more effective integrated pest management of this globally important structural pest.

6.5 Chapter summary

X-ray CT analysis revealed the 3D nest-gallery system of drywood termites and the importance of the anatomical differences among growth rings and knots to foragers. The nest-gallery excavations demonstrated continual adaptation to anatomical constraints by foragers in selecting favorable areas of less dense wood fiber inside timbers. The present results confirmed the findings of previous studies that reported drywood termites

possess the ability to assess the internal quality of growth rings, knots, and wood-fiber density within timbers, for exhibiting preferential feeding and selectivity within timbers.

CHAPTER

7

CONCLUSIONS

Conclusions

The evaluation of the colony-founding activities of *I. minor* following the nuptial flight on six commercial timber species suggested that *I. minor* reproductives showed timber preferences. The order of preferred timber species was as follows: hinoki, spruce, western red cedar, sugi, Douglas-fir, karamatsu. The current result showing nesting preferences among these six commercial timbers corresponded to previous report on feeding preferences (Indrayani *et al.* 2006).

The reproductives of *I. minor* expressed nest-site selectivity on a preferred part of the timbers, i.e, on the springwood part of the annual growth rings on the sapwood part of the timbers. The reproductives of *I. minor* also showed selectivity in determining their nest-site location in response to the timber arrangement, namely by preferring the CG area. The results corresponded to previous reports which suggested that *I. minor* royal pairs like wood cracks, crevices or holes as sites at which to excavate the first royal chamber.

In extending the nest-gallery, *I. minor* also expressed selectivity in foraging by selecting favorable excavation areas, and showed adaptability with respect to the timber environment. The incipient colony following the nuptial flight to have 0 – 5 colony members in the first year, in which nest extension followed a certain pattern: selective excavation in the springwood part of the particular growth ring where the entrance holes were excavated in a direction either perpendicular or parallel to the longitudinal axis of the timber.

In the absence of primary reproductives, the colony showed dynamic change in its caste composition through the emergence of replacement reproductives. The results also suggested that replacement reproductives can emerge from the pseudergate stage. However, the sexes of the replacement reproductives, the time interval before they emerge and the suitable conditions required to facilitate the emergence of replacement reproductives are not yet fully understood.

During colonization and foraging within wood, the internal structures of fibers and growth rings and other anatomical properties influenced the way drywood termites established their nest-gallery. The nest-gallery excavations demonstrated continual adaptation by foragers to anatomical constraints in selecting favorable areas of less dense wood fiber inside the timbers. The colony also exhibited defense mechanisms with which to protect the colony, such as by sealing a tunnel leading to the outer environment using cement pellets. The sticky hydrated pellets were observed in the chambers inside the nest-gallery, even in the first six months of new nest establishment.

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