Asssesment of Initial Colony Founding by Swarming Reproductives of the Western Drywood Termite, *Incisitermes minor*

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Asssesment of Initial Colony Founding by Swarming Reproductives of the Western Drywood Termite, *Incisitermes minor*

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Abstract. Colony founding activities in drywood termite is difficult to assess, since the colony forage and nest inside the wood. Non-destructive observation using X-ray computed tomography was used to evaluate nest-gallery establishment and colony breeding of the incipient colonies. The infested timbers were marked by year of infestation and were left on its original position to allow termite colonies develop in natural environment. Selected timbers were subjected for X-ray scanning analysis in Kyushu National Museum, Japan, in which colony founding from 5 royal pairs in the first year development was monitored. The CT data of the first six months colony founding indicated two different outcome in colony founding; two colonies distributed the energy to breed and slowed down in foraging simultaneously and other three concentrated in foraging. The CT scan after one year showed that only one royal pair showed no breeding activity, while the other four had been observed to have 2 –5 new colony members. Colony extraction also confirmed that colony size in the first and second year varied from 0 – 5 new colony members. The new colony members were mostly in larval instars, sometimes with the presence of one small soldier. Colony founding through nuptial flight is a risky process, as 59% of the observed chambers were failed colony founding, resulted from the dead of a royal pair or being abandoned (empty chamber).

1. Introduction

The western drywood termite, *Incisitermes minor* (Hagen) (Kalotermitidae) is considered to be the most invasive drywood termite in the world [1], and one of the five most economically important termites in the USA [2]. The colonies live entirely within a piece of sound and drywood [3,4], and thus, they can be easily transported around the world as a result of human activities. This invasive species was reported originally from the southwestern USA and northern Mexico [3], and spread all over Canada [5], China [6], and Hawaii [7], and more than half of the prefectures in Japan are listed as infested areas [8-11].

Although it is an economically important pest, there is very little scientific literature on the nesting biology of *I. minor*. Himmi et al. [12] has reported in situ nest-gallery development by foraging groups of *I. minor*, but in situ development of nest gallery by swarming reproductives is not yet reported. The establishment of a new colony following the dispersal of alates during swarming has been described as "vulnerable solitary founding" [13]. The level of failure is high, as the queen must brood and forage at the same time. Harvey [14] 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 [15].

The present report aimed to monitor 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 results provide important information regarding the nesting biology of the royal pairs in the very early stage of colony founding.

2. Materials and methods

2.1 Timbers set up

Evaluation of nest-founding activity of *I. minor* reproductives was carried out at four infested houses in Wakayama Prefecture, Japan. In total, 60 pieces of Sitka spruce (*Picea sitchensis* Bong. Carriere) timbers and 40 pieces sugi (*Cryptomeria japonica* D. Don) timbers were laid with gaps of ~10 mm between them in the highly infested attic area of the houses (**Figure** 1). All timbers were made up of a combination of sapwood and heartwood with dimensions of 50 (R) x 50 (T) x 1000 mm (L). The timbers were arranged 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 [11].

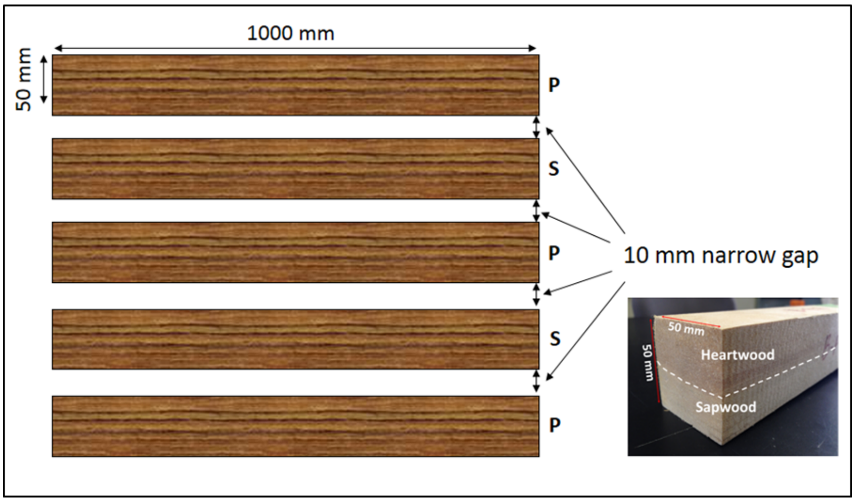


Figure 1. The arrangement of timbers with "P-S-P-S-P" arrangement and narrow gap (~10 mm) between timbers in each of test area, which consists of three Spruce timbers (P) and two Sugi timbers (S). In total, 20 test areas were set up in August 2012 at four highly infested houses.

The first evaluation was conducted two months after *I. minor* swarming season at Wakayama, on November 15, 2012. Due to low result of new infestations, we evaluated the arrangement of timber positions. In the first set up, Sitka spruce and sugi timbers were adjacently laid each other in straight position with narrow gap (~10 mm) between two timbers (Figure 1). We decided to modify the lay out in the following spring (March 2013), prior to a swarming season of *I. minor* in Wakayama. Figure 2 shows the modification in lay out of timbers. The modification was made by arranging "a close gap" in the edge of two timbers and "an open gap" in the other edge. Close gap is a position in which two edge of timbers are touch each other. Open gap is a position in which two edge of timbers are away from each other by ~10 mm gap.

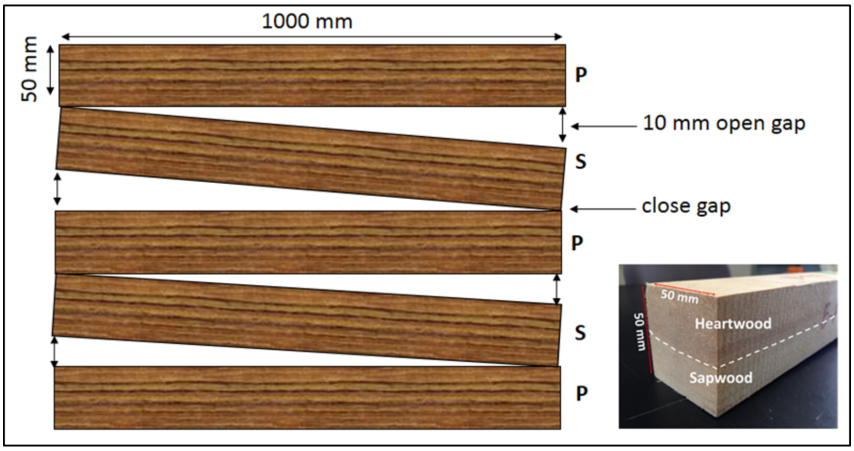


Figure 2. The modification of timbers set up by arranging the lay out to have a closed gap (CG) between two timbers at one end and an open gap (OG) between the two timbers at the other end. The CG is the area where the edges of the two timbers are touching each other, while the OG is the area where edges of the two timbers are separated by a ~10 mm gap. S: Sugi timber, P: Spruce timber.

2.2 Data analysis

The monitoring was conducted once a year, in November, around two months after swarming season. The infested timbers were brought back to the laboratory and were prepared for Computed Tomography (CT)-Scan analysis. The timbers were transported to Fukuoka, Japan, and were scanned by a large-scale X-ray CT (Y.CT Modular, YXLON International GmBH, Germany) at Kyushu National Museum.

The monitoring of 5 newly excavated nests was proceeded in biannual X-ray CT scanning analysis. The digital X-ray data were reconstructed into three-dimensional (3D) images, two dimensional (2D) section image and series of virtual cuts using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH Germany), with digital data thickness 1 mm, 1024 x 1024 pixels, 3.17 2D image slice per mm. The 3D images of *I. minor* nest were reconstructed from digital image data and displayed as discrete values of a density function F (x, y, z), which was representing physical property.

The statistical analysis was carried out by Repeated Measures ANOVA ($P < 0.05$) using SPSS PASW (Ver. 18.0) (SPSS Inc., U.S.A). We relied on the volume of five scanned nests as the dependent variable to measure the development over time stages (the initial stage, the half year and the one year stage), and generated the differences under those three time stages.

3. Results and discussion

3.1 Nest founding activities

The first monitoring on November 15, 2012 found many alate wings were found on the test areas, indicating the high swarming activities. Two types of infestation were found in three spruce timbers: one timber was infested by a group of pseudergates, and two timbers were infested by primary reproductives. No attack was found in sugi timbers. The second monitoring was conducted in November 2013, indicated that lay out modification accomplished better results as the number of infestations were increased significantly. Six Sitka spruce timbers and four sugi timbers were found to be infested by *I. minor* reproductives. All the infestations were found in the close gap areas in sapwood part of the timbers. The details of infestations are presented on Table 1.

Table 1. Nest founding record by swarming repoductives of *I. minor* on the timbers

No.	Timber specimen	Year of collection	Details of Infestation									
			Type of Infestation		Infestation spot at the timber							
			Rp	W	Og	Cg	Sw	Hw	Bo	Sd	Up	Bm
1	Sitka spruce	2012		1	○*		○					○
2	Sitka spruce	2012	4		○*		○			○		
3	Sitka spruce	2012	1		○*		○					○
4	Sitka spruce	2013	1			○	○			○		
5	Sugi	2013	1			○	○					○
6	Sitka spruce	2013	1			○	○					○
7	Sitka spruce	2013	1			○	○			○		
8	Sitka spruce	2013	2			○	○			○		○
9	Sugi	2013	1			○	○			○		
10	Sugi	2013	1			○	○			○		
11	Sitka spruce	2013	1			○	○			○		
12	Sugi	2013	1			○	○			○		
13	Sitka spruce	2013	1			○	○					○

*Timber lay out in 2012 do not have a close gap (Cg) area.

Note:

Rp	=	Pairing Reproductive	Up	=	Upper surface of timber
W	=	Pseudergates (false worker)	Bm	=	Bottom surface of timber
Sw	=	Sapwood	Og	=	Open gap
Hw	=	Heartwood	Cg	=	Close gap
Bo	=	Border	○	=	Infestation event
Sd	=	Side surface of timber			

Figure 3 shows nest founding actvities of a pairing reproductive of *I. minor*. The pair were assessing a spruce timber by chewing several spots before selecting a certain site and working together to excavate a royal chamber on that site. The assessment of nest site location may related to the feeding biology of drywood termite, which is suggested to be influenced by both the physical and chemical properties of the wood [16-18]. Himmi et al. [19] suggested that *I. minor* has preferences on nest-site selection, i.e., on the softer part of the timber [18] which related to physical properties of the timber [20,21]. The results supported previous reports, as *I. minor* reproductives showed preference in establishing the nest on the sapwood (Table 2). With respect to the timber arrangement, *I. minor* reproductives preferred the Cg area (Table 1). Previous studies have reported that *I. minor* reproductive love wood cracks, crevices, and holes as locations in which to establish the royal chamber [22,23]. 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.

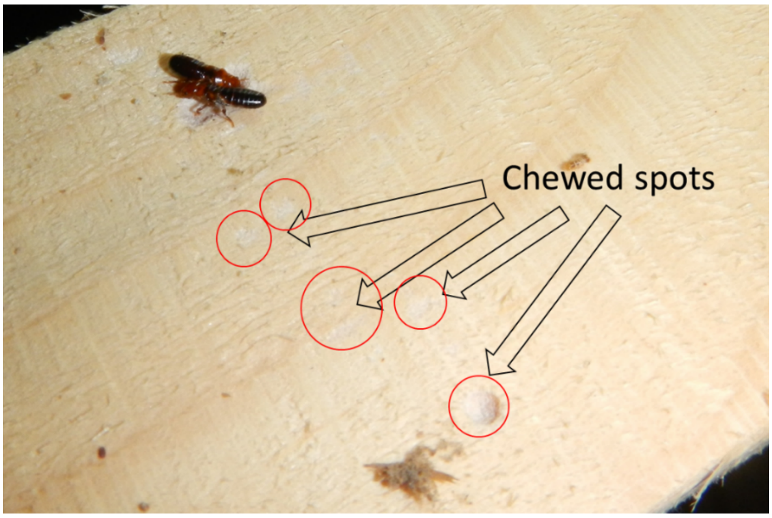


Figure 3. Nest founding activities: a pairing reproductive are working together to search a spot to establish the royal chamber in a Sitka spruce Timber.

3.2 Initial development of the nest-gallery

The first year development of drywood termite nest-gallery is recorded using X-ray CT scan. After assessing the preferred spot on timber for establishing a nest, a pairing reproductive of *I. minor* excavated a tiny hole and sealed it from inside using cement pellet. This tiny sealed-hole is often referred as a royal chamber. In current report, we presents CT data of a royal chamber that was established by a pairing reproductive from nuptial flight in a Sitka spruce timber. Figure 4 and Figure 5 illustrate the first year development of drywood termite nest-gallery in 3D and 2D CT images, respectively. The initial royal chamber (Figure 4.a) was excavated in an entrance slope (Figure 5.a – c). The chamber was extended in sloping vertical direction in springwood part of particular growth ring where the entrance hole was excavated (Figure 5.d – f) in the first six months, and further developed in vertical direction, parallel to vertical axis of that of springwood ring (Figure 5.g – i).

The initial cashew-nut shape of royal chamber (Figure 5.b) with ~ 4.6 mm and 41.4 mm^3 in diameter and volume (Figure 6), respectively, had been extended to be a new spacious chamber (Figure 5.e) with the maximum diameter of ~10.6 mm and the volume of 209.8 mm^3 (Figure 6) in the first six months. 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 in the one-year CT images. The royal pair excavated the nest-gallery on the springwood of a particular growth ring in which the entrance hole was established. The extension was indicated by the accretion in depth and length value (Figure 6). In the first year development, the fecal pellets were stored in the excavated chamber, and not yet disposed to outside of the nest. The first new colony member (body length ~ 2.1 mm) was observed in the first six months, which indicated that the royal pair distributed their energy in foraging and breeding.

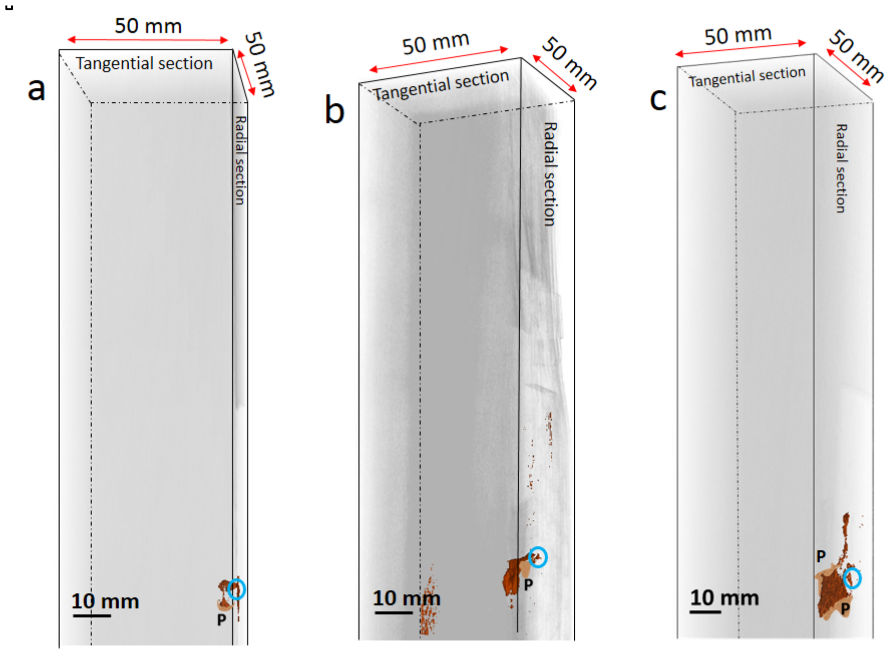


Figure 4. Three-dimensional CT images of nest-gallery development of *I. minor* in a Sitka spruce timber: (a) initial breeding chamber; (b) half year; (c) one year. P: fecal pellets. Entrance hole is marked by circular blue color. Orange color represents space inside the chamber. Termite presence is indicated by uncolored area inside the chamber.

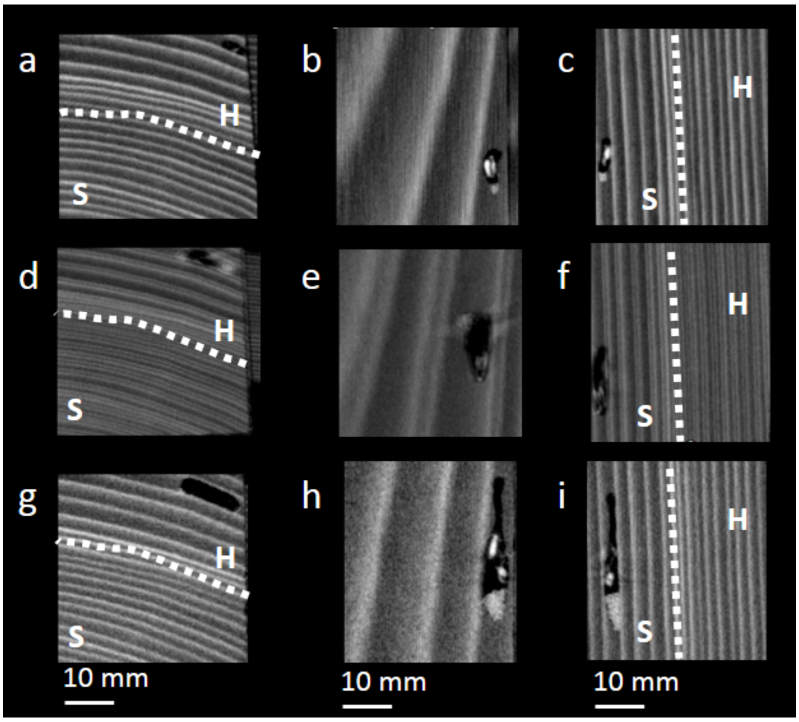
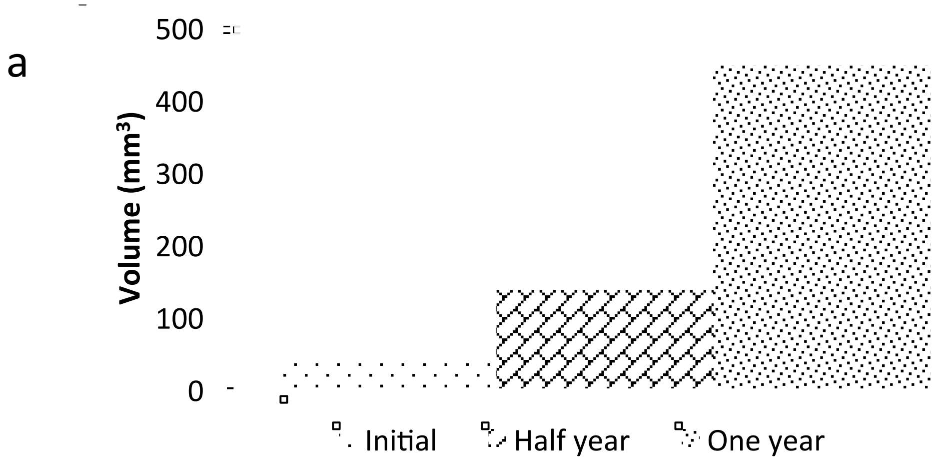


Figure 5. Two-dimensional CT images of the breeding chamber development in Timber C: (a – c): Initial breeding chamber; (d – f): half year; (g – i): one year. S: sapwood; H: heartwood. Cross section: a, d, g; Tangential section: b, e, h; Radial section: c, f, i. Springwood is indicated by darker color in CT images. Summerwood is indicated by lighter color in CT images.

After one year, more spacious chamber (17.5 mm and 445.8 mm^3 in diameter and volume, respectively) was excavated. No further development was observed in parallel direction to the axial system of the timber, which was shown by the stagnant value of the depth (Figure 6.b) as the chamber has reached the edge of timber (Figure 2.20.g). One exploratory tunnel was excavated in the vertical axis of spring growth ring. The queen and the king grew from initial 6.2 mm and 5.8 mm body length, to 6.7 mm and 5.9 mm after one year, respectively. The royal pair bred a new member, spotted with body length ~2.2 mm. The colony had a royal pair and two labor forces after one year development.



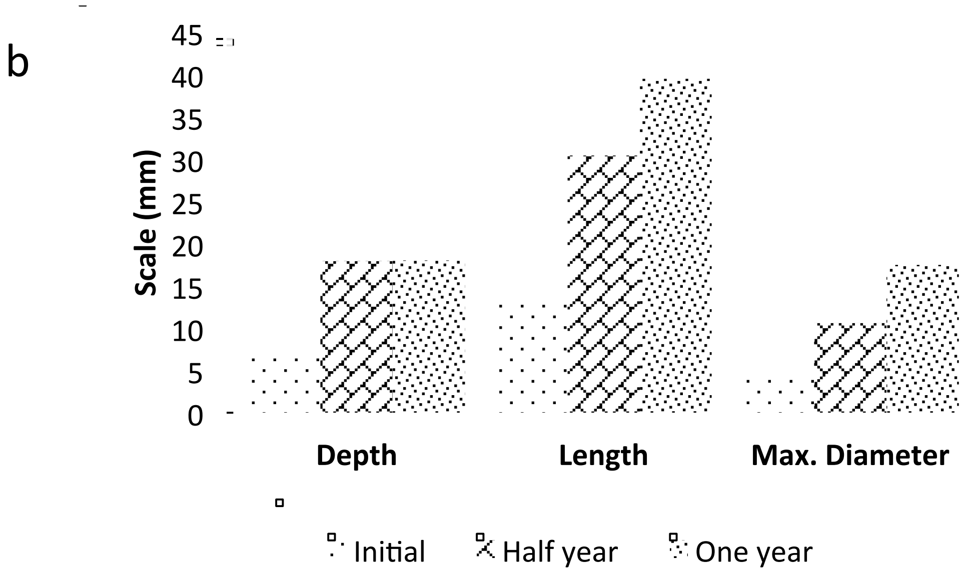


Figure 6. The properties of first year development of the nest gallery. (a) accretion in volume; (b) depth, length and maximum diameter.

Qualitative generalization of stage development of the nest-galleries was carried out using a repeated measures ANOVA ($P < 0.05$), by relying on volume properties of 5 scanned nests as a dependent variable. A repeated measures ANOVA with a Greenhouse-Geisser correction determined that the volume differed significantly ($p < 0.0005$) between time stages (initial, half year and one year). The average accretion in volume between time stages: initial, half year and one year development are 31.9 mm3, 201.7 mm3, and 447.6 mm3, respectively. Post hoc tests using the Bonferroni correction also revealed that biannual accretion in volume from initial establishment to half year and one year later were significantly different ($p = 0.001$ and $p < 0.0005$, respectively).

Moreover, the volume of breeding chambers after one year was also significantly different from the previous six months (p = 0.002).

The monitoring of five scanned nest-galleries provides important biological feedback of nesting biology of drywood termite. Harvey [14] argued that a nine-months inactivity period was a necessity in early stage of colony establishment, generated by tremendous fund of energy spent by royal pair in swarming, divesting wings, mating and excavating royal chamber. On the present study, the pairing reproductives in all chambers showed no hibernation period in the first six months and continuously excavated the galleries, in contrast with that of suggested by Harvey. One year CT analyses also confirmed that the reproductives continued foraging, which were indicated by significant accretion in volume and structure of the chambers. The chambers were developed in a certain pattern: cavernously excavated in a particular annual growth ring where the breeding chamber was established.

The results of present study are too early to draw any conclusive argumentation related to certain period of inactivity phase. All the naturally-infested timbers were kept in conditioned room (28 ± 2^0C; RHs $80 \pm 10\%$), thus the pairing reproductives never experienced any seasonal changing. Even though either temperature or humidity was reported as essential factors to influence feeding activities of *I. minor* [24-26], we argue that hibernation period of primary reproductive in the early stage of colony establishment is a biological fact that is need to be carefully evaluated.

In the second field monitoring in November 2013, we encountered a spruce timber infested by a pairing reproductive, which had volume properties approximately equal to that of a half-year chamber (Himmi, unpublished data), indicating that timber must be infested earlier than the common nuptial flight in September [11]. Thus the pairing reproductive were experiencing natural seasonal changing. If an inactivity period lasted for nine months as described by Harvey [14], the chamber should result in the same profile with that of initial royal chamber, and remain undeveloped.

There are no adequate literature about the actual time interval of primary reproductive in starting laying eggs after establishing the first royal chamber, neither in natural seasonal changing nor in laboratory assessment. The only comparative report was provided by Atkinson [27] who recorded seasonal changes in caste composition of 38 *I. minor* colonies dissected from their natal nest. However, he did not provide the adequate information whether the observations were carried out in actual seasonal changing, or in a conditioned temperature and humidity. He estimated that eggs were not encountered in 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, which indicated that the oviposition occurred between November 2012 and April 2013.

4. Conclusion

The results showed that the royal pairs of *I. minor* can start breeding new colony members in the first six months since successful nest founding activities, and by the end of the first year, an incipient colony can have 0 – 5 new members. All the nests were established by pairing reproductives on the sapwood. The excavation of nest-galleries in the first year showed a preference for the springwood part of the particular growth rings where the entrance holes were excavated, which may related to foraging efficiency based on physical properties of the timbers.

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5. References

1. Evans TA, Forschler BT, Grace JK (2013) Biology of invasive termites: a worldwide review. Annu Rev Entomol 58:455-474. doi:10.1146/annurev-ento-120811-153554
2. Su NY, Scheffrahn RH (1990) Economically important termites in the United States and their control. Sociobiol 17:77-94
3. Light SF (1934) The distribution and biology of the common drywood termite, *Kalotermes minor*: Distribution and means of identification. In: Kofoid CA (ed) Termites and termite control. University of California Press, Berkeley, pp 210 - 216
4. Evans TA, Inta R, Lai JCS (2010) Foraging choice and replacement reproductives facilitate invasiveness in drywood termites. Biol Invasions 13 (7):1579-1587. doi:10.1007/s10530-010-9915-4
5. Grace JK, Cutten GM, Scheffrahn RH, Kevan DKM (1991) First Infestation by *Incisitermes minor* of a Canadian Building (Isoptera: Kalotermitidae). Sociobiol 18 (3):299-304
6. Xie Y, Li Z, Gregg WP, Li D (2001) Invasive species in China: an overview. Biodeversity and conservation 10:1317-1341
7. Haverty MI, Woodrow RJ, Nelson LJ, Grace JK (2000) Cuticular hydrocarbons of termite of the Hawaiian islands. J Chem Ecol 26 (5):1167-1191
8. Yasuda I, Kinjo K, Yaga S (2003) A new record of *Incisitermes minor* (Hagen) from Okinawa island (in Japanese). Jpn J Entomol 6 (2):103-104
9. Harunari M, Tomioka Y (2004) Occurrence and control of exotic termite, *Incisitermes minor* (Hagen), in Yokohama (in Japanese). House Househ Insect Pests 26 (2):107-113
10. Indrayani Y, Yoshimura T, Fujii Y, Yanase Y, Okahisa Y, Imamura Y (2004) Survey on the infestation of houses by *Incisitermes minor* (Hagen) in Kansai and Hokuriku areas. Jpn J Appl Entomol Zool 15 (4):261-268
11. Yoshimura T (2011) Strategies towards the integrated management of the invasive dry-wood termite, *Incisitermes minor* (in Japanese). Mokuzai Gakkaishi 57 (6):329-339
12. Himmi SK, Yoshimura T, Yanase Y, Oya M, Torigoe T, Akada M, Imadzu S (2016) Nest-gallery development and caste composition of isolated foraging groups of the drywood termite, *Incisitermes minor* (Isoptera: Kalotermitidae). Insects 7 (3). doi:10.3390/insects7030038
13. Cronin AL, Molet M, Doums C, Monnin T, Peeters C (2013) Recurrent evolution of dependent colony foundation across eusocial insects. Annu Rev Entomol 58:37-55. doi:10.1146/annurev-ento-120811-153643
14. Harvey PA (1934) The distribution and biology of the common drywood termite, *Kalotermes minor*: Life history of *Kalotermes minor*. In: Kofoid CA (ed) Termites and Termite Control. University of California Press, Berkeley, pp 217-233
15. Lee S-H, Su N-Y (2010) Simulation study on the tunnel networks of subterranean termites and the foraging behavior. J Asia-Pac Entomol 13 (2):83-90. doi:10.1016/j.aspen.2010.01.003
16. Kasseney BD, Deng T, Mo J (2011) Effect of wood hardness and secondary compounds on feeding preference of *Odontotermes formosanus* (Isoptera: Termitidae). J Econ Entomol 104 (3):862-867. doi:10.1603/ec10216
17. Sajap AS, Sahri MH (1983) Responses to wood and wood extractives of *Neobalanocarpus heimii* and *Shorea ovalis* by the drywood termite, *Cryptotermes cynocephalus* (Isoptera: Kalotermitidae). Pertanika 6 (3):28-31
18. Himmi SK, Yoshimura T, Yanase Y, Mori T, Torigoe T, Imazu S (2016) Wood anatomical selectivity of drywood termite in the nest-gallery establishment revealed by X-ray tomography. Wood Science and Technology 50 (3):631-643. doi:10.1007/s00226-016-0800-x
19. Himmi SK, Yoshimura T, Yanase Y, Oya M, Torigoe T, Imazu S (2014) X-ray tomographic analysis of the initial structure of the royal chamber and the nest-founding behavior of the drywood termite *Incisitermes minor*. J Wood Sci 60 (6):453-460. doi:10.1007/s10086-014-1427-x
20. Himmi SK, Yoshimura T, Yanase Y, Torigoe T, Akada M, Ikeda M, Imazu S (2018) Volume visualization of hidden gallery system of drywood termite using computed tomography: a new

approach on monitoring of termite infestation. In: McLellan B (ed) Sustainable future for human security: Environment and resources. vol 2. Springer Nature Singapore, Singapore, pp 61-68. doi:10.1007/978-981-10-5430-3_6

21. Choi B, Himmi SK, Yoshimura T (2017) Quantitative observation of the foraging tunnels in Sitka spruce and Japanese cypress caused by the drywood termite Incisitermes minor (Hagen) by 2D and 3D X-ray computer tomography (CT). Holzforsch 71 (6):535. doi:10.1515/hf-2016-0140
22. Harvey PA (1934) Colonization of the common dry-wood termite in wooden structures. In: Kofoid CA (ed) Termites and Termite Control. University of California Press, Berkeley, pp 239-265
23. Cabrera BJ, Scheffrahn RH (2001) Western drywood termite, *Incisitermes minor* (Hagen) (Insecta: Isoptera: Kalotermitidae). Univ Fla IFAS Ext (EENY248):1-7
24. Cabrera BJ, Rust MK (1994) The effect of temperature and relative humidity on the survival and wood consumption of the western drywood termite, Incisitermes minor (Isoptera, Kalotermitidae). Sociobiol 24 (2):95-114
25. Cabrera BJ, Rust MK (1996) Behavioral responses to light and thermal gradients by the western drywood termite (Isoptera: Kalotermitidae). Environ Entomol 25 (2):436-445
26. Indrayani Y, Yoshimura T, Yanase Y, Fujii Y, Imamura Y (2006) Evaluation of the temperature and relative humidity preferences of the western dry-wood termite *Incisitermes minor* (Hagen) using acoustic emission (AE) monitoring. J Wood Sci 53 (1):76-79. doi:10.1007/s10086-006-0817-0
27. Atkinson TH (1994) Seasonal changes in composition of colonies of the western drywood termite, *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae). Ann Entomol Soc Am