Damage on living trees and ecological characteristics of the Ryukyu drywood termite, *Neotermes sugioi* Yashiro in Okinawa Island

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CONTENTS

Chapter 1
Introduction

Research Backgrouud

Termites are important house pests (Lee, 2002; Lee et al., 2007). Additionally, they can be harmful to crops, trees in forests, and city environments (Tsunoda, 1998; Lee, 2014). In subtropical and tropical zones, termite pest control in trees and crops is an extremely important research topic because termite activity, invasion to trees, and heartwood rot by fungi (followed by termite damage) are enhanced in warmer regions (Iwata, 2006; Lee, 2014). On the other hand, in temperate and subarctic zones such as Japan, Europe, and North America, termite damage to crops and trees is often overlooked (Iwata, 2006).

The greening of urban sites through planting ornamental or/and popular trees enhances the value of urban landscape and the citizens' quality of life. Because governmental economical resources are often used for the planting programs, damage to planted trees decreases the value of urban areas. Studies on urban forestry and termite management are therefore needed, but little information is available. Lee (2014) reported three subterranean termite species attacking 11 tree species in Malaysia. In Japan, Iwata and Kodama (2007) reported two subterranean termite species, *Coptotermes formosanus* (Rhinotermitidae) and *Reticulitermes speratus* (Rhinotermitidae) attacking trees. *C. formosanus* were found in 57 species (34 families) of trees, including conifers and broadleaves (Iwata and Kodama, 2007), all well-known park trees, roadside trees, and garden trees that had been transplanted. In addition, Azuma and Aoki (2016) reported on the damage to street trees in Center of Hiroshima City by *C. formosanus*. In the USA it is best known as a pest of buildings in urban areas, however *C. formosanus* also attacks live trees along streets and in urban parks, suggesting it may be able to invade forests in the USA (Evans et al., 2019).

However, there are only a few reports on tree damage by drywood termites. Kalshoven (1959) described the damage in the teak tree *Tectona grandis* Linnaeus f. by the drywood termite *Neotermes tectonae* (Kalotermitidae) in Indonesia. Li et al. (2016) reported on the termite pest status of two tree species in Taiwan and the spatial distribution on trees. The two dominant termite species, *Odontotermes formosanus* (Termitidae) and *Reticulitermes flaviceps* (Rhinotermitidae), had subterranean nests and infested trees from bottom up. The two primitive termites *Neotermes koshunensis* (Kalotermitidae) and *Glyptotermes satsumensis* (Kalotermitidae) had low infestation rates and are most likely to infest trees by alates from top down. The niche segregation in trees of three termite families, Kalotermitidae, Rhinotermitidae, and Termitidae, was distinct. In Japan, Iwata and Kodama (2007) reported heartwood damage in young broadleaf trees by *G. satsumensis*. Tomioka et al. (2014) reported a case of western drywood termite *Incisitermes minor* (Kalotermitidae) damage to living Yoshino cherry trees *Cerasus* × *yedoensis* (Matsumura), Masamune and Suzuki 'Somei-yoshino' and ginkgo trees *Ginkgo biloba* Linnaeus, which are Japanese people favorites and thus transplanted as park trees, roadside trees, and garden trees.

The Ryukyu drywood termite *Neotermes sugioi* (Kalotermitidae; Yashiro et al. 2019) is a drywood termite that might be a pest for ornamental plants in Japan. Individuals of this species nest within a limited scope of a tree's branches and trunk (Maki and Abe, 1986; Abe, 1989) and invade from the branches of living trees (Maki and Abe, 1986) expanding their nesting space toward the roots while damaging the inside of the tree; eventually, the invaded tree is consumed from the inside and withers while standing (Fig. 1.1). Although this termite has not yet been recognized as a pest, Sugio and Miyaguni (2017) reported it from Taiwan cherry tree *Cerasus campanulata* Masamune and Suzuki, Indian coral tree *Erythrina variegata* Linnaeus, and Taiwan banyan Ficus *microcarpa Linnaeus* f., which are representative species planted in parks, historical sites, and alongside roads in Okinawa. They are potentially an important species to study in terms of its effects on urban forestry and living trees; however, accumulation of basic ecological information is insufficient. Thus, detailed investigation on the damage exerted by this termite on living trees and ecological characteristics is needed for improving urban forestry in Okinawa.

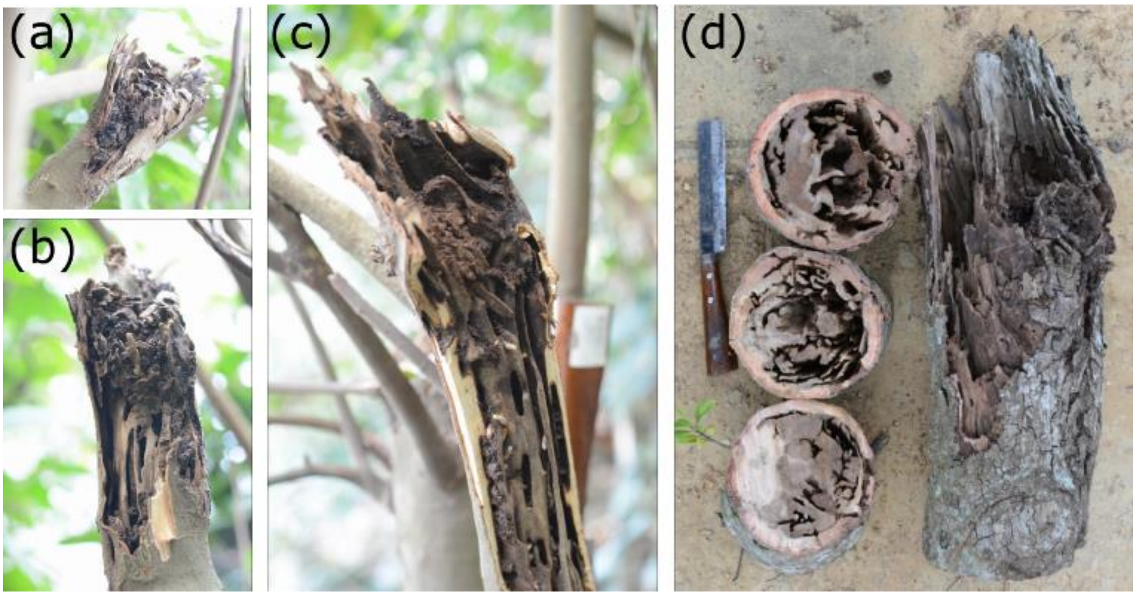


Fig. 1.1 Damage and traces of damage to a tree by *Neotermes sugioi*. (a) Excrements in a nest section. (b) Traces of damage. (c) Internal structure (vertical) of a nest section. (d) Internal structure (horizontal) of a nest section.

Biology of the Ryukyu drywood termite, Neotermes sugioi Yashiro

Termites of the genus *Neotermes* (Kalotermitidae) are distributed in tropical and subtropical regions, including the Ryukyu Archipelago (Krishna et al., 2013). In the Ryukyu–Taiwan Island arc, only one member of the genus, *Neotermes koshunensis*, had been identified (Ikehara, 1966; Krishna et al., 2013). However, recently, a systematic study using molecular and morphological methods found that some *N. koshunensis* populations are composed of a second cryptic species, *Neotermes sugioi* Yashiro, 2019 (Yashiro et al., 2019). These two species are distributed allopathically in the Ryukyu–Taiwan Island arc, with the former found only in Taiwan, and the latter found only in the Ryukyu Archipelago (Yashiro et al., 2019).

This species nests in dead trees and the dead branches of living trees, which serve as a source of both food and shelter (one-piece termites, sensu Abe 1987; Korb and Lenz 2004). This species is classified as a drywood termite; however, they cannot eat hard dried wood. Usually, their nests are found in “green” (or living) trees (Fig. 1.2). This species has a linear caste development pathway, whereby all castes differentiate from pseudergates (older larvae, functional worker caste) after molting (Roisin 2000; Katoh et al. 2007). The colony of this species consists of the following castes, adult queen and king (primary reproductives), neotenic king (secondary king), pseudergates (older larvae, functional worker caste), nymphs (first nymphs and pre-alate nymphs), alates and soldiers (pre-soldiers and soldiers) (Katoh et al., 2007; Miyaguni et al., 2013b).

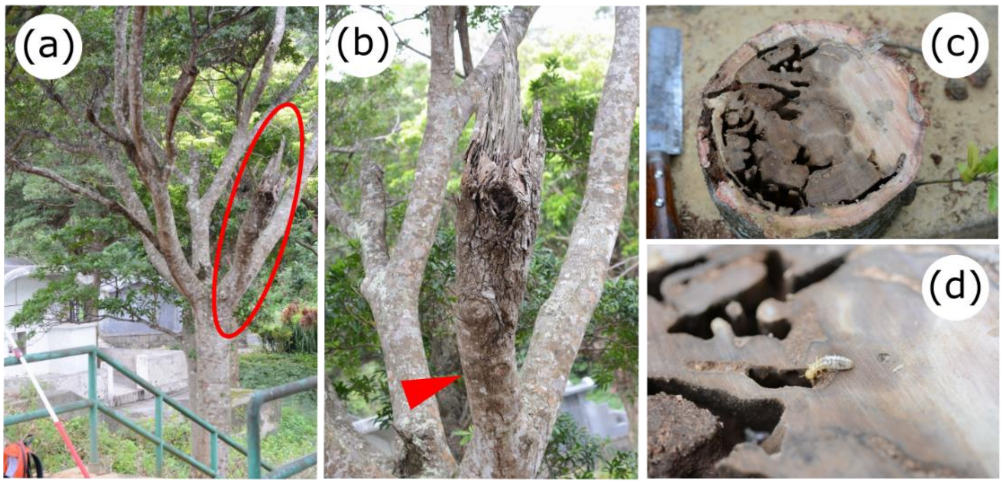


Fig. 1.2 The living environment of the *Neotermes sugioi* colony. (a-b) termite nest (red ellipse) in green tree, *Elaeocarpus sylvestris* var. *ellipticus* (Hara 1951); (c) cross-section of branch with nest that was cut (shown with a red arrow in b); (d), pseudergate of *N. sugioi*.

Objective and outline of the study

The Ryukyu drywood termite *Neotermes sugioi* is a termite that might be a pest for ornamental plants in Japan. The termite is potentially an important species to study in terms of its effects on urban forestry and living trees; however, accumulation of basic ecological information is insufficient. Thus, detailed investigation on the damage exerted by this termite on living trees and ecological characteristics in Okinawa Island. The study is divided into six chapters.

Chapter 1 introduces the background, objective and outline of the study. The chapter also explains the biology of *N. sugioi*. Chapter 2 presents the frequency composition of the reproductive castes, the size of wood with termite gallery, the population size of colonies, and the relative position of the reproductive and non-reproductive castes within nests, and the collection rate of the incipient colonies of this termite based on the exhaustive branch sampling of *Leucaena leucocephala*. Chapter 3 presents the termite damage to Taiwan cherry trees planted in parks, historical sites, and alongside roads in the southern part of Okinawa Island, and estimate the dispersion pattern of infested trees at each site. The transmission and the management of *N. sugioi* are discussed. Chapter 4 presents the distribution of *N. sugioi* in the south and central area of Okinawa Island in detail. Thereafter, the termite distribution and the soil distribution were compared. Based on the results of this study, the northern limit and the characteristics of distribution of this termite species will be discussed. Chapter 5 presents the seasonality of disperser production and the phenology of dispersal flight of *N. sugioi* in the field colonies, and the alates from five isolated colonies under semi-field conditions. It is also discussed how meteorological factors influence the dispersal flight patterns of this termite species. Chapter 6 provides the conclusion of the study.

Chapter 2
Colony structure and caste distribution in living trees

2.1 Introduction

This termite eats both sapwood and heartwood in xylem, and the nest-gallery reaches the cambium of trees (Maki and Abe 1986, Sugio et al., 2018b). The xylem of branch tips is often consumed as the gallery of the termites expands, causing the branches to be structurally weakened greatly (they can often easily be crushed by human hands). Therefore, the termite nests may be divided when the areas are affected by typhoons. Moreover, the necrotic tissue around the nest gallery including cambium of tree may be caused by fungal decay and it may result in the death of trees (Scheffrahn, 2011). They are potentially an important species to study in terms of its effects on urban forestry and living trees; however, accumulation of basic ecological information is insufficient. The accumulation of information on basic ecological knowledge about this termite will be useful for the understanding of the diversification of life history in termites and its selective advantage, and potentially for the management of trees.

In this study, data obtained from 255 mature *Neotermes sugioi* colonies, collected from August 1983 to May 2014 in Okinawa Island were analyzed. Since no genetic survey has been conducted, the term ‘colony’ is synonymous with ‘nest’ in this study (Krishna, 1969). It was investigated that the frequency composition of the reproductive castes, the size of wood with termite gallery system and the population size of colonies, and the related position of the reproductive and the non-reproductive castes within colonies. The collection rate of the incipient colonies (Maki and Abe, 1986) of this termite was also investigated based on the exhaustive branch sampling of *Leucaena leucocephala*.

2.2 Materials and Methods

Collection of colonies

In drywood Kalotermitid termites, such as *Incisitermes minor,* which is an important pest making nests in architectural timber (Lewis, 2003; Lewis and Forschler, 2014), the six-sided fecal pellets are a mark of the presence of a termite colony. In *Neotermes sugioi*, the fecal pellets are also sometimes found, but clay-like feces are more visible as the mark of termite colonies in the field. Also, probably reflecting the difference in the size of the mandibles (Weesner, 1969), the surface of the wood left uneaten by this termite is smoother than that of relatively smaller termites, such as *Coptotermes formosanus.*

A total of 255 *N. sugioi* colonies nesting in dead branches, limbs and trunk of living trees were collected from nature forests, nature park, woods in residential areas, public parks in south and central of Okinawa Island from August 1983 to May 2014. The survey was conducted as follows, walking each field and a careful visual inspection to detect characteristic marks of termite damage and excrement traces on the branches, limbs and trunk of trees that had been damaged by termites (Fig. 2.1). In order to get the entire colony, branches with termite colony were cut longer and confirmed that there was no nest-gallery for termite in the cut surface on the root side of the branches. Then, the root side of the branches were cut carefully and the parts with no nest-gallery was removed. There are about 60 different tree species in the central and southern area where this termite is found on Okinawa Island (Aramoto et al., 1992). However, this study was able to identify only some nesting tree species, and I identified tree species in 45 of the 255 colonies.

In this study, destructive method (Roonwal, 1973; Matsuura et al., 2007) are used to investigate the structure and composition of the colonies. Wood colonized by termite colonies were brought to the laboratory and the mean size of their root-side circumference was measured. Then, the branches were cut into 10 cm length blocks from the tip side. Therefore, the number of blocks in each colony showed the length of the colony. The blocks of each colony were carefully cut into smaller pieces (thin enough to ensure that there was no gallery that could hide termites) on a wide tray and all nest members and wood dusts with termite eggs were collected on the tray. I did not identify any independent colonies that nested in the same piece of wood but which were not connected by the nest gallery. Then, the tray was then covered by a large piece of moistened filter paper and left in the dark for a few minutes. The termites cling to the filter paper and were dropped onto another tray. This process was repeated and termites that did not cling to the filter paper and damaged termites were picked up with tweezers or an insect tubular aspirator. The wood dust with termite eggs were passed through sieves with different mesh sizes (2×2 mm, 1×1 mm and 0.5×0.5 mm) and separated into the large dust component with no eggs and the small dust component with eggs. Eggs were picked up and counted using an insect tubular aspirator under a magnification loupe. Nest members such as a primary queen, primary king, neotenic king (secondary king), alates, pre-soldiers, and soldiers were visually classified based on their morphological characteristics (Roisin, 2000; Miyaguni et al., 2013a). Sexual dimorphism in the sternite structure was used to determine sex (Miyaguni et al., 2012). Larvae (1st to 5th instar), pseudergates (6th instar), nymphs, and pre-alate nymphs were classified based on head width, pronotum length, and wing bud length (Katoh et al., 2007) under a stereomicroscope and loupe. Then, termites were picked up with tweezers or an insect tubular aspirator and counted. The caste and the developmental stages were determined for all termites (even damaged termites).

In the study field where south and central area of Okinawa Island, the average breast height diameter of trees is about 7 cm (it is estimated about 22 cm when converted to circumference) in natural forest (Aramoto, et al., 1992). In this study, the average root side circumference of the collected wood with termite nests (cm) was 23.3 ± 7.9 cm and the median was 22 cm. Although 255 termite colonies were collected, the number of colonies for which reliable data was available was 133 for the measurement of the size of the root side circumference of branches, 166 for the measurement of the number of colony blocks (the length of each colony), 141 for the measurement of the number of individuals per colony, and 251 for the measurement of the pattern and frequency of reproductives (primary queen, primary king and neotenic king) in each colony. The relationships between the number of individuals of each colony and the number of colony blocks (n = 110), the number of individuals of each colony and the size of the root side circumference of branches (n = 92), and the number of colony blocks and the size of the root side circumference of branches (n = 126) were analyzed by Spearman's rank correlation coefficient.

Relative position of reproductive and non-reproductive castes in nest

The material inhabited by drywood termites serves as a source of both food and shelter for the termites (Romano and Acda, 2017). Since, the xylem at the tip of the termite nest is more consumed and structurally weakened (Fig. 2.1 and 2.2) (Yanase et. al. 2014), the wood on the root side of the branch is more likely to escape destruction than the tip, and is safer for the queen and king. Based on the aforementioned estimation, the relative position of reproductives in nests were analyzed in 127 monogamous colonies (colonies headed by a primary queen and a primary king or a primary queen and a secondary king) with data obtained from the block numbers of each colony collected by the method described above. Colony blocks were ordered from root to tip and the frequency of the presence reproductives was investigated in each block (Matsuura et al., 2007). Differences in the relative position of male and female reproductives were statistically analyzed by paired *t*-tests using the R software, version 3.2.2 (R Development Core Team, 2015). Moreover, in colonies A and B (named 850812-A and 850821-A, respectively), the number of eggs, larvae, pseudergates, nymphs, pre-alate nymphs, alates, pre-soldiers, and soldiers was also investigated in every block to understand the location of non-reproductive castes in the colonies.

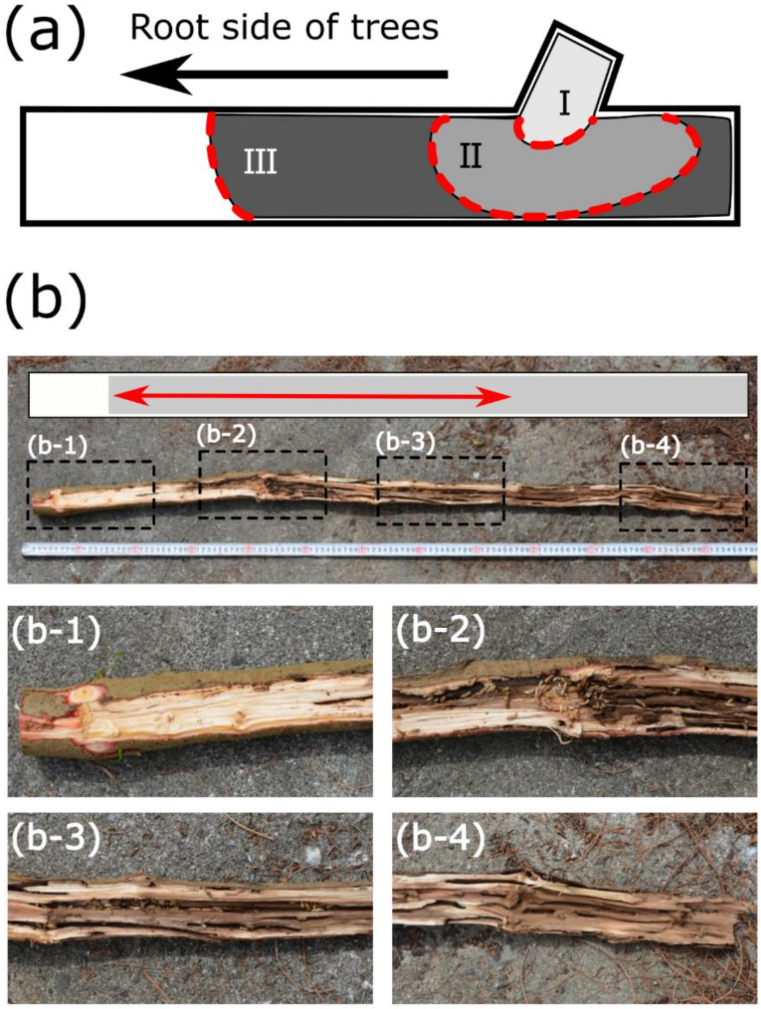


Fig. 2.1 The structure of termite colony areas in the branches. (a) A model of damage direction and colony area expansion in drywood termites. The colony area expands as shown from I to III in the figure. The red dashed line shows the supply area of food resources at each stage (Maki and Abe, 1986). (b) Photograph of the vertical cross-sectional structure of *Neotermes sugioi* nests. The gray area in the figure indicates the damaged area and the double-ended arrow indicates the area of the colony where termites were found. b-1: There are almost no termites (no nest-gallery) at the base of the termite colony and the woody part is alive and fresh. b-2: There are many individuals in the center of the colony area. b-3: Some termites are observed at the boundary of the tip of the colony area. b-4: The nest-gallery has already been abandoned by termites and no termites are found (most of the branches were consumed by termites and died). As a border between abandoned galleries and occupied galleries, there are sometimes termite dung barricades (Maki and Abe, 1986; note, there was a barricade between b-3 and b-4, but it was broken by this survey).

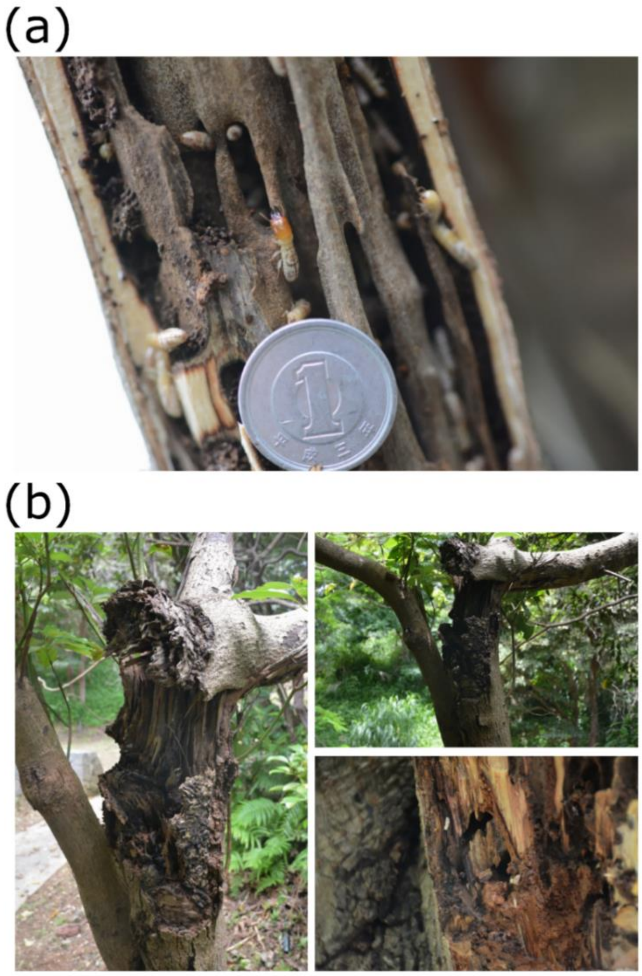


Fig. 2.2 Photograph of *Neotermes sugioi* in the field. (a): Inside a colony (the diameter of the coin is 20 mm). (b): Tree with broken trunk caused by *N. sugioi* infestation; the termite nest has divided. *N. sugioi* was found in the lower nest, although a part of the gallery in the lower nest was used by ants. Both nests were separated by termite mud dung barricades. There were no termites in the upper nest (the nest gallery was occupied by ants).

Survey of ravaged colonies by the typhoon

This survey was conducted at the Sueyoshi Park (26.2292N, 127.7145E) in 1983. Sueyoshi Park is an urban park in Naha City, Okinawa, and consists of open park areas and natural forest. Around the Aja River that flows through the park and the south side of the park are artificial green parks with paved roads, but in the mountains on the north side, there are forests and historic sites. On September 25–26, 1983, Okinawa Island was hit by a typhoon (Japan Meteorological Agency, https ://www.jma.go.jp/jma/index.html). In part of the forest in the northern area of the park, there is an old, unpaved road (width 2 m and a length of 30 m). The preliminary survey was conducted just before the typhoon arrived, making sure there were no broken branches and limbs on the road or in the bushes on either side of the road. After the typhoon had passed, the post-survey was conducted again on 29 September and 3 October, and collected a total of 25 branches and limbs with a diameter of > 3cm and a length of >50 cm that had fallen on the ground, and were presumed to have been knocked down by the typhoon. The largest of the collected wood was 17 cm in diameter and 350 cm in length. The collected branches were taken to the laboratory and examined.

Collection of incipient colonies by exhaustive branch sampling

The survey was conducted at the woods of *Leucaena leucocephala* in Tsuhanaha (26.231639N, 127.757974E) and University of the Ryukyus (26.246035N, 127.763468E) in Nishihara, Okinawa on August 21 and 22, 2015. In each site, all trees in 5 m × 30 m area were investigated and a total 256 branches of *L. leucocephala* were collected from over 100 trees. These thin branches (about 10–25 mm in diameter, about 100–300 mm in length) had young leaves even though their tips were broken, and did not have the characteristic damage marks or excrement traces indicative of termite infestation. The collected branches were taken to the laboratory and carefully dismantled. When termites were found, members were classified into the following castes: primary king, primary queen, 1st to 6th instar larvae, pre-soldier, soldier, and egg (Roisin, 2000; Katoh et al., 2007; Miyaguni et al., 2012; Miyaguni et al., 2013a, 2013b).

2.3 Results

Size of wood with termite-gallery system and size of population in colonies

The population and wood size of the colonies, and the relationship between them, are shown in Fig. 2.3. The average number of individuals in each colony was 2,058.2 ± 1,695.0 (mean ± standard deviation) and the median was 1,700 individuals (Fig. 2.3a). The average number of branch blocks was 5.9 ± 3.2 and the median was 6 blocks (Fig. 2.3a). The average root-side circumference of the branches was 23.3 ± 7.9 cm and the median was 22 cm (Fig. 2.3a). The colony population was positively correlated with both the number of colony blocks ($n = 110$, $\rho = 0.453$, $p < 0.001$) and the size of the root-side circumference of branches ($n = 92$, $\rho = 0.620$, $p < 0.001$) (Fig. 2.3a). The relationship between the number of colony blocks and the size of the root-side circumference of branches was not significant ($n = 126$, $\rho = 0.07$, $p = 0.422$) (Fig. 2.3b).

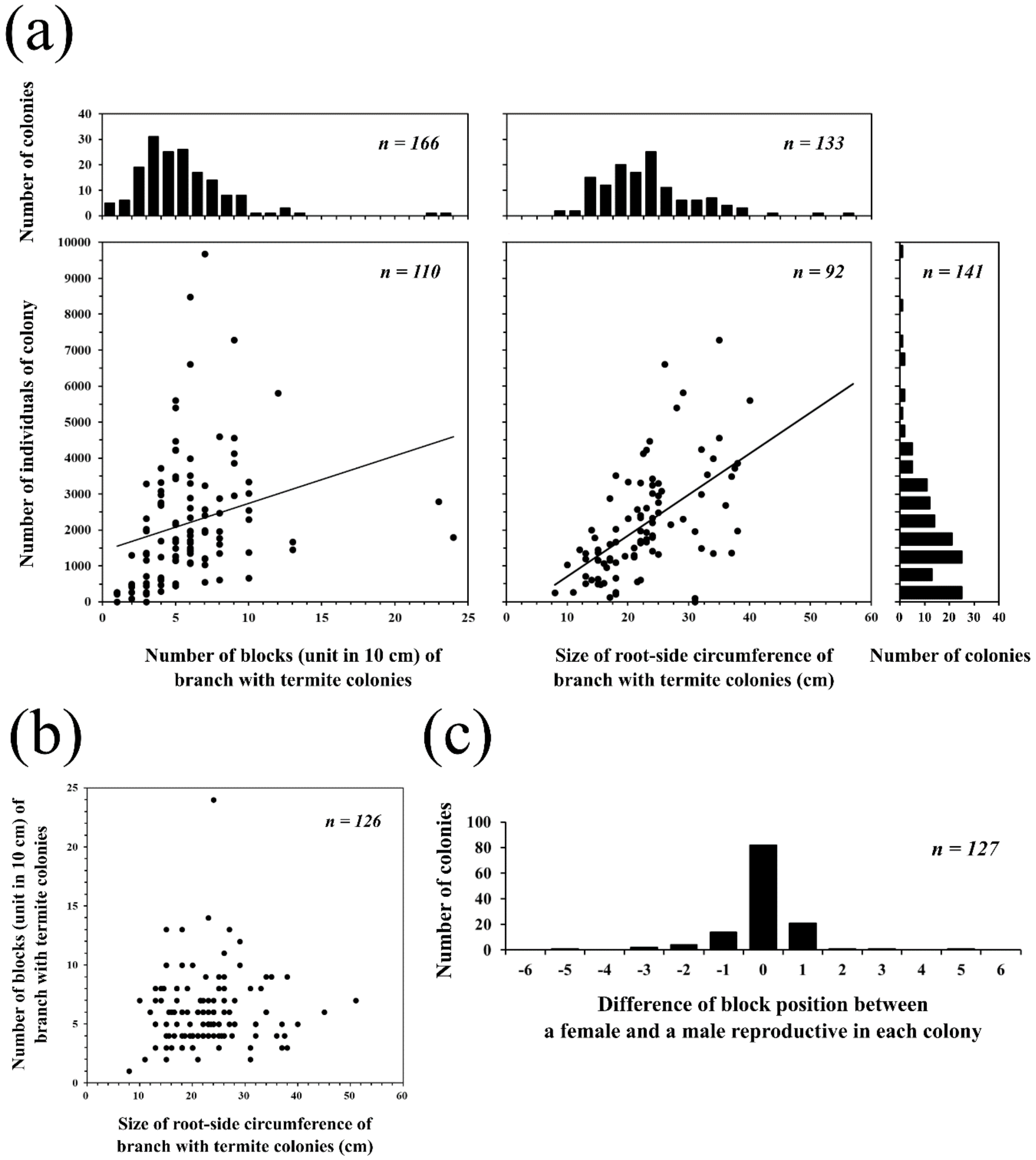


Fig. 2.3 Colony size characteristics (population and wood size) and the position of *Neotermes sugioi* reproductives. (a) The frequency distribution of the colony population, the thickness and the length of the colony, and the relationship between the population and the wood size of the colony. Owing to missing data, the number of samples in the frequency distribution and the number of samples in the scatter plot do not match. (b) The relationship between the thickness and the length of the colony. (c) Relative positional relationship of blocks with male reproductives (primary kings and male neotenics) and female reproductives (primary queens); a positive number indicates that males were mostly found in the tip side of the block, and a negative number indicates that males were mostly found in the root side of the block.

Pattern-frequency of colony reproductives

Table 2.1 shows the composition and frequency of the reproductive castes in *Neotermes sugioi* colonies. Colonies headed by primary queens and primary kings were most common. All colonies of this type were monogamous. Colonies with primary queens and neotenic (secondary) kings as the reproductive caste were the next most frequent type. Most colonies of this type were monogamous; this also applied to some of the colonies headed by a primary queen and two or three neotenic kings ($n = 2$ and $n = 1$, respectively). The frequency of colonies headed only by primary queens, primary kings, or neotenic kings was similar. Colonies with three reproductive castes (primary queen, primary king, and neotenic king), colonies with a primary king and a neotenic king but no female reproductive, and orphaned colonies (i.e., no reproductives) were infrequent.

Table 2.1 Pattern-frequency of reproductives in *Neotermes sugioi* colonies.

Pattern of repdoductives					*n*	Rate in total (%)
Primary queen	+	Primary king			170	67.7
Primary queen			+	Neotenic king	33	13.1
		Primary king	+	Neotenic king	2	0.8
Primary queen	+	Primary king	+	Neotenic king	4	1.6
Primary queen					13	5.2
		Primary king			12	4.8
				Neotenic king	11	4.4
No reproductives					6	2.4
				Total	**251**	**100**

Relative position of reproductive and non-reproductive castes in nest

The frequency distribution of the position of blocks with female reproductives (primary queens) and those of blocks with male reproductives (primary kings and neotenic kings) for each number of blocks are shown in Tables 2.2 and 2.3. The modal value of the location of primary queens was found on the central or root-side block, except for the group with six blocks. The modal value of male reproductives (primary kings and neotenic kings) was found on the central or root-side block in all groups. Female and male reproductives were found in the same block in 82 colonies (64.6% of the colonies investigated in this study) (Fig. 2.2c) and no statistical difference was detected between the location of female and male reproductives (Paired *t*-test, *df* = 126, *t*-value = -0.173, *p* = 0.863).

Table 2.2 Relative position of queens relative to the wood size of colonies.

Number of blocks of termite colony	Number of colonies	Position of female reproductives (queens) in the blocks													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	5													
2	3	3	0												
3	15	4	9	2											
4	22	4	10	8	0										
5	19	3	4	9	3	0									
6	22	2	4	4	9	3	0								
7	12	1	1	4	3	1	2	0							
8	11	0	3	4	3	1	0	0	0						
9	6	0	1	2	1	0	1	1	0	0					
10	7	0	3	0	1	1	1	0	1	0	0				
11	0	0	0	0	0	0	0	0	0	0	0	0			
12	1	0	0	0	0	0	1	0	0	0	0	0	0		
13	3	1	0	0	0	1	1	0	0	0	0	0	0	0	
14	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Total	**127**	**23**	**35**	**33**	**21**	**7**	**6**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

Table 2.3 Relative position of male reproductives (kings and neotenic kings) relative to the wood size of colonies.

Number of blocks of termite colony	Number of colonies	Position of male reproductives (kings or neotenic kings) in the blocks													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	5													
2	3	3	0												
3	15	3	9	3											
4	22	2	14	3	3										
5	19	2	7	6	4	0									
6	22	1	10	4	4	3	0								
7	12	2	0	3	4	1	2	0							
8	11	0	5	1	4	1	0	0	0						
9	6	0	0	2	0	2	1	1	0	0					
10	7	0	2	0	2	2	0	0	0	1	0				
11	0	0	0	0	0	0	0	0	0	0	0	0			
12	1	1	0	0	0	0	0	0	0	0	0	0	0		
13	3	0	1	0	0	0	2	0	0	0	0	0	0	0	
14	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Total	**127**	**19**	**48**	**22**	**22**	**9**	**5**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**

Relative position of non-reproductive castes in colonies

Table 2.4 shows the caste composition per block in two sample colonies. I collected 795 eggs, 520 larvae, 2,551 pseudergates, 44 nymphs, 10 pre-alate nymphs, 152 alates, 6 pre-soldiers, and 55 soldiers from colony A, which contained one primary queen and one primary king. From colony B, I collected 246 eggs, 188 larvae, 913 pseudergates, 15 nymphs, 16 pre-alate nymphs, 236 alates, 3 pre-soldiers, 15 soldiers, one primary queen, and one primary king. In both colonies the primary queen and primary king were found in the second block of the root. Eggs and most larvae were collected from the same block or the block closest to the reproductives. Pseudergates were distributed over most blocks, but most individuals were found in the same blocks as reproductives. Nymphs and pre-alate nymphs were hardly found in blocks near the tip of the colony. Alates in colony A were found in the same blocks or the blocks closest to reproductives. Alates in colony B, were found in the same blocks or blocks nearby reproductives and the blocks on the tip side of the colony. Pre-soldiers were in the same blocks or blocks nearby reproductives in both colonies. Soldiers were distributed widely in both colonies.

Table 2.4 Caste composition per block in two sample colonies (850812-A and 850821-A).

Caste	Number of individuals in each block									
	1	2	3	4	5	6	7	8	9	10
Colony A (850812-A)										
Queens	0	1	0	0	0	0	0	0	0	0
Kings	0	1	0	0	0	0	0	0	0	0
Eggs	0	352	443	0	0	0	0	0	0	0
Larvae	1	219	300	1	0	0	0	0	0	0
Pseudergates	141	1,142	782	103	143	121	98	18	2	1
Nymphs	3	1	22	7	2	9	0	0	0	0
Pre-alate nymphs	1	5	4	0	0	0	0	0	0	0
Alates	0	72	79	0	0	1	0	0	0	0
Pre-soldiers	0	4	2	0	0	0	0	0	0	0
Soldiers	0	2	9	5	13	19	6	1	0	0
Total	**146**	**1,799**	**1,641**	**116**	**158**	**150**	**104**	**19**	**2**	**1**
Colony B (850821-A)										
Queens	0	1	0	0	0	0	0	0	0	0
Kings	0	1	0	0	0	0	0	0	0	0
Eggs	0	230	16	0	0	0	0	0	0	0
Larvae	1	109	66	12	0	0	0	0	0	0
Pseudergates	18	448	263	125	12	5	11	15	14	2
Nymphs	0	5	3	7	0	0	0	0	0	0
Pre-alate nymphs	0	0	6	5	2	0	0	0	3	0
Alates	0	18	20	30	8	0	0	1	157	2
Pre-soldiers	0	3	0	0	0	0	0	0	0	0
Soldiers	0	2	1	0	1	0	5	4	2	0
Total	**19**	**817**	**375**	**179**	**23**	**5**	**16**	**20**	**176**	**4**

Tree species with termite nests

Forty five colonies with identified nesting tree species of *N. sugioi* were collected from nature forests, nature park, woods in residential areas, public parks in south and central of Okinawa Island from August 1983 to June 1988. The following 17 species with termite nests were identified: *Ligustrum japonicum*, *Gardenia jasminoides*, *Cinnamomum yabunikkei, Macaranga tanarius*, *Viburnum odoratissimum*, *Mallotus philippensis*, *Ficus virgate*, *Elaeocarpus sylvestris* var. *ellipticus*, *Litsea japonica*, *Schima liukiuensis*, *Ficus erecta*, Elaeagnus x *reflexa*, *Callicarpa japonica* var. *luxurians*, *Melia azedarach*, *Machilus thunbergii*, *Daphniphyllum teijsmannii*, *Ardisia sieboldii* (Table 2.5).

Table 2.5 Tree species with termite nests.

Botanical name	*n*
Ligustrum Japonicum	12
Gardenia jasminoides	6
Cinnamomum yabunikkei	5
Macaranga tanarius	3
Viburnum odoratissimum	3
Mallotus philippensis	2
Ficus virgata	2
Elaeocarpus sylvestris var. *cllipticus*	2
Litsea japonica	2
Schima liukiuensis	1
Ficus erecta	1
Elaeagnus x reflexa	1
Callicarpa japonica var. *luxurians*	1
Melia azedarach	1
Machilus thunbergii	1
Daphniphyllum teijsmannii	1
Ardisia sieboldii	1
Total	**45**

Survey of ravaged colonies by the typhoon

Seven colonies of *N. sugioi* found in 25 samples, each in a different branch or limb (Table 2.6). In these woods, nest galleries were widely found in the broken scars on the root side, so this colony was a fragmentation of a colony on a tree. In four of the seven colonies (No. 1, 3, 5, and 6), the nest gallery was found in whole the woods. In the other three colonies, the termite nest gallery did not extend to the tip of the branches of the timber, and there was a living tissue with fresh twigs at the ends of the branches. It was presumed that these colonies had a nest with a nest gallery extending from the branch to the limb, which was broken at the limb. In addition, two branches had a damage mark caused by this termite on the broken scar, but no termites were found. In these two branches, the termite nest gallery did not extend to the tip of the branches of the timber, and there was a living tissue with fresh twigs at the ends of the branches.

Table 2.6 Population composition in *Neotermes sugioi* colonies found in ground wood knocked down from trees during a typhoon on September 25–26, 1983.

Colony no.	Reproductives				Egg	Lava	Pseud-ergate	Nymph	Pre-alate Nymph	Alate	Pre-solder	Solder	Total
	Adult		Neotenic										
	F	M	F	M									
1	0	0	0	0	144	413	1,270	2	0	0	0	8	1,837
2	1	0	0	0	81	977	1,185	315	0	7	1	95	2,662
3	1	0	0	0	732	1,044	2,675	52	0	1	7	175	4,687
4	0	0	0	1	0	1	42	0	0	0	0	0	44
5	0	0	0	1	0	5	136	0	0	0	0	14	156
6	0	0	0	3	0	0	102	4	0	0	0	13	122
7	1	1	0	0	0	274	1,349	42	0	0	2	54	1,723

Symbols; Q: Queen, K: King, mNeo: male Neotenic (Miyaguni et al., 2013b)

Collection of incipient colonies by exhaustive branch sampling

Of the 256 *L. leucocephala* branches, 29 (11.3%) contained incipient colonies. Of these colonies, 28 were headed by a single queen and a single king, and in one colony a single queen, but no king, was found (Table 2.7). The termites were found from a broken tip and a very small gap of branches (Fig. 4a-c). In many cases, the plant tissue surrounding the space where the termites lived was black and necrotic or dead, but living tissue remained around it. The entrance of colonies was often found from a broken branch tip (Fig. 2.4d), but in some cases, the entrance of colonies was found from a very small hole (approximately 2 mm diameter) in the node of the branch (Fig. 2.4e).

Table 2.7 Population composition in incipient colonies of *Neotermes sugioi*.

Colony code	Date	Number of individuals											Colony size
		Q	K	E	1L	2L	3L	4L	5L	6L	PS	S	
P60-01	2015/8/21	1	1	0	0	0	0	0	0	0	0	0	2
P60-02	2015/8/21	1	1	6	0	0	0	0	0	0	0	0	2
P60-03	2015/8/21	1	1	1	0	0	0	0	0	0	0	0	2
P60-04	2015/8/21	1	1	3	0	0	0	0	0	0	0	0	2
P60-05	2015/8/21	1	1	3	3	2	6	3	4	0	0	0	20
P60-06	2015/8/21	1	1	0	0	0	0	0	0	0	0	0	2
P60-07	2015/8/21	1	1	8	3	6	3	10	0	0	0	2	26
P60-08	2015/8/21	1	1	4	1	5	1	0	0	0	0	0	9
P60-09	2015/8/21	1	1	15	4	1	2	8	0	0	0	1	18
P60-10	2015/8/21	1	1	0	0	0	0	0	0	0	0	0	2
P60-11	2015/8/21	1	1	3	0	0	0	0	0	0	0	0	2
P60-12	2015/8/21	1	1	7	2	4	0	0	0	0	0	1	9
P60-13	2015/8/21	1	1	3	0	0	0	0	0	0	0	0	2
P60-14	2015/8/21	1	1	6	0	0	0	0	0	0	0	0	2
P60-15	2015/8/21	1	1	0	2	3	4	0	0	0	0	1	12
P60-16	2015/8/21	1	1	0	1	4	0	0	0	0	0	0	7
P60-17	2015/8/21	1	1	0	2	8	0	0	0	0	0	0	12
P62-01	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2
P62-02	2015/8/22	1	1	0	0	2	6	7	0	0	0	0	17
P62-03	2015/8/22	1	0	0	0	0	0	0	0	0	0	0	1
P62-04	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2
P62-05	2015/8/22	1	1	0	0	1	2	0	0	0	0	0	5
P60-18	2015/8/22	1	1	0	1	1	0	0	0	0	0	1	5
P60-19	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2
P60-20	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2
P60-21	2015/8/22	1	1	0	0	5	11	7	0	0	0	3	28
P60-24	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2
P60-25	2015/8/22	1	1	2	1	5	10	8	0	0	0	2	28
P60-26	2015/8/22	1	1	0	0	0	0	0	0	0	0	0	2

Symbols; Q: Queen, K: King, E: Egg, 1L to 6L: 1st instar larva to 6th instar larva, PS: pre-soldier, S: Soldier.

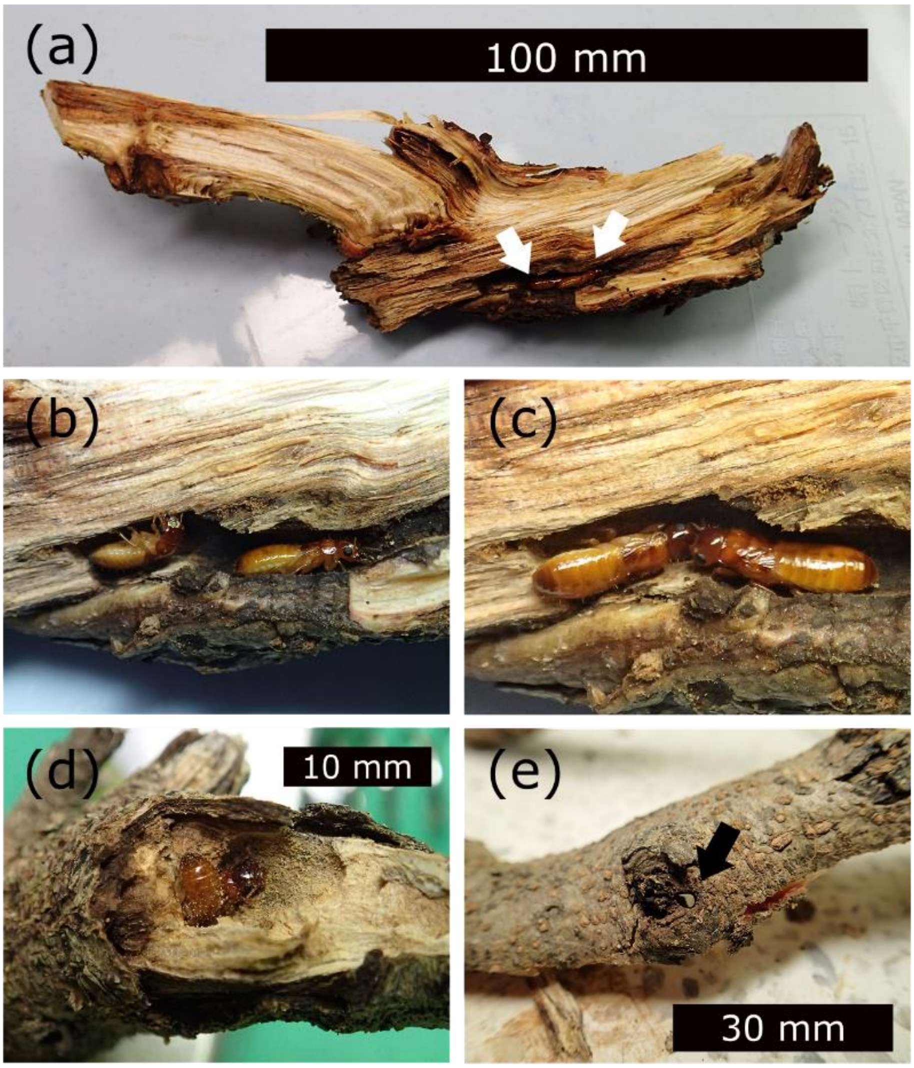


Fig. 2.4 Reproductives in incipient colonies of *Neotermes sugioi*. (a) : a primary queen and a primary king in a branch (white arrows show the reproductives); (b) and (c): closeup of the branch in photo (a); (d): a reproductive pair that protrudes from the branch tip; (e): A hole (black arrow) in the middle of a branch connected to a termite nest .

2.4 Discussion

The results on the population and wood size of *Neotermes sugioi* colonies support and reinforce the results of previous studies on this termite. Maki and Abe (1986) reported that, out of 85 investigated colonies, over 60 colonies had populations of less than 1,000 individuals. In this study, colonies were collected not only from branches but also from trunks; the population size did not exceed 10,000 (Fig. 2.3a). It seemed that colonies with 1,000 to 4,000 individuals were common for this species in this study, even if there was a sampling bias (Fig. 2.3a). Compared to other drywood termites, colony size of this termite appears to be larger (Rust et al., 1979; Jones et al 1995; Korb and Schmidinger, 2004; López et al., 2006). The results indicate that population size has more to do with the size of the root-side circumference of branches (i.e., the thickness of the branches) than the length of the colony blocks. Moreover, it seemed that the average length of the colony branches was less than 100 cm (Fig. 2.3a) and it was not related with the size of the root-side circumference of branches (Fig. 2.3b). These results suggest the possibility that the development of food resources for the increase of a termite population is limited by the surface of the colony area and it will ultimately be limited by the cross section of trees (Maki and Abe, 1986; Sugio et al., 2018b) (Fig. 2.1a). Then, if the upper limit population consumes resources at a constant rate, the colony area will move toward the root of the tree but the increase in colony population may stop. The relationship between increasing populations of termite colonies and food resources needs further investigation as the termites may use acoustic signals to measure tree resource size (Evans, et al. 2005; Evans, et al. 2007).

The study found that monogamous colonies were common, although a few half-orphaned colonies (colonies where there is a queen or a king but not both) were also found (Table 2.1). These results corroborate the results of previous studies on drywood termites (Maki and Abe, 1986). The relative position of reproductives was mainly in the center of the nest or toward the root-side area of the nest (Tables 2.2 and 2.3) in many colonies. Most reproductive pairs were found in the same block area of the colony (Fig. 2.3c). This looks like the reproductives are preparing for the destruction of the nest. Okinawa experiences several typhoons every year (Japan Meteorological Agency, https ://www.jma.go.jp/jma/index.html) and it is the main cause of disturbance in existing colonies. In fact, we found some broken nest branches on the ground that had been split from trees by a typhoon that occurred on September 25–26 1983; sometimes reproductives were found in wood from the broken nest branches (Fig. 2.2b, Table 2.5). Although, termites may have moved quickly in the gallery during the observations, the presence of nymphs and eggs that are unable to move rapidly found with the reproductives at the root side of nest (Table 2.4) supports our idea that the reproductives are usually at the root side of nest. The position of pseudergates, nymphs, alates and soldiers may need careful consideration. For the distribution of termites within a colony in the field, complementary studies with a non-destructive method, e.g.,using X-rays (Himmi et al., 2014; Himmi et al., 2016a, 2016b), may be needed. The genetic testing will be needed in the future to detect whether the colony has undergone fusion and replacement of reproductives (Booth et al., 2010).

Given the range of nest-gallery system in this termite, it will not be difficult to estimate the location of the reproductive center of termite colonies in the field (Lewis et al., 2004). Drilling holes in trees and injecting non-repellent dust formulations of pesticides may be effective in achieving this task (Lewis and Rust 2009; Rust and Venturina, 2009; Lewis and Forschler, 2014). However, considering the corrosion of dead termites, fungal corrosion, and the possibility of other insects invading the gallery after the termites have gone, the removal of an entire branch infested with termites by pruning and application of a patch/paint to the pruning scars (Lewis and Forschler, 2014) may be a simple method to manage this species. Moreover, incipient colonies were found in 11.3% of thin *L. leucocephala* branches with no obvious signs of termite damage. The regular pruning of dead branches to enhance the appearance of trees in parks might help to remove small (early-stage) nests of drywood termites (Fig. 2.4). However, it seems that it is necessary to apply a patch to prevent new termites from getting into the wound caused by pruning.

For enhancing the value of urban landscape, Okinawan citizen's quality of life, and tourism, the planting of popular trees is encouraged and promoted. However, proactive tree health and pest prevention procedures after initial planting are not routine at this time (Lee, 2014). The following research requires a detailed study of the tree species with high economic value.

2.5 Chapter summary

In this study, I observed 255 colonies (nests) of the drywood termite *Neotermes sugioi*, collected in the field on Okinawa Island, and reported the frequency composition of the reproductive castes, the size of wood with termite gallery, the population size of colonies, and the relative position of the reproductive and non-reproductive castes within nests. Most colonies were headed by a primary queen and a primary king. However, colonies headed only by primary queens, primary kings, or neotenic kings, each accounted for approximately 5% of the colonies. A colony size of 1,000– 4,000 individuals (2058.2 ± 1695.0 [mean ± SD]) was common and the average length of colony branches was less than 100 cm. A population size has more to do with the size of the thickness of the branches than the length of the colony blocks. Queens and kings were found in the same or nearby nest areas, and more predominantly in the central or root side of nest wood areas. The termites may experience colony fragmentation and reproductive loss as a consequence of typhoons. This looks like the reproductives are preparing for the destruction of the nest by the annual typhoon. Given the range of nest-gallery system in this termite, it will not be difficult to estimate the location of the reproductive center of termite colonies in the field. Therefore, the removal of an entire branch infested with termites by pruning and application of a patch/paint to the pruning scars may be a simple method to manage this species. Moreover, incipient colonies were found in 11.3% of thin *L. leucocephala* branches with no obvious signs of termite damage. The regular pruning of dead branches to enhance the appearance of trees in parks might help to remove small (early-stage) nests of drywood termites.

Chapter 3

Termite damage on Taiwan cherry tree *Cerasus campanulata*

3.1 Introduction

Chapter 2 showed that *Neotermes sugioi* could be a pest for living trees and suggested ways to manage them so that damage would not spread. Individuals of this species nest within a limited scope of a tree's branches and trunk (Maki and Abe, 1986; Abe, 1989) and invade from the branches of living trees (Maki and Abe, 1986) expanding their nesting space toward the roots while damaging the inside of the tree; eventually, the invaded tree is consumed from the inside and withers while standing (Fig. 3.1). This termite has not yet been recognized as a pest, but reported it from Taiwan cherry tree *Cerasus campanulata* Masamune and Suzuki, that is representative species planted in parks, historical sites, and alongside roads in Okinawa (Sugio and Miyaguni, 2017).

The three most abundant tree species along the roads in Japan are ginkgo (*G. biloba*), cherry tree varieties (*Cerasus* spp.), and Japanese zelkova *Zelkova serrata* (Thunberg) Makino (Iizuka, 2009). However, only cherry tree varieties are planted in Okinawa Prefecture, and Taiwan cherry tree, a flowering tree representative of Okinawa, is frequently planted in gardens, parks, and roadsides. As this type of cherry tree is the first to bloom in Japan, it is helpful for attracting tourists during winter (Sakai, 2014). Therefore, Taiwan cherry trees are proactively planted in parks or along streets by local governments and civilian volunteers, and are a suitable tree variety for evaluating the damage caused by termites.

Understanding how termite pests are transmitted within a site enables their efficient management and control. Subterranean termites attack tree roots from underground using belowground tunnels and aboveground shelter tubes extended from colonies (Abe, 1987; Shellman-Reeve, 1997; Su and Scheffrahn, 1998). In this case, due to the extension of the belowground colony area, termite damage to trees will be transmitted by direct contact with the foraging territory (Su and Scheffrahn, 1998), resulting in a clumped dispersion pattern of infested trees. In this study, such infection transmission mode is defined as "Contact Transmission Model" (Fig. 3.2a). There is little information on the arrangement of drywood termite colonies in the environment. They do not build belowground tunnels and aboveground shelter tubes, so the infection of the trees requires founding a new colony by disperser termites (alates) released from infested trees (Maki and Abe, 1986). Such an infection transmission mode is defined as "Droplet Transmission Model" (Fig. 3.2b). In this case, a random or uniform distribution pattern of infested trees will be found for long-range dispersal (Fig. 3.2b), and a clumped dispersion pattern of infested trees will be found for short-range dispersal (Fig. 3.2b). Under the clumped dispersion pattern, the prevalence of infested trees around any randomly chosen infested tree will be higher than that around a randomly chosen healthy tree.

In this chapter, I report the termite damage to Taiwan cherry trees planted in parks, historical sites, and alongside roads in the southern part of Okinawa Island, and estimate the dispersion pattern of infested trees at each site. The transmission and the management of *N. sugioi* are discussed.

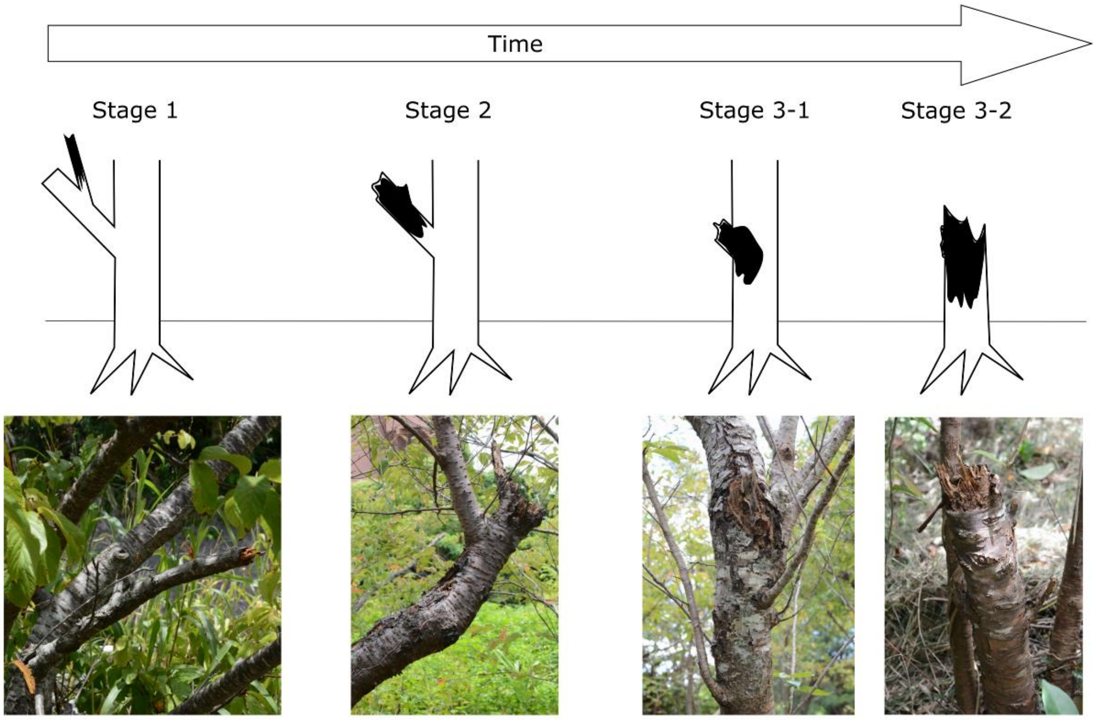
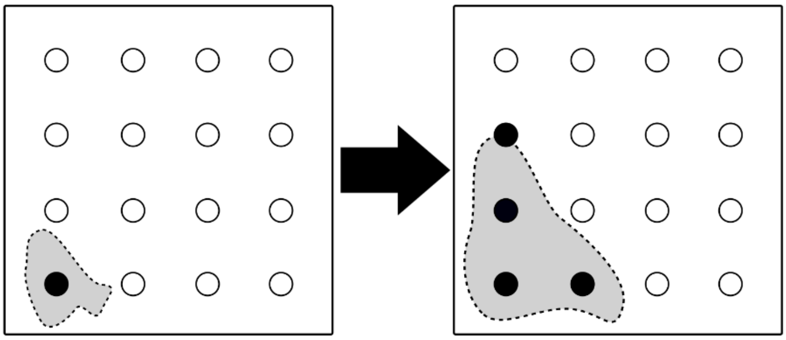


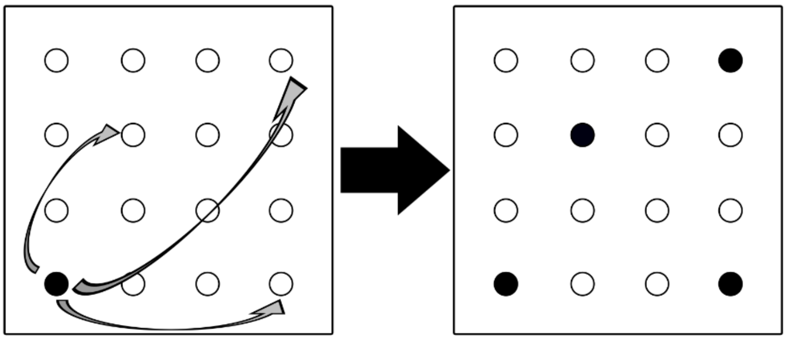
Fig. 3.1 *Neotermes sugioi* hypothesized damage progress (upper) and examples of each damage stage. The black area in the figure shows the extent of the termite nest.

(a) Contact transmission model (Subterranean termites)



(b) Droplet transmission model (Drywood termites)

Longer dispersal



Shorter dispersal

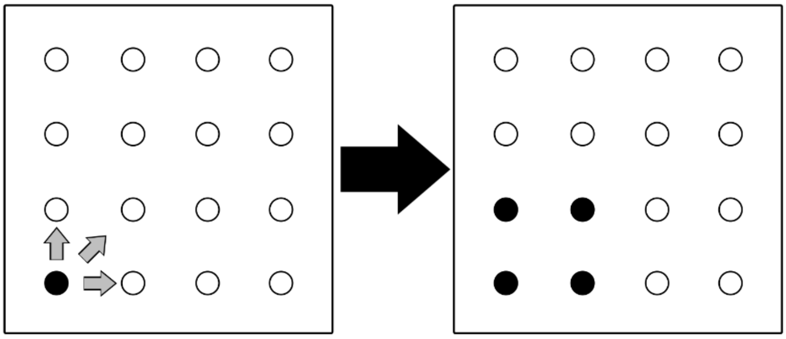


Fig. 3.2 Termite pest transmission model in subterranean termites (a: Contact transmission model) and drywood termites (b: Droplet transmission model) within small sites. Black circles show trees infected with (attacked by) termites and white circles show healthy trees (with no termites). The shaded area surrounded by the broken line in (a) corresponds to the foraging territory of subterranean termites. Grey arrows in (b) show the flight distance of disperser termites (alates) released from infested trees.

3.2 Materials and Methods

Examination of termite damage

In this study, 1,076 Taiwan cherry trees from 36 sites (Table 2.1) in the central-southern area of Okinawa Island were surveyed from March to December 2016. At each study site, careful visual inspection of the trees was conducted to find characteristic marks of termite damage and traces of excrements on the trees that had been damaged by termites (please see Fig. 1.1 in chapter 1 and Fig. 4.3 in chapter 4). When damage marks or traces of excrements were found, a minimal part of the damaged section was cut off using pruning scissors or a saw to confirm the presence of termites and their species.

Because drywood termites inhabit the material they damage (Romano and Acda, 2017), it is possible to use damage indicators to assess the rate of damage progression and the number of termite colonies nesting in the tree. Therefore, I sorted and documented the rate of damage progression based on the following three stages (Fig. 3.1): stage 1, the scope of the damage does not extend beyond a secondary divergent branch from the trunk; stage 2, the scope of the damage does not extend beyond a primary divergent branch from the trunk; stage 3, the scope of the damage has penetrated the trunk.

If the damaging species was a subterranean termite (e.g., *Coptotermes formosanus* or *Reticulitermes okinawanus*), only the presence of damage was documented. The location of the study site was recorded using handheld global positioning system (GPS) units (GPSMap 60CS, Garmin Ltd., Southampton, UK). The location of each tree within the study site was recorded, but, because the GPS data for 80 of the trees at Site P18 were accidentally lost after the end of the study, data analysis was conducted excluding this site and using the data collected for 996 trees at 35 sites.

Estimation of the dispersion pattern of infested trees within sites

Except for site P18, where trees' GPS data were accidentally lost, one damaged cherry tree was randomly selected from every study site with two or more damaged trees (n = 14). The distance between this tree and all other cherry trees at the study site was calculated based on the GPS data of trees and on the Pythagorean theorem, and then sorted in ascending order. The number of sites at which the cherry tree closest to the damaged cherry tree was also a damaged tree was counted. For comparison, one undamaged tree was randomly selected at each study site. Using the method described above, the number of sites at which the cherry tree closest to this undamaged cherry tree was a damaged cherry tree was also counted. The distance between two trees (the randomly chosen damaged/undamaged tree and the tree closest to this randomly chosen tree) differed among sites but it was always less than 10 m, approximately. Then, using Fisher’s exact test, the proportion of sites where a damaged tree was found closest to the randomly chosen damaged tree was compared with the proportion of sites where a damaged tree was found closest to the randomly chosen undamaged tree. Analyses were performed using R software, version R 3.2.2 (R Development Core Team, 2015)

3.3 Results

Termite damage

The only termite species that caused damage to Taiwan cherry trees were *Neotermes sugioi* and *Coptotermes formosanus*. Damage by *N. sugioi* at each study site is shown in Fig. 3.3 and Table 3.1. Cherry trees that sustained damage from *N. sugioi* were confirmed at 21 of the 36 study sites. The damaged trees at these 21 sites were 3.6 on average, with a maximum of 19 and a minimum of 1. The proportion of damaged trees at each site was 18.4% on average, with a maximum of 50.0% (five trees in 10), and a minimum of 2.5% (four trees in 160). *N. sugioi* damage was confirmed in 76 of the 1,076 trees examined (maximum 19, minimum 1 per site). Regarding the number of damaged trees classified according to the rate of damage progression, there were 29 trees at stage 1 (38.2%), 32 at stage 2 (42.1%), and 15 at stage 3 (19.7%). Ten Taiwan cherry trees were found damaged by *C. formosanus* at six sites (maximum three, minimum one per site). No damage was confirmed by subterranean termites of the genus *Reticulitermes*, reported to damage trees in Japan, or damage by drywood termites that are house pests such as *Cryptotermes domesticus* and *Incisitermes minor*.

Estimation of the dispersion pattern of infested trees within sites

Excluding site P18, in the study sites where two or more damaged trees were found ($n = 14$), damaged trees were closest to a randomly-selected damaged tree at seven sites (50.0%) and closest to a randomly-selected undamaged tree at only one site (7.1%). The number of sites where the tree closest to the randomly chosen damaged tree was a damaged tree was significantly higher than the number of sites where the tree closest to the randomly chosen undamaged tree was a damaged tree (Fisher's exact test, $p < 0.05$; Fig. 3.4a). A few representative nesting arrangements are shown in Fig. 3.4b.

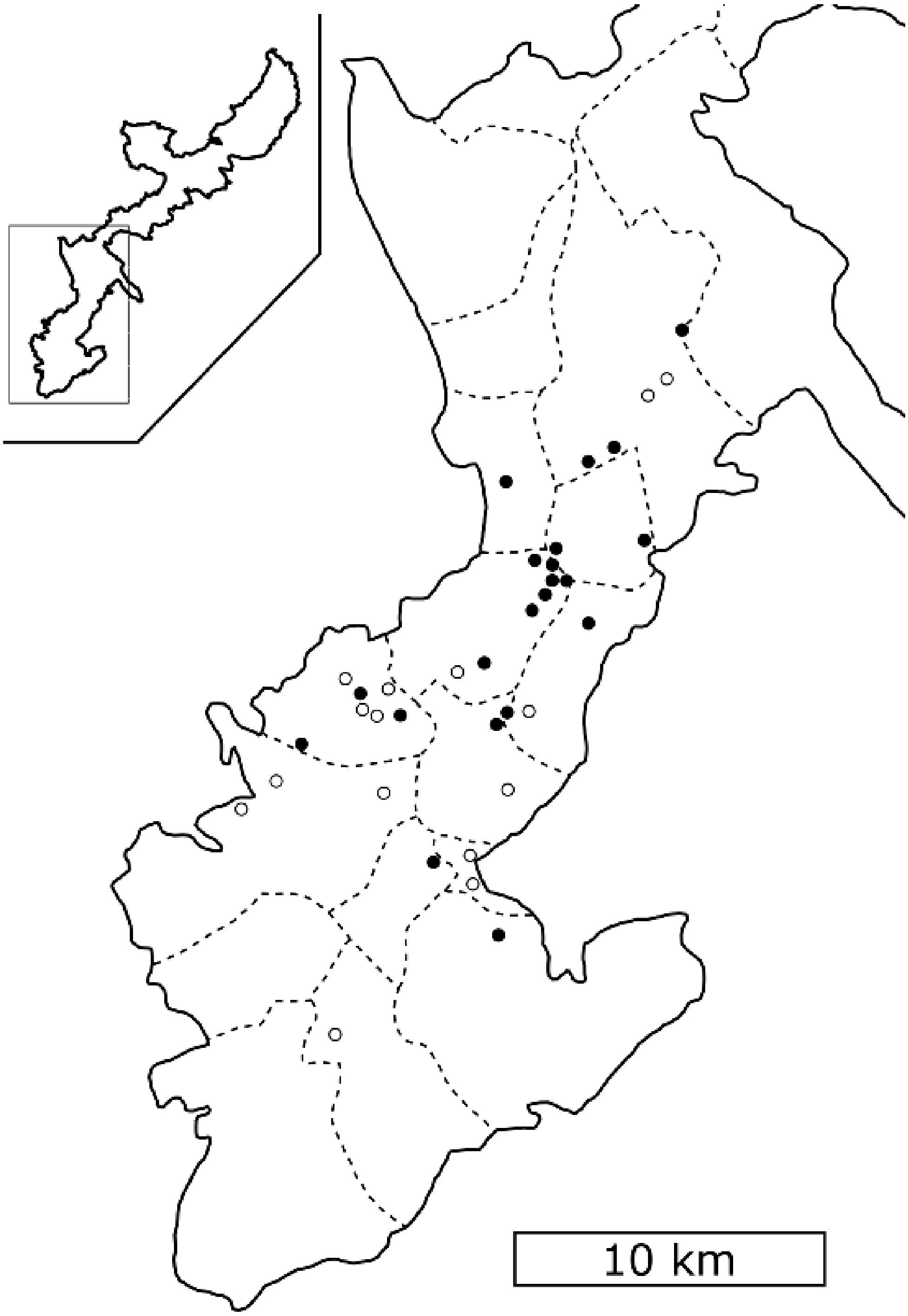


Fig. 3.3 Study sites in the central-southern area of Okinawa Island. Black circles indicate sites at which *Neotermes sugioi* nests were confirmed in Taiwan cherry trees. White circles indicate sites at which no *N. sugioi* nests were confirmed in Taiwan cherry trees. Dotted lines indicate municipality limits.

Table 3.1 Termite (*Neotermes sugioi* and *Coptotermes formosanus)* damage to Taiwan cherry tree (*Cerasus campanulata)* at each study site.

Site	GPS		*n*	*N. sugioi*				*C. formosanus*	
				Number of trees at each damage stage			% of the damaged trees	Number of trees with termite nests	% of the damaged trees
	North	East		Ⅰ	Ⅱ	Ⅲ			
P01	26.35486	127.82186	15	0	3	0	20.0	1	6.7
P02	26.34034	127.81341	14	0	0	0	0.0	0	0
P03	26.33911	127.81032	41	0	0	0	0.0	0	0
P04	26.32631	127.79659	22	4	1	1	27.3	0	0
P05	26.32186	127.78975	10	1	0	0	10.0	0	0
P06	26.31493	127.76879	24	1	0	0	4.2	0	0
P07	26.26618	127.76339	93	1	0	2	3.2	0	0
P08	26.26126	127.75167	7	0	0	0	0.0	0	0
P09	26.28105	127.77737	42	4	2	0	14.3	0	0
P10	26.28027	127.77675	18	0	1	1	11.1	0	0
P11	26.29326	127.77685	13	4	1	0	38.5	0	0
P12	26.29329	127.78349	10	0	5	0	50.0	0	0
P13	26.28834	127.78216	7	0	2	1	42.9	0	0
P14	26.28807	127.78457	12	0	0	2	16.7	0	0
P15	26.29445	127.78251	160	1	3	0	2.5	0	0
P16	26.30441	127.80852	5	0	2	0	40.0	1	20
P17	26.24627	127.77230	16	0	0	0	0.0	0	0
P18	26.27327	127.78637	80	5	9	5	23.8	0	0
P19	26.24644	127.76559	56	3	0	0	5.4	0	0
P20	26.24179	127.76229	64	0	2	1	4.7	0	0
P21	26.22838	127.77032	18	0	0	0	0.0	0	0
P22	26.25631	127.71377	6	0	0	0	0.0	0	0
P23	26.23701	127.69883	28	0	1	0	3.6	0	0
P24	26.25053	127.72442	73	4	0	0	5.5	2	2.7
P25	26.25462	127.72665	48	0	0	0	0.0	0	0
P26	26.24651	127.72086	5	0	0	0	0.0	0	0
P27	26.24607	127.72250	3	0	0	0	0.0	0	0
P28	26.24984	127.71917	22	1	0	0	4.5	0	0
P29	26.22807	127.69393	81	0	0	0	0.0	0	0
P30	26.22326	127.67751	2	0	0	0	0.0	0	0
P31	26.22373	127.72242	21	0	0	0	0.0	1	4.8
P32	26.20720	127.75468	15	0	0	0	0.0	2	13.3
P33	26.19834	127.75610	22	0	0	0	0.0	3	13.6
P34	26.18989	127.76038	2	0	0	1	50.0	0	0
P35	26.20543	127.74053	13	0	0	1	7.7	0	0
P36	26.15216	127.70485	8	0	0	0	0.0	0	0

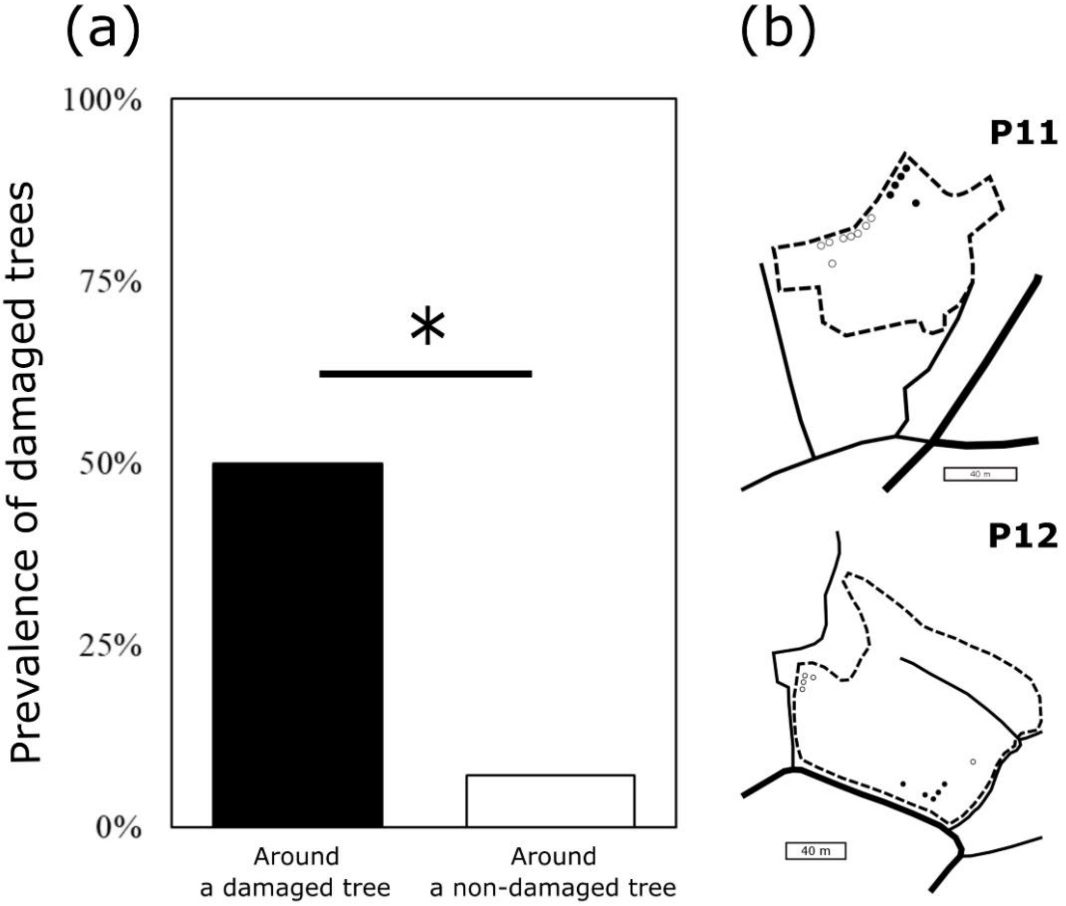


Fig. 3.4 Relationship between locations with Taiwan cherry trees damaged by *Neotermes sugioi* and undamaged. (a) Probability that a damaged tree is found close to a tree damaged by *N. sugioi* (black bar) and to an undamaged tree (white bar). Asterisks indicate a statistically significant difference ($p < 0.05$) according to Fisher's exact test. (b) Locations of damaged and undamaged trees at Site P11 and Site P12. Dotted lines indicate the scope of the survey within each site. Solid lines indicate major roads near each site or footpaths and small roads within each site. Black circles indicate the location of Taiwan cherry trees damaged by *N. sugioi*. White circles indicate the location of undamaged Taiwan cherry trees.

3.4 Discussion

Neotermes sugioi damage to Taiwan cherry trees was confirmed at 58.3% of the study sites (Fig. 3.3). At the site with the greatest proportion of damage, 50% of the cherry trees were damaged (Table 3.1). Furthermore, 7.1% of all Taiwan cherry trees in the 36 study sites were damage, and the damage exerted by *N. sugioi* was much worse than the damage exerted by *Coptotermes formosanus*, known as a house pest (Table 3.1). These results indicate that *N. sugioi* is a major harmful pest for Taiwan cherry trees in Okinawa. As it is difficult to detect tree damage by termite during the early stages of nesting in termite colonies, nests might have been overlooked in previous surveys. Furthermore, the damage levels in trees might have been under-assessed in the present study as the survey was done with minimal destruction of Taiwan cherry trees.

The results of analysis showed that damaged trees are highly likely to exist near damaged trees (Fig. 3.4). These results support the Droplet Transmission Model, assuming the short flight range of dispersers in *N. koshunensis* (Fig. 3.2b), in agreement with the extremely short flying range predicted for alates of drywood termites (Abe, 1989). This also means that the infectivity of this termite is likely to be restricted.

Because of the cryptic nature of subterranean termites, these are usually difficult to control. The detection of a small foraging group on trees may indicate the presence of an underground colony containing several millions of foragers (Su and Scheffrahn, 1998). However, the absence of termites in trees does not guarantee their absence belowground. In these cases, chemical control, including soil treatment techniques with effects in a wide range of species, is the most common method used against subterranean termites (Su and Scheffrahn, 1998; Lee, 2014). On the other hand, chemical control may not be effective against drywood termites (Cowie et al., 1989). Based on our study and on the ecological characteristics of these termites, physical control, such as the removal of the whole branch with termite nests, might be a feasible strategy for controlling dry-wood termites (Logan et al., 1990).

For example, the colonies of *N. sugioi* nesting on tree branches can be detected by periodic inspection of pest control professionals, which probably do not require sophisticated techniques. Even in areas where many trees are infested with termites, most prominent nests that release many dispersers seem to be removable, which also has the effect of protecting existing healthy trees from termite infection (Figs. 3.2b and 3.4). Removing the small nests that are difficult to detect is probably feasible, along with regular pruning of dead branches. Frequent health checks and management of trees would eliminate termite nests, thereby contributing to keep the value of urban landscapes. Our results on the low infectivity of the dry-wood termite *N. sugioi* might accelerate the implementation of such management plans.

Based on the results of the present study, *N. sugioi* can be called a major pest of Taiwan cherry trees in Okinawa. Furthermore, because damage has been recognized in other representative trees in Okinawa, a more detailed survey of the basic ecology of this species is required. *N. sugioi* is distributed only in the Ryukyu Archipelago (Yashiro et al., 2019), but its detailed distribution and the species of trees with termite nests are poorly studied. Then, since they do not build belowground tunnels and aboveground shelter tubes, so the infection of the trees requires founding a new colony by alates released from infested trees. However, there is little information on the dispersal flight. The following research requires a more detailed investigation of the distribution of this species on Okinawa Island, and the phenology of dispersal flight.

3.5 Chapter summary

Termites are typical house pests that can also be harmful pests for living trees, although this topic has not received much attention. To clarify the damage to trees caused by *Neotermes sugioi*, and the ecological characteristics of such damage, a study on Taiwan cherry trees (*Cerasus campanulata*) grown in parks, historical sites, and streets in Okinawa Island was conducted. *N. sugioi* damage to Taiwan cherry trees was confirmed at 58.3% of the study sites. At the site with the greatest proportion of damage, 50% of the cherry trees were damaged. Furthermore, 7.1% of all Taiwan cherry trees in the 36 study sites were damaged, and the damage exerted by *N. sugioi* was much worse than the damage exerted by *Coptotermes formosanus* (16.7% of the study sites and 0.9% of all Taiwan cherry trees), known as a house pest. These results indicate that *N. sugioi* is a major harmful pest for Taiwan cherry trees in Okinawa. Furthermore, the probability of the presence of damaged trees close to other damaged trees was significantly higher than the probability of the presence of damaged trees close to undamaged trees, implying that destructive colonies spread from damaged trees to other trees nearby. Therefore, care for trees in the initial stages of termite invasion seems to be the best method for preventing the spread *N. sugioi* damage to other trees in the same area.

Chapter 4

The distribution range with special references to soil characteristics

4.1 Introduction

Chapter 3 showed that *Neotermes sugioi* can be called a major pest of Taiwan cherry trees in Okinawa. Furthermore, because damage was also recognized in other representative trees in Okinawa, a more detailed survey of the basic ecology of this species is required. *N. sugioi* is distributed only in the Ryukyu Archipelago (Yashiro et al., 2019), but its detailed distribution and the species of trees with termite nests are poorly studied.

Yasuda *et al.* (2000) reported that this termite was found in the forests of the south and central area in Okinawa Island, whereas it was not found in the northern area of Okinawa Island. Moreover, they reported that this species was not distributed in areas further north than Okinawa city. However, since only a few information of their study is useable, the distribution, including the northern limit, of this species in Okinawa Island is poorly understood. This chapter reports the results of a detailed survey of the distribution and the species of trees with termite nests of *N. sugioi* in the south and central area of Okinawa Island.

4.2 Materials and Methods

A survey of termite distribution was conducted at 257 sites including forests, woods, public parks, burial grounds, ancient castles, and roadside trees in south and central area of Okinawa Island from March 2007 to May 2009 (Fig. 4.1A). The time limit to investigate a termite nest was fixed at 30 minutes at the site. However, investigations were closed at the site when a nest was once found in less than 30 min. GPS data of investigated sites were recorded using a registering-type GPS (WGS84, Pokenavi Light; Empex Instruments Inc., Tokyo, Japan).

4.3 Results

The nests of *Neotermes sugioi* were found at 115 of the 257 sites studied (44.7%) (Fig. 4.1A and Table 4.1). *Neotermes sugioi* were distributed in the region extending from 26°05′00″N to 26°25′00″N, located in the south and central area of Okinawa Island. This species was difficult to find at the northern end of the studied area and was not found further north beyond 26°25′00″N (Fig. 4.1A). In addition, only a few colonies were found beyond 26°07′30″ N at the southern end of Okinawa Island (Fig. 4.1A).

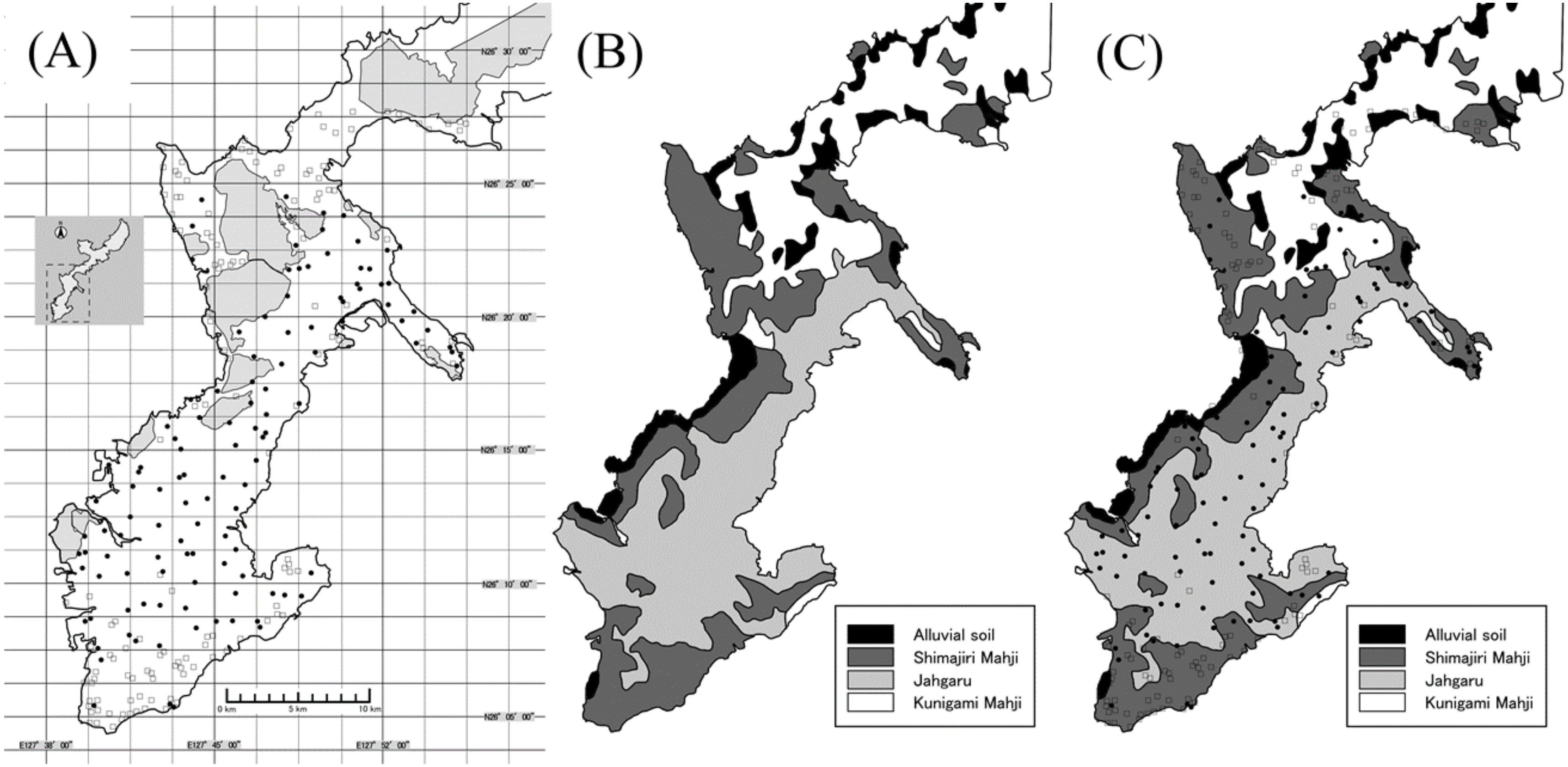


Fig. 4.1 Distribution of *Neotermes sugioi* in the south and central area of Okinawa Island (A), soil distribution in the same region (B) (modified from Miyamaru, 2013), and the merging of A and B (C). Black circles show the presence of *N. sugioi,* whereas white squares show the absence of this termite. Painted areas on (A) show military areas (therefore not investigated).

The 257 study sites were divided into six types of habitats, and the presence rates at each location are shown in Table 4.1. The order of termite presence was Ancient castle (60.0%), Forest (58.3%), Public park (49.1%), Burial ground (48.6%), Woods (35.1%), and Roadside trees (34.6%). Landscape examples of each habitat are shown in Fig. 4.2.

Table 4.1 Presence of *Neotermes sugioi* by habitats.

Classification of habitats	*n*	Presence	Absence	% of presence
Ancient castle	10	6	4	60.0
Forest	24	14	10	58.3
Public park	53	26	27	49.1
Burial ground	70	34	36	48.6
Woods	74	26	48	35.1
Roadside trees	26	9	17	34.6
Total	**257**	**115**	**142**	**44.7**

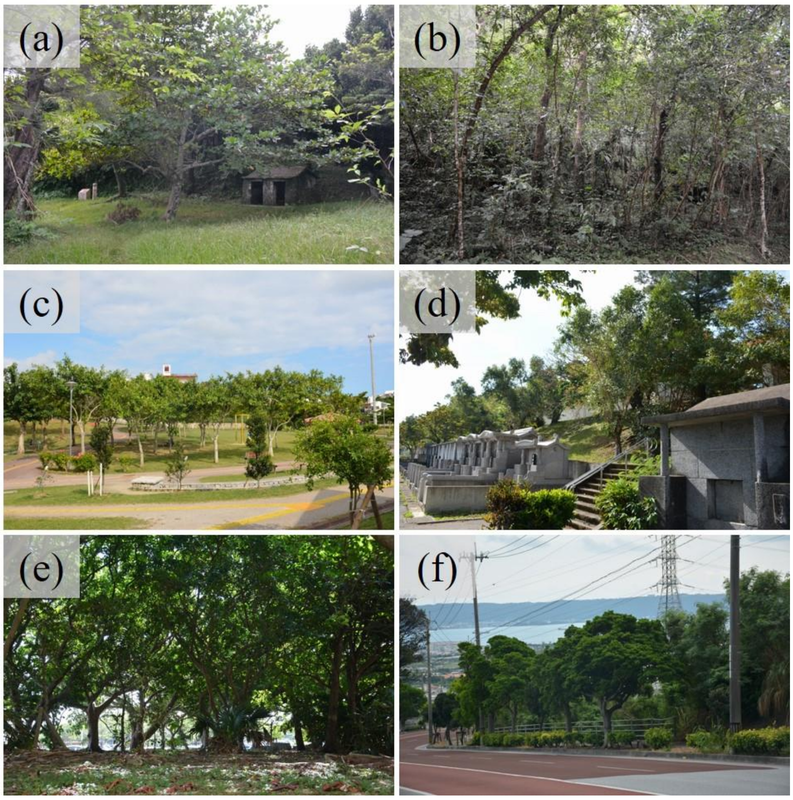


Fig. 4.2 Example photograph of six habitats. (a) Ancient castles (Nakagusuku); (b) Forest (Urasoe); (c) Public park (Ginowan); (d) Burial ground (Urasoe); (e) Wood (Nishihara) (f) Roadside (Nishihara).

Although some tree species were not identified, the following 12 tree species with the termite nests were identified: *Cerasus campanulata*, *Leucaena leucocepha*, *Cerbera manghas*, *Elaeocarpus sylvestris* var. *ellipticus*, *Ficus microcarpa*, *Acacia confusa*, *Ehretia acuminata*, *Erythrina variegata*, *Syzygium jambos*, *Delonix regia*, *Morella rubra*, *Bauhinia variegate* (Table 4.2). Photograph of these trees are shown in Fig. 4.3 to 4.11.

Table 4.2 Number of nesting trees identified.

Botanical name	*n*
Cerasus campanulata	16
Cerbera manghas	8
Leucaena leucocephala	4
Elaeocarpus sylvestris var. *ellipticus*	5
Ficus microcarpa	3
Acacia confusa	2
Ehretia acuminata	1
Erythrina variegata	1
Syzygium jambos	1
Delonix regia	1
Morella rubra	1
Bauhinia variegata	1
Total	**44**

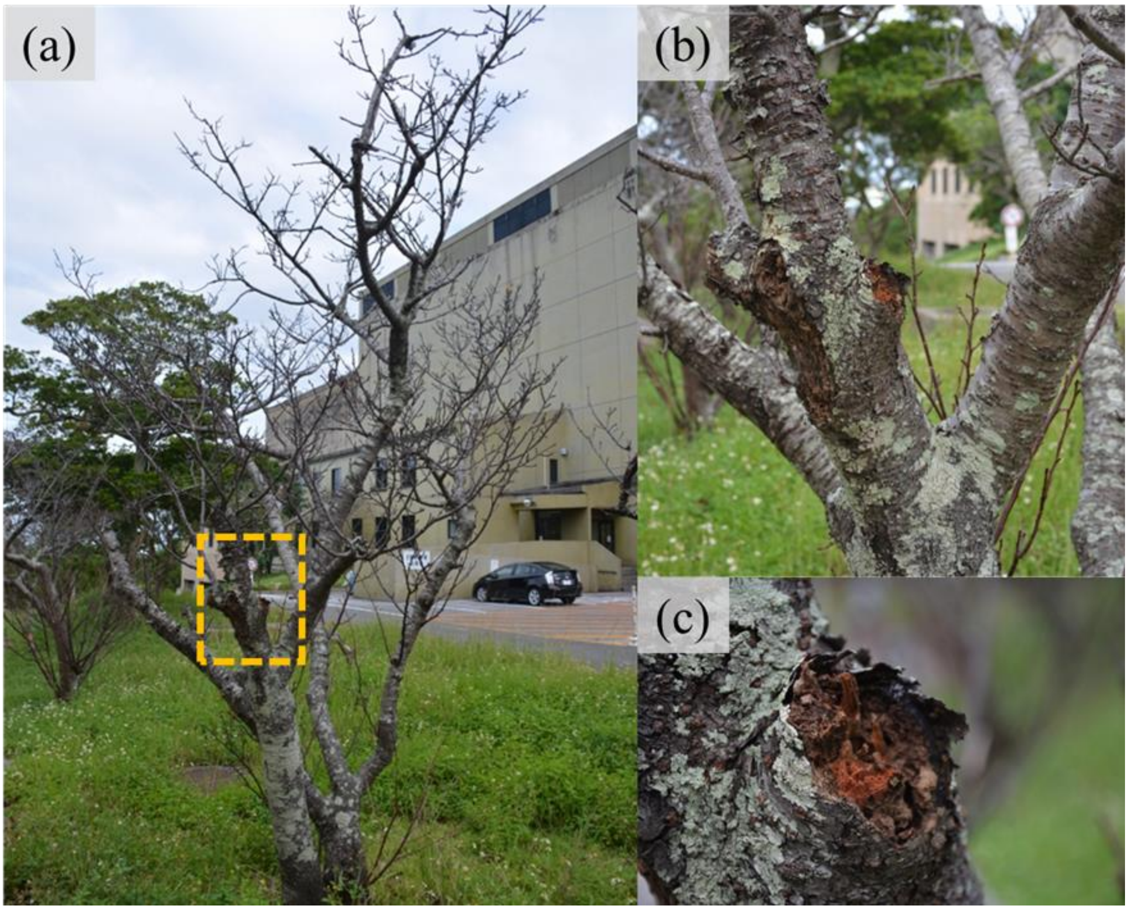


Fig. 4.3 Photographs of *Cerasus campanulata* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *C. campanulata*; (b) Closeup of the termite colony; (c) Damage mark by *N. sugioi*.

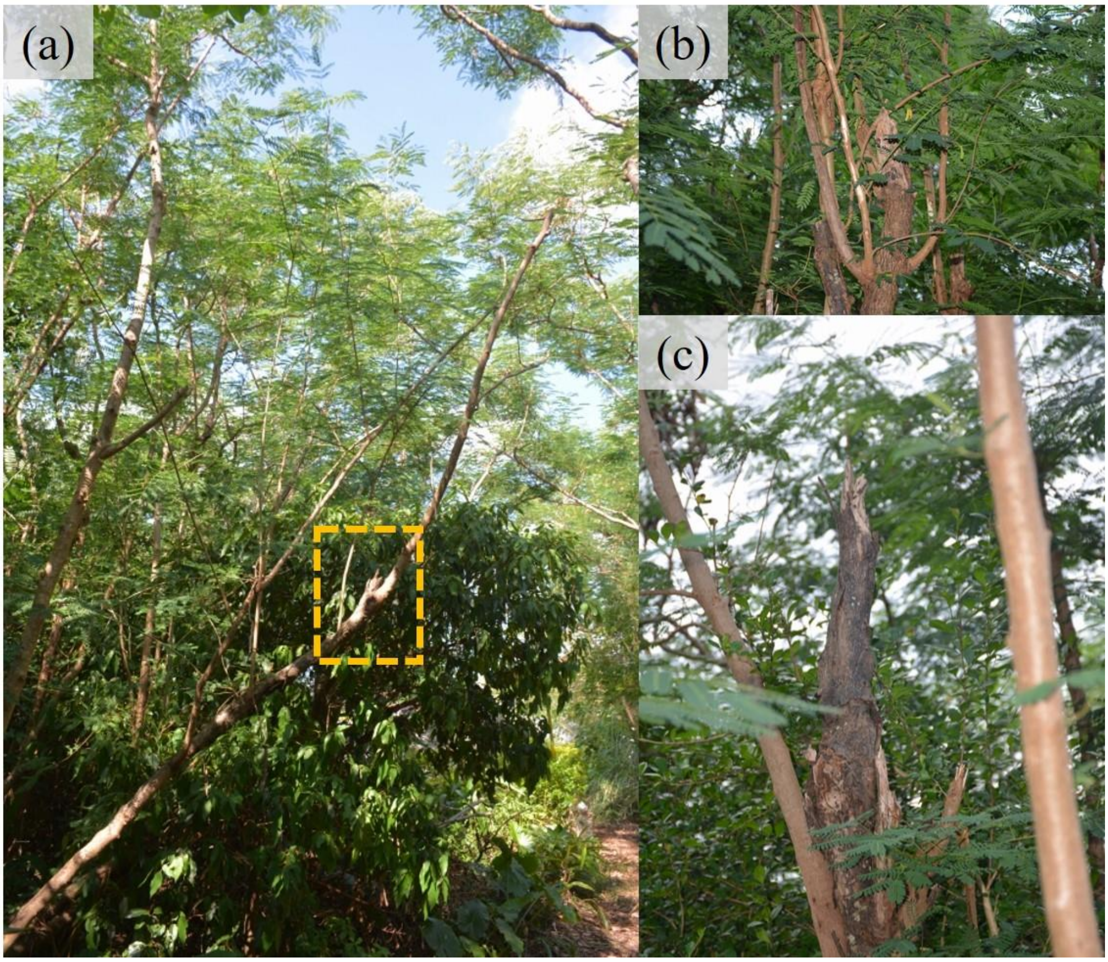


Fig. 4.4 Photographs of *Leucaend leucocephala* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *L. leucocephala*; (b, c) Closeup of the termite colony.

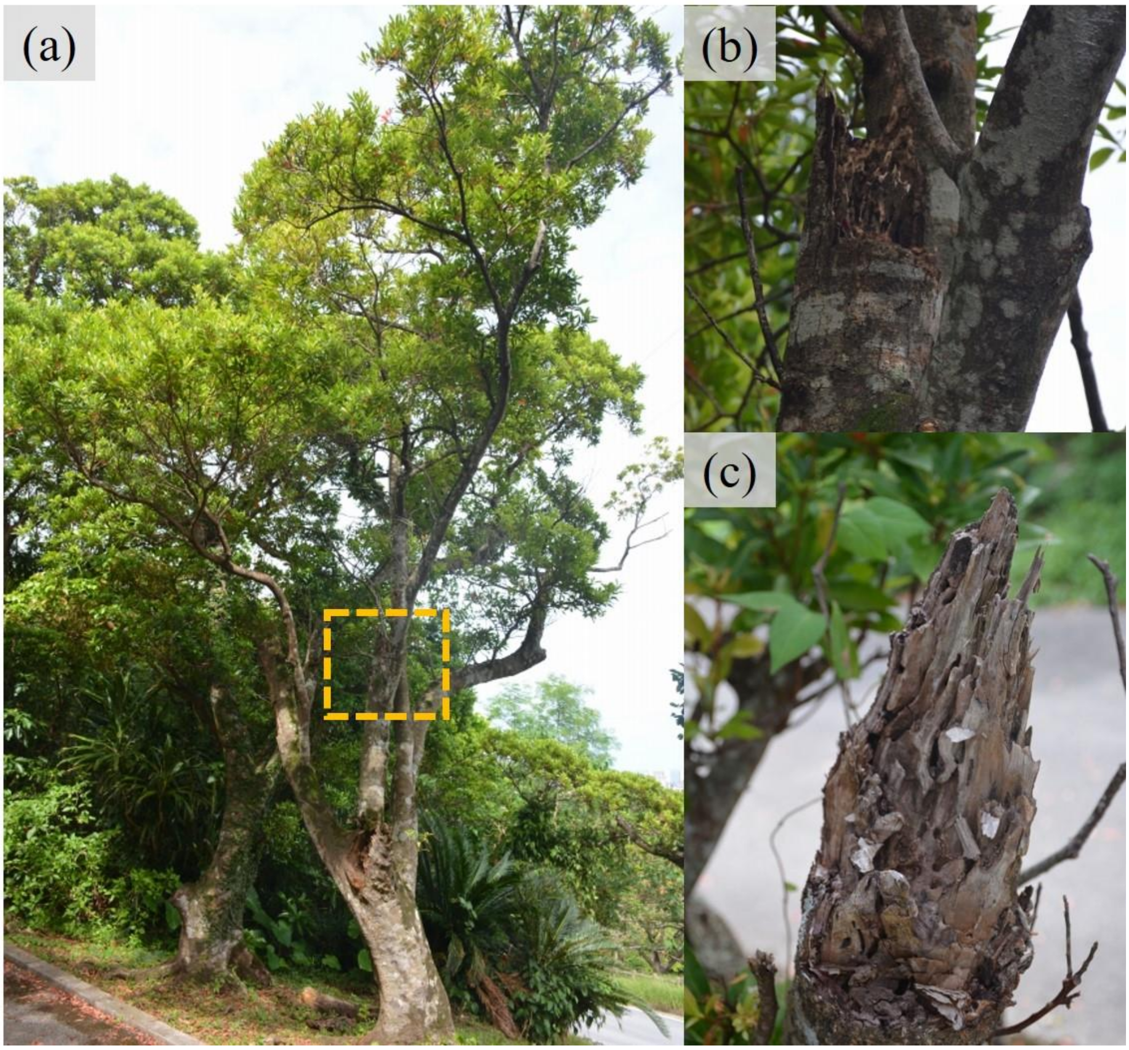


Fig. 4.5 Photographs of *Elaeocarpus sylvestris* var. *ellipticus* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *E. sylvestris*; (b) Closeup of the termite colony; (c) Damage mark due to feeding by *N. sugioi*.

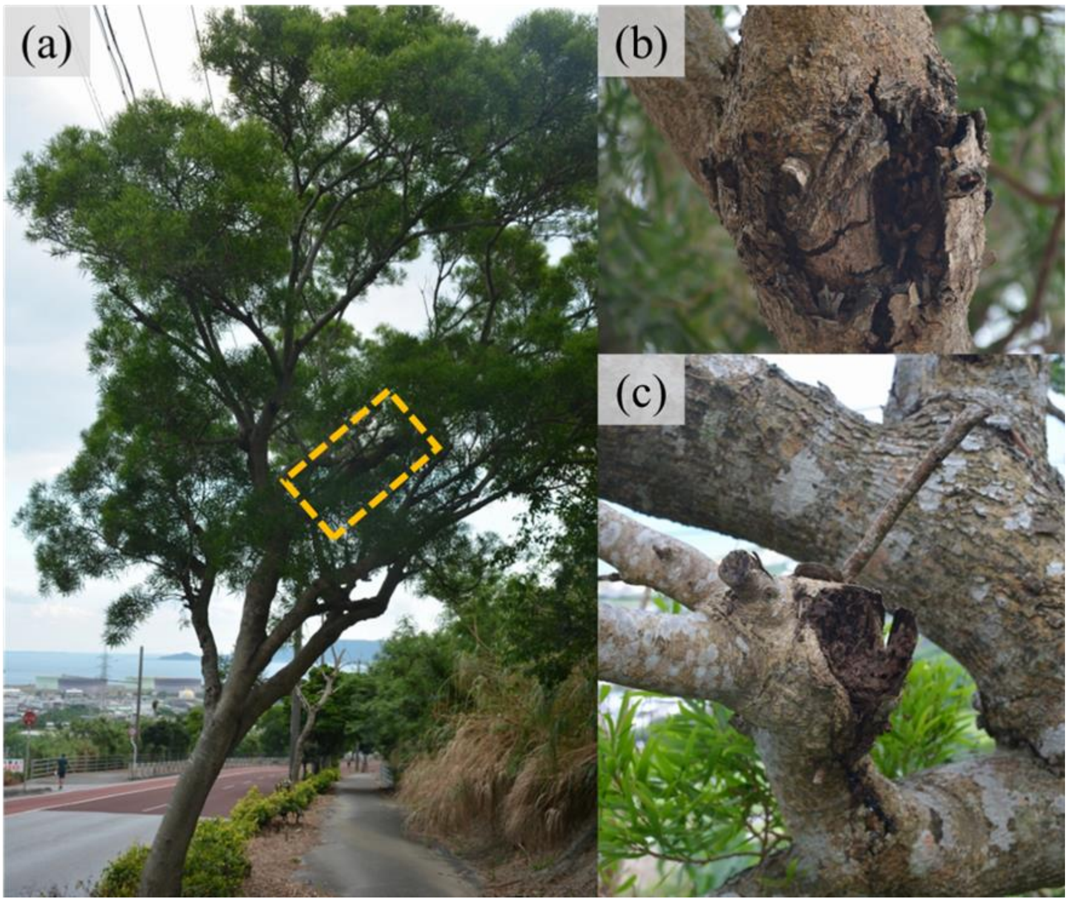


Fig. 4.6 Photographs of *Acacia confusa* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *A. confusa*; (b) Closeup of the termite colony; (c) Damage mark by *N. sugioi*.

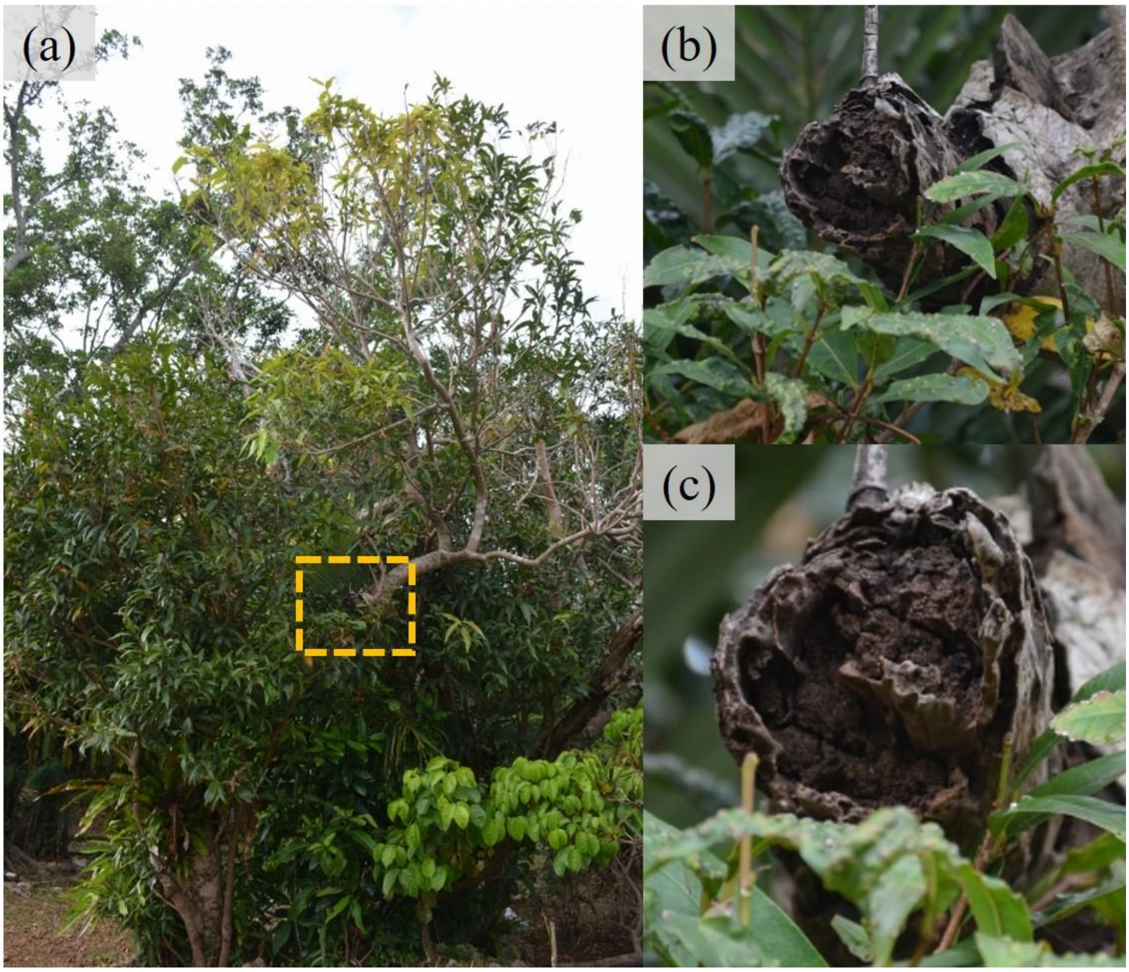


Fig. 4.7 Photographs of *Syzygium jambos* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *S. jambos*; (b) Closeup of the termite colony; (c) Damage mark by *N. sugioi*.

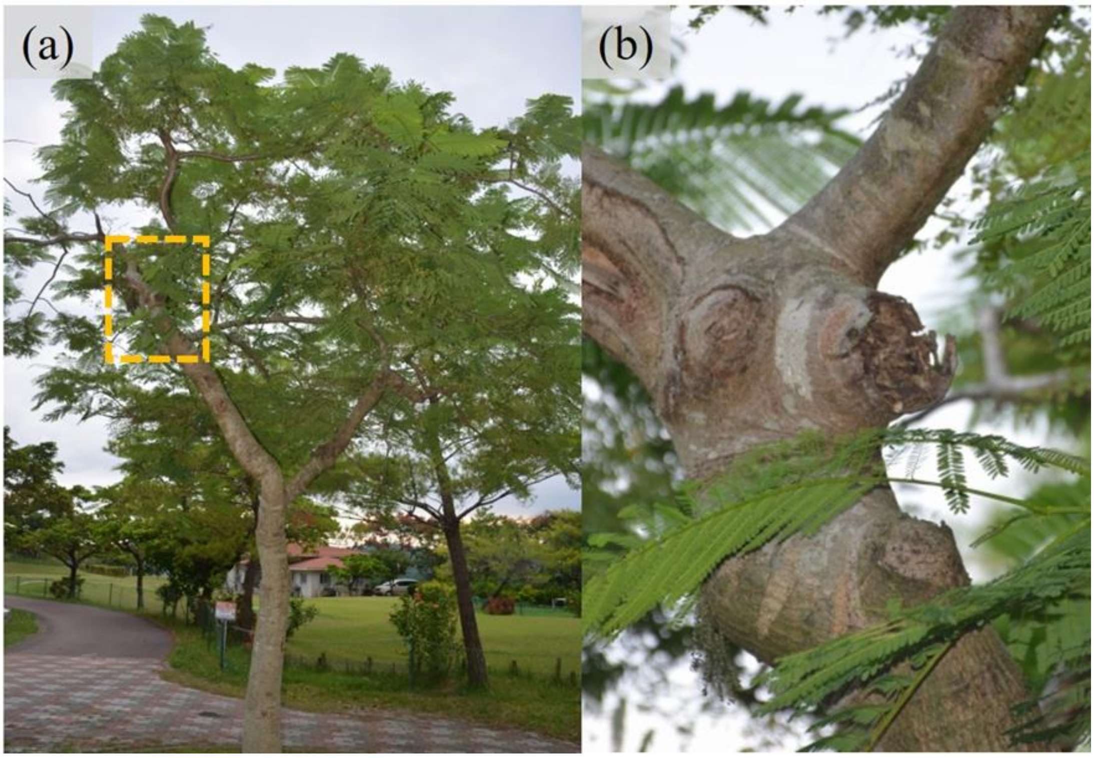


Fig. 4.8 Photographs of *Delonix regia* with *Neotermes sugioi* colony. The yellow broken square show the presence of termite colony. (a) Whole tree of *D. regia*; (b) Closeup of the termite colony.

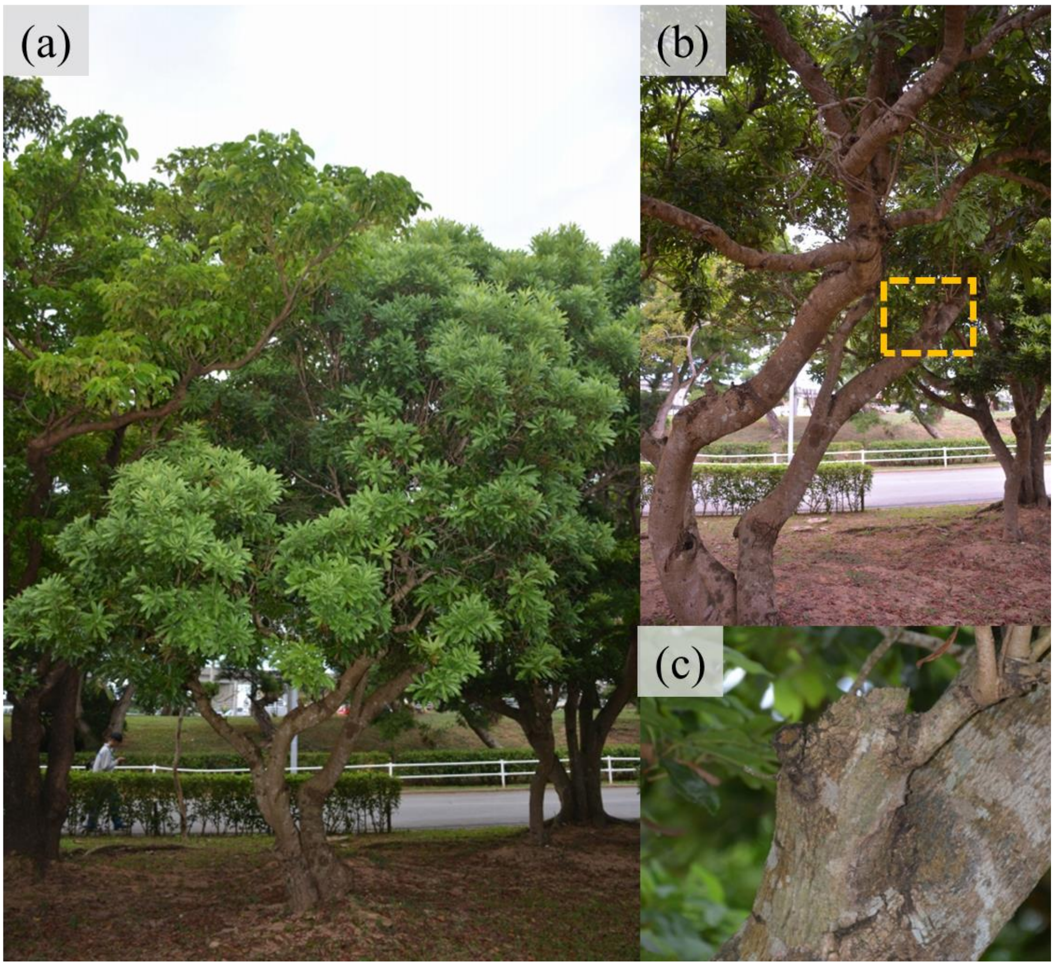


Fig. 4.9 Photographs of *Morella rubra* with *Neotermes sugioi* colony. The yellow broken square show the presence of the termite colony. (a) Whole tree of *M. rubra*; (b) Closeup of the termite colony; (c) Damage mark by *N. sugioi*.

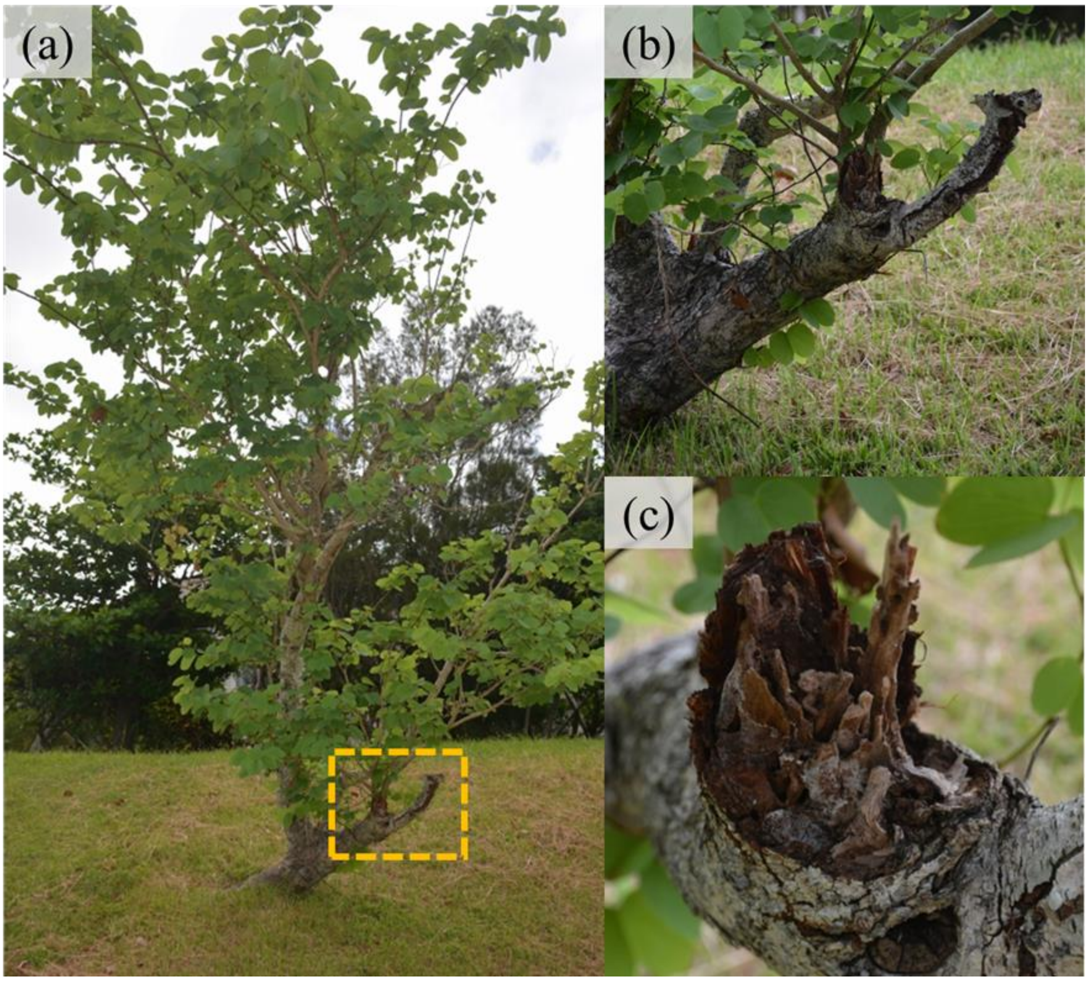


Fig. 4.10 Photographs of *Bauhinia variegata* with *Neotermes sugioi* colony. The yellow broken square show the presence of the termite colony. (a) Whole tree of *B. variegata*; (b) Closeup of the termite colony; (c) Damage mark by *N. sugioi*.

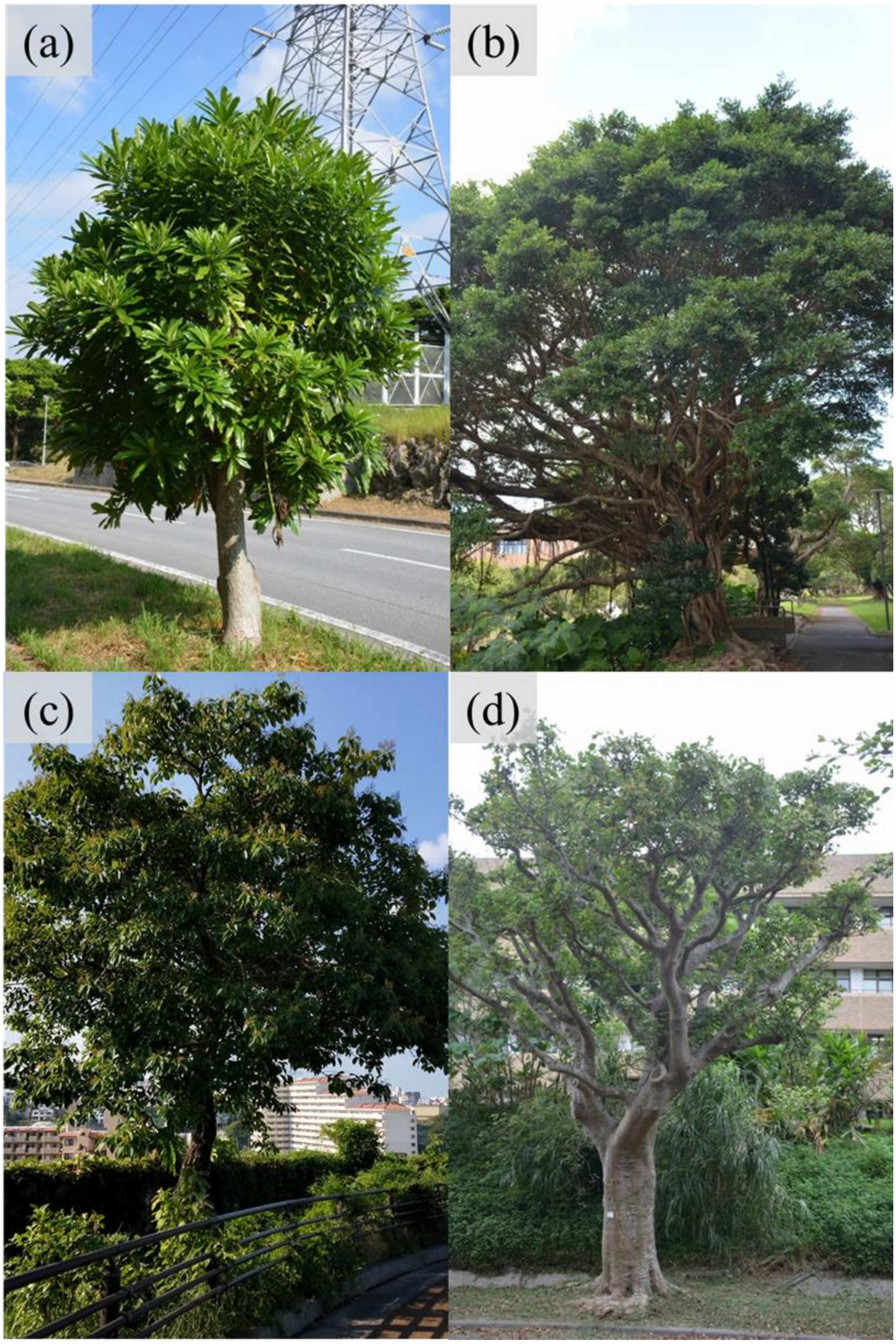


Fig. 4.11 Photographs of the other four tree species. (a) *Cerbera manghas*, (b) *Ficus microcarpa*, (c) *Eheria acuminata*, (d) *Erythrina variegate*.

4.4 Discussion

Neotermes sugioi were distributed in the region extending from 26°05′00″N to 26°25′00″N, located in the south and central area of Okinawa Island. This species was difficult to find at the northern end of the studied area and was not found further north beyond 26°25′00″N (Fig. 4.1A). Therefore, it was suggested that 26°25′00″N is the northern limit for this termite, and it is supported by the findings of Yasuda *et al.* (2000). Although more study is required, the factor of temperature may be important to explain the distribution of this species in this area.

On the other hand, this species found only a few colonies beyond 26°07′30″ N at the southern tip of Okinawa Island (Fig. 4.1A). Because *N. sugioi* was found on many islands south of Okinawa Island, including Ishigaki Island, Iriomote Island, and Yonaguni Island (Ikehara, 1966), the zone in the southern end of Okinawa Island, where this termite is not present, may not be explained by temperature only. Yasuda et al. (2000) suggested that *N. sugioi* was found in forest areas with Ryukyu limestone, whereas it was not found in forest areas not containing limestone. Because the termite distribution might be influenced by the soil distribution, the termite distribution and the soil distribution in Okinawa Island were compared for understanding of the blank zone of this termite in the southern end area.

In Okinawa Island, Kunigami mahji (red-yellow soil; Maejima, 2016), Jahgaru (immature soil; Maejima, 2016), Shimajiri mahji (dark-red soil and immature soil; Maejima, 2016), and alluvial soil are the major soil types (Fig. 4.1B) (Miyamaru, 2013; National Agriculture and Food Research Organization, 2017). *N. sugioi* was found at many sites in the areas with Jahgaru soil, which is widely distributed in the middle and southern area of Okinawa Island (Fig. 4.1C). However, in the southern end area, it seems that the blank zone of *N. sugioi* occurred owing to the presence of Shimajiri mahji (Fig. 4.1C). Because *N. sugioi* does not contact with soil, the relationship between the soil characteristics and the distribution of this termite remains unclear.

This study supported the description about the northern distribution of *N. sugioi* in a previous study (Yasuda et al., 2000). Meanwhile, our investigation found a zone in the southern area of Okinawa Island where this termite species is absent. Further, the present study will require the more information about the relation between the soil type (especially Shimajiri mahji and Jahgaru) and the life history of termites for understanding for characteristics of distribution of this termite species.

In addition, 12 tree species with the termite nests could be confirmed in this survey. On the other hand, 17 species were reported in Chapter 2, but only one species was common to both. Therefore, there are a total of 29 tree species with the termite nests.

4.5 Chapter summary

In the present study, it was examined the distribution of *Neotermes sugioi* on Okinawa Island in the Ryukyu Archipelago, Japan, which represents the northern limit of its distribution. Termite nests were found at 115 of the 257 sites investigated. The results suggest that 26°25′00″N is the northern limit for this species. However, new zone lacking this species was found in the southern end of the island. The comparison of the termite distribution and the soil distribution suggested that the presence/absence of this species might be related with the soil distribution of Shimajiri mahji and Jahgaru. In addition, 15 tree species with the termite nests were confirmed, and therefore a total of 31 species of nesting trees were identified. In addition, 12 tree species with the termite nests were identified, and together with other results, a total of 29 species of nesting trees were revealed.

Chapter 5
Characteristics of dispersal flight and dispersal strategy

5.1 Introduction

In termites, colonies have limited mobility, and thus there is an annual emergence of alates among both males and females. Alate termites form monogamous pairs that establish new colonies in the vicinity of their landing site (Nutting 1969; Jones et al. 1988; Lepage and Darlington 2000). Chapter 3 showed that *Neotermes sugioi* do not build belowground tunnels and aboveground shelter tubes, so the infection of the trees requires founding a new colony by alates released from infested trees. Therefore, understanding of the phenology of this termite alates will be helpful their management and control.

The most common strategy used by termites to establish new colonies is through the production and flight of alates (winged imagos), with species-specific flight patterns occurring (reviewed by Nutting 1969). The dispersal pattern of termite alates is associated with their "life type," rather than their phylogenetic status. For example, the flight pattern of the most primitive termite, *Mastotermes darwiniensis* (Mastotermitidae), is similar to that of the more derived termites belonging to the families of Rhinotermitidae and Termitidae (Nutting 1969; Neoh and Lee 2009a, 2009b; Nalepa et al. 2001; Sangamma and Chimkod 2012). These termites are termed "separate-piece nesters" (*sensu* Abe 1987; Eggleton and Tayasu 2001). The alates of termites from this group exhibit a very concentrated flight period (in some cases, it is just a few days) that is distinct from other periods over the course of the dispersal flight season, which lasts several months (Medeiros et al. 1999; Nalepa et al. 2001; Martius 2003; Bourguignon 2009; Neoh and Lee 2009a, 2009b).

The families of Archotermopsidae and Kalotermitidae have the longer dispersal flight season, with a lower peak in flight activity, followed by a gentle increase (and subsequent decrease) in flight activity, because these termites continuously release small numbers of alates (Jones et al. 1988; Martius et al. 1996; Medeiros et al. 1999; Martius 2003; Huang et al. 2004a, 2004b; Huang et al. 2007; Bourguignon 2009; Howell et al. 2009). These termites are called "single-piece nesters" (*sensu* Abe 1987; Eggleton and Tayasu 2001). The dispersal flight pattern of alates from these colonies is poorly understood, because studies on the phenology of these termites in relation to their life history characteristics remain limited. Thus, information on the evolution and diversity of the dispersal strategies of alates in relation to the life cycle remains limited. Since *N. sugioi* also belongs to this group, there is little information on the dispersal flight. Here, this chapter report the seasonality of disperser production and the phenology of dispersal flight of *N. sugioi* in the field colonies, and the alates from five isolated colonies under semi-field conditions. In addition, it will be discussed whether weather factors affect the dispersal flight patterns of this termite species.

5.2 Materials and Methods

5.2.1 The phenology of dispersal flight in the field colonies

Study site and trapping methods in the field

Alates were collected by using a light trap for 452 days from April 23, 1983 to July 17, 1984 in the subtropical laurel forest of the Ryukyu limestone area in Nishihara (26.24785°N, 127.76114°E), Okinawa, Japan. Of note, the colony-sampling points (explained in a later paragraph) were at least 1 km (this was based on their estimated flight distance, Abe 1989) away from the light trap point of 1983 to 1984; thus, the results of the light trap surveys were not affected by collection of colonies. The light trap was prepared by installing a 20 W fluorescent light and a 20 W black light over a plastic vessel (40 × 30 × 6 cm) containing water. The light trap was lit every day from 18:00 to 06:00 the next morning during the survey period, except for September 24–26, 1983, when a typhoon struck Okinawa Island. Trapped termites were collected every morning. The males and females were counted separately (Miyaguni et al. 2012). To ascertain the period during which dispersal flight occurred, two fixed-time surveys were conducted (on May 29 and June 4, 1983), during which the traps were checked every hour for 24-h, collecting alates from 08:00 in the morning until 08:00 the next morning. The number of alerts collected by the light trap was assumed to reflect the dispersal flight activity in the field.

Pattern of disperser production in the field

To demonstrate seasonal changes in the disperser production of the *Neotermes sugioi* colony, seasonal changes in the ratio of each caste in the field colonies were investigated. A total of 134 wild colonies were collected during 1983–2012 around Nakagusuku and Nishihara, were transferred to our laboratory. Colony members were removed from each wild colony by cutting the wood into small pieces. The number of caste members was counted, including small larvae, pseudergates, early nymphs, pre-alate nymphs, alates, pre-soldiers, soldiers, queens, kings, and male neotenics (Roisin 2000; Katoh et al. 2007; Miyaguni et al. 2013b). In the analyses, the number of individuals from all colonies was pooled by collection month, without considering the year of collection. Then, the ratio of pre-alate nymphs and alates to monthly termite members was calculated. Because alates differentiate from pseudergates via early nymphs and pre-alate nymphs, it is important to calculate the number of pre-alate nymphs and alates in relation to the sum of the four castes when estimating the timing of disperser production.

Data analysis of the factors affecting flight activity in the field

Previous studies have reported the dispersal flight behavior of termites by analyzing how the number of trapped alates is correlated with meteorological factors, based on the assumption that meteorological factors trigger termite flight and flight activity (Nutting 1969; Neoh and Lee 2009a, 2009b). However, the number of trapped alates is limited by the number of alates in field colonies. The number of trapped alates might increase because the increase in the production of alates is independent of a weather-based trigger. Moreover, the production of dispersers might be related to long-term environmental conditions. In this study, data analysis was conducted at three different stages to study when alates are released by the colonies.

In the first stage, the relationship between the production of dispersers (alates and pre-alate nymphs) inside the colony and long-term environmental conditions was investigated. The monthly means of air temperature, atmospheric pressure, relative humidity, precipitation and wind velocity during 1983–2012 at the Naha Meteorological Observation Point (located 10 km southwest of the study location) were obtained from the database of the Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html). The relationship between the ratio of alates inside the colony and each environmental condition each month was analyzed by Pearson's product moment correlation coefficient. The relationship between the ratio of pre-alate nymphs and each environmental condition each month was also analyzed using the same method.

In the second stage, the relationship between the release of alates from the colony and environmental conditions was investigated by generalized linear models (GLM) with a Negative Binominal error distribution. The number of trapped alates from April 25 to November 4 in 1983 was used as the response variable. The daily means of the air temperature, atmospheric pressure, relative humidity, precipitation, and wind velocity during same period at the Naha Meteorological Observation Point were used as the explanatory variables.

In the third step, the number of trapped alerts was described using the GLM with Poisson error distribution and log link function. In the third stage, the number of trapped alates was explained using the GLM with Poisson error distribution and log link function. For explanatory variables, the monthly ratio of alates and meteorological factors were included. The number of alates caught in the light trap during the survey period in 1983 was re-counted to obtain the numbers of alates that were trapped each month, which were used as a response variable. The monthly means of the air temperature, relative humidity, atmospheric pressure, wind velocity, and total precipitation from April 1983 to March 1984 at the Naha Meteorological Observation Point were used. All analyses were performed using R software, version R 3.2.2 (R Development Core Team 2015).

5.2.2 The phenology of dispersal flight in experimental colonies

Observation of alate emergence in experimental colonies and data analysis

In late May 1985, five dead branches (A, B, C, D, and E; 56–75 cm in length) containing the nests of *Neotermes sugioi* were collected in Bengadake Park (26.21765N, 127.73066E; Torihori, Syuri, Naha City, Okinawa, Japan) from an area of approximately 250 m in diameter. Each branch was isolated using a plastic case (65 × 40 × 20 cm) with a mesh cover. Within these cases, each branch was placed diagonally, so that the upper section of the colony (from which alates emerge and fly) was positioned against a wall of the case. To trap the alates, a wide pad soaked with water was placed under of the upper section of the colony in each plastic case. Cases were placed on a table installed in a grove with no streetlights at the University of the Ryukyus (Okinawa) for 183 days from June 1st to November 30th, 1985, which included the most of core flight season. Accordingly, environmental conditions such as temperature and relative humidity resembled those of the termite natural environment. During the investigation period, trapped alates were collected each morning and the numbers of males and females were determined (Miyaguni et al. 2012). Furthermore, a single-day fixed-time survey was conducted on July 30th to check traps at hourly intervals for 24 h, collecting alates from 08:00 in the morning until 08:00 on the following morning. The synchronicity of alate emergence among the colonies was analyzed based on correlations between the number of alates emerging from each pair of colonies. For synchrony over the entire flight season, the correlation between the number of alates that emerged in all pairs of colonies was analyzed using Spearman’s rank correlation coefficient based on all survey data (183 days) collected during the study period. With respect to the monthly changes in synchrony, the correlations between the number of alates that emerged in all pairs of colonies in each month was analyzed using Spearman’s rank correlation coefficient. All analyses described here and below were performed using R software, version R 3.2.2 (R Development Core Team 2015).

Colony composition in experimental colonies

After the investigation period, each branch was promptly cut into small blocks in the laboratory, and the number of individuals in each caste were counted. If there is synchrony in the emergence of alates among colonies, it was hypothesized that similarities in the stage or size of colonies may result in alates emerging at the same time. It was investigated the relationship between the ranking of long-term correlation coefficients (Spearman rank) among the colonies (10 pairs) described above and ranking of the following five factors: (1) the difference in the total number of individuals; (2) the number of pseudergates (Maki and Abe 1986); (3) the number of produced alates (including both emerged and pre-emergence alates); (4) the total number of colony members; and (5) the proportion of produced alates among the total population. Individuals of this species nest within a limited scope of a tree's branches and trunk and invade from the branches of living trees, expanding their nesting space toward the roots while consuming the internal plant tissues (Maki and Abe 1986; Sugio et al. 2018b). At the time of dismantling the branches on completion of the experiment, it was confirmed that there were sufficient woody parts (i.e., potential food resources; Korb and Lenz 2004) without colony nesting on the root side of the branches.

Relationship between alate flight and meteorological factors in experimental colonies

The biological responses to environmental factors can be divided into seasonality factors that affect selection of the seasons of alate production or dispersal flights (Nutting 1969; Sugio et al 2018a), and trigger factors that induce alate flight from colonies (Nutting 1966; Nutting 1969). Preliminary analyses indicated that for all colonies, there were important relationships between alate flight and three environmental factors (Table 5.1). Although consistent with previous results concerning seasonal factors, important relationships are seldom detected in short-term (monthly) analysis (Table 5.2). Therefore, this method appeared to be unsuitable for the identification of factors promoting synchronous alate emergence among colonies in the short term, particularly for those species with continuous dispersal.

Determination of the conditions for flight non-occurrence when flight is highly expected and vice versa is useful to understand flight triggers factors. Based on the results of the field and experimental colonies, seasonal flight activity was classified into three phases, namely high season (June and July), mid-season (August and September), and low season (October and November). In addition, daily flight activity was divided into three grades, namely, high (alate emergence occurred in four or all five of the experimental colonies), moderate (alate emergence occurred in two or three colonies), and low (alate emergence occurred only in a single colony or not at all). In order to determine the factors influencing alate dispersal, environmental conditions were investigated by analyzing the following four groups: high season-high flight activity (HS-HFA, n = 36 days), high season-low flight activity (HS-LFA, n = 15 days), low season-high flight activity (LS-HFA, n = 9 days), and low season-low flight activity (LS-LFA, n = 44 days). Data for daily temperature, atmospheric pressure, relative humidity, and wind velocity at 21:00 from June 1st to November 30th (1985), recorded at the Naha Meteorological Observation Point (located 10 km southwest of the study location), were obtained from the database of the Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html). On the basis of a preliminary analysis, which indicated that temperature and humidity had significant effects on flight activity (Table 5.3), it was thus compared the temperature and relative humidity distribution among the HS-HFA, HS-LFA, LS-HFA, and LS-LFA groups (Chouvenc et al. 2017).

Table 5.1. Correlation coefficients between the number of alates emerging in each experimental colony of *Neotermes sugioi* and meteorological factors.

Colony	Spearman rank correlation coefficient			
	Temperature	Atmospheric pressure	Wind velocity	Relative humidity
A	0.373*	-0.343*	0.186	0.250*
B	0.480*	-0.320*	0.088	0.291*
C	0.441*	-0.477*	0.088	0.314*
D	0.321*	-0.222*	0.064	0.134
E	0.389*	-0.333*	0.091	0.216*

*: Significance determined by Bonferroni correction ($p < 0.05$). Daily values of the four environmental factors were obtained from May 1st to November 30th, 1985, using the database of the Naha Meteorological Observation Point (located 10 km southwest of the study location) provided by the Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html). The relationship between the daily number of alates emerging from each colony and the four meteorological factors was determined using Spearman rank correlation analysis based on the entire survey data (183 days) of the study period. Analyses were performed using R software, version R 3.2.2 (R Development Core Team 2015).

Table 5.2. Correlation coefficients between the number of alates emerging from each experimental colony of *Neotermes sugioi* and meteorological factors in each month.

Month	Colony					Average [SD]	Min	Max
	A	B	C	D	E			
Temperature								
Jun	0.184	0.701*	0.108	0.080	0.007	0.216 [0.278]	0.007	0.701
Jul	-0.112	-0.505	-0.299	-0.306	-0.391	-0.323 [0.144]	-0.505	-0.112
Aug	0.068	-0.103	0.004	-0.097	0.221	0.019 [0.134]	-0.103	0.221
Sep	0.294	0.231	0.006	0.063	0.293	0.177 [0.134]	0.006	0.294
Oct	0.418	-0.023	0.459	0.208	0.381	0.289 [0.199]	-0.023	0.459
Nov	0.459	0.385	0.624*	0.463	0.361	0.458 [0.103]	0.361	0.624
Atmospheric pressure								
Jun	-0.153	-0.165	-0.264	0.124	-0.316	-0.155 [0.170]	-0.316	0.124
Jul	0.204	-0.032	0.014	0.431	0.385	0.200 [0.210]	-0.032	0.431
Aug	0.155	-0.245	-0.029	-0.182	0.297	-0.001 [0.227]	-0.245	0.297
Sep	0.096	-0.083	-0.161	-0.387	0.003	-0.106 [0.184]	-0.387	0.096
Oct	-0.149	0.135	-0.096	0.107	0.329	0.065 [0.192]	-0.149	0.329
Nov	-0.326	-0.128	-0.275	-0.326	-0.246	-0.260 [0.081]	-0.326	-0.128
Relative humidity								
Jun	-0.367	-0.101	-0.288	-0.533	-0.279	-0.314 [0.157]	-0.533	-0.101
Jul	-0.166	0.238	0.089	0.017	0.046	0.045 [0.145]	-0.166	0.238
Aug	-0.073	0.089	-0.219	-0.273	-0.332	-0.162 [0.170]	-0.332	0.089
Sep	0.268	0.307	0.312	0.319	0.263	0.294 [0.026]	0.263	0.319
Oct	0.295	0.025	0.427	0.150	-0.015	0.176 [0.185]	-0.015	0.427
Nov	0.368	0.275	0.405	0.364	0.296	0.342 [0.054]	0.275	0.405
Precipitation								
Jun	-0.128	-0.321	-0.118	-0.250	-0.015	-0.166 [0.120]	-0.321	-0.015
Jul	0.002	0.251	0.093	0.076	0.116	0.108 [0.091]	0.002	0.251
Aug	-0.075	0.255	0.034	0.149	-0.141	0.044 [0.161]	-0.141	0.255
Sep	0.183	0.159	0.166	0.122	0.168	0.160 [0.023]	0.122	0.183
Oct	0.203	-0.052	0.294	0.117	-0.149	0.083 [0.182]	-0.149	0.294
Nov	-0.165	-0.165	-0.137	-0.165	-0.133	-0.153 [0.016]	-0.165	-0.133

*: Significance determined by sequential Bonferroni correction ($p < 0.05$). The data in this table are based on the daily values of the four environmental factors from May 1st to November 30th, 1985, using the database of the Naha Meteorological Observation Point (located 10 km southwest of the study location) provided by the Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html). The relationship between the daily number of alates emerging from each colony and four daily meteorological factors was determined using Spearman rank correlation analysis for each month. Analyses were performed using R software, version R 3.2.2 (R Development Core Team 2015).

Table 5.3. Summary of generalized linear models (with Binomial distributions) on the daily fight activity (high or low) of colonies of *Neotermes sugioi* in high season (June and July) and in low season (October and November).

	Estimate	SE	*Z* -value	*P* -value
In high season				
Intercept	135.388	161.826	0.837	0.403
Temperature	0.203	0.295	0.689	0.491
Atmospheric pressure	-0.113	0.156	-0.725	0.469
Relative humidity	-0.300	0.115	-2.616	< 0.01
Daily wind velocity	-0.054	0.270	-0.199	0.842
In low season				
Intercept	-124.902	212.423	-0.588	0.557
Temperature	0.601	0.248	2.420	< 0.05
Atmospheric pressure	0.108	0.206	0.520	0.603
Relative humidity	0.035	0.065	0.542	0.588
Daily wind velocity	-0.448	0.291	-1.537	0.124

The seasonal flight activity of *N. sugioi* was classed into three parts as follows: high season (June and July), middle season (August and September) and low season of flight (October and November). Then, daily flight activity was classed into three grades as follows: high (Alate emergence was caused in four or all experimental colonies), middle (it was caused in three or two colonies), low (it was caused in only a colony or was not caused in any colony). The daily flight activity was significantly negatively correlated with relative humidity in high season of flight, and then, it was significantly positive correlated with temperature in low season of flight.

Analysis of sex ratio in experimental colonies

Given the frequent small-scale flight events of *N. sugioi* alates, the daily sex ratios among flown alates show considerable fluctuation, and accordingly the patterns are difficult to generalize. Therefore, months were divided into three periods, namely, early (1st to 10th), mid- (11th to 20th), and late (21st to 30th/31st), and summed the daily alate dispersals during each period of each month as a single flight event ($n = 18$). Events with < 10 individuals were excluded from the statistical analysis within each colony but were used for analysis of the total number of alates. The sex ratio for each event was arranged on a timeline (Chouvenc et al. 2017).

5.3 Results

5.3.1 The phenology of dispersal flight in the field colonies

Flight pattern and sex ratio

In 1983, dispersal flight started in late April and ended in early November (Fig. 5.1). The number of alates increased gradually until numbers peaked on June 29 (209 alates, 6.6% of all alates in this year), after which numbers declined gradually until the end of the season (November). From May 27 to August 8 (74 days), dispersal flight occurred daily. The two fixed-time surveys showed that dispersal flight started after 20:00, and ended at 02:00 or 04:00 the next morning (Fig. 5.2). The largest hourly catch was trapped between 20:00 and 21:00. I observed that the alates of *Neotermes sugioi* flew near the trap, even though tandem behavior (when males maintained almost constant contact with the posterior of the female's abdomen using his antennae until finding a suitable nesting site; Nutting 1969; Stuart 1969) was not observed. In total, 3169 alates (1764 females and 1405 males) were collected. The (male) sex ratio was 0.443; thus, it was significantly skewed towards females (binomial test, $P < 0.0001$) (Fig. 5.1).

In 1984, dispersal flight began on May 6 and peaked in June, as observed in 1983 (Fig. 5.1). This survey was discontinued on July 17, 1984. In this year, equal numbers of male and female alates (757 females and 757 males) were collected. Of note, the biased sex ratio recorded in 1983 started after dispersal flight peaked in June (Fig. 5.1).

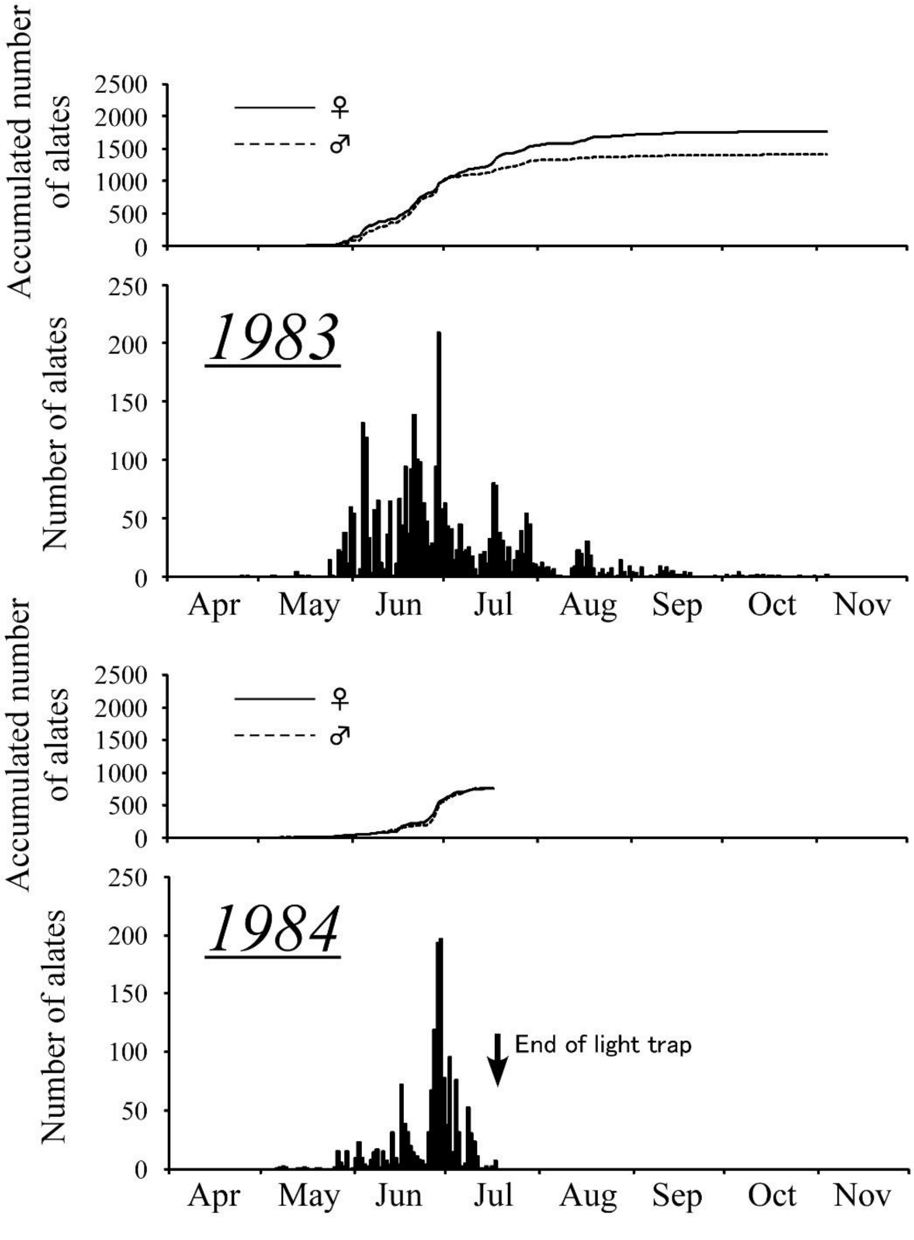


Fig. 5.1 Pattern of flight activity. Black bars show the number of trapped alates each day. *Solid* and *broken* lines show the accumulated number of female and male alates, respectively, across the season.

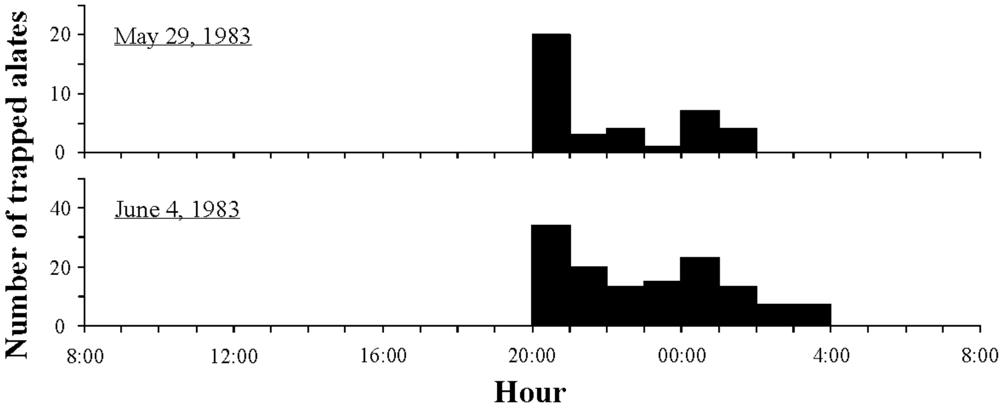


Fig. 5.2 Flight duration of alates during two 24-h periods.

Pattern of disperser production in field colonies

Seasonal changes in the ratio of pre-alate nymphs and alates to monthly numbers of termite members in the field are shown in Fig. 5.3. The ratio of alates increased rapidly from May, peaked in June, and decreased gradually until October. During this period, a high percentage of colonies contained alates. Some colonies (1, 1, and 5 colonies in December, January, and February, respectively) retained 1–173 alates, even in winter (December–February). The ratio of pre-alate nymphs increased rapidly and peaked in May, decreasing rapidly after June. The ratio of alates exceeded that of pre-alate nymphs in August and September. In the colonies where alates were retained, the number of alates exceeded pre-alate nymphs in zero out of 11 colonies in May, six out of 23 colonies in June, three out of seven colonies in July, nine out of 10 colonies in August, and five out of eight colonies in September. The ratio of pre-alate nymphs also exhibited a small peak in October. In the colonies that retained alates, the number of pre-alate nymphs exceeded alates in five out of six colonies in October. Furthermore, some colonies (two, one, and eight colonies in December, January, and February, respectively) retained one to 552 pre-alate nymphs, even in the winter (December–February), reflecting our observations of alates (Table 5.4).

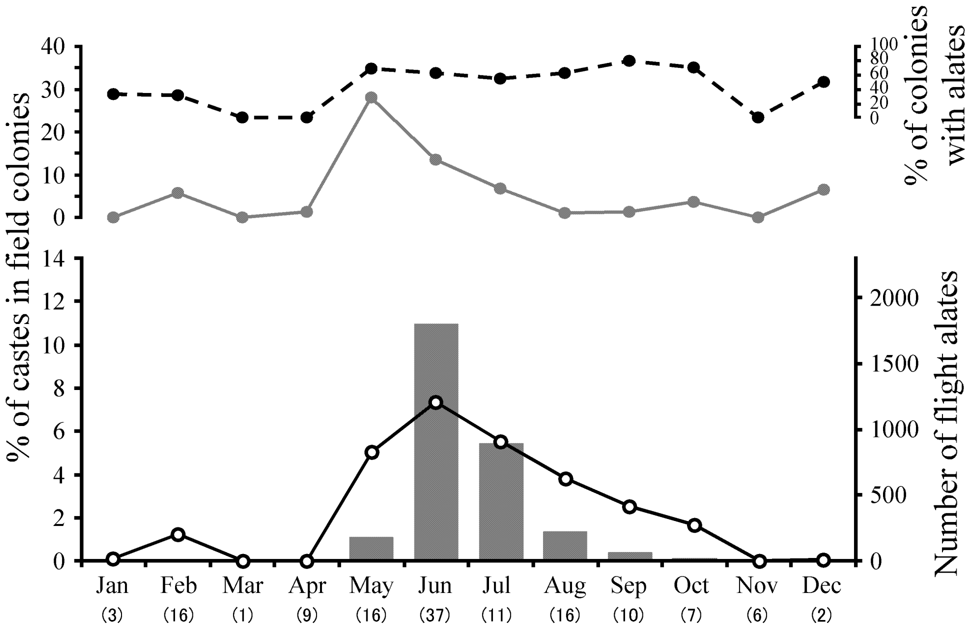


Fig. 5.3 Seasonal change in the ratio of dispersers in field colonies. Solid lines show the ratio of dispersers (black represents alates, while gray represents pre-alate nymphs on the left side of the lower vertical axis). The broken line shows the ratio of colonies with alates (on the right side of the upper vertical axis). Numbers in parentheses denote sample size, i.e., the numbers of colonies collected each month (on the right side of the upper vertical axis). Gray bar shows the monthly number of dispersal flight alates collected by the light trap in 1983 (on the right side of the lower vertical axis).

Table 5.4 Seasonal change in disperser production in the field colonies of *Neotermes sugioi* (based on the 134 colonies collected in 1983 to 2012).

Month	Number of colony	Number of individuals				% of each caste			
		Alate	Pre-alate nymph	Nymph	Pseudergate	Alate	Pre-alate nymph	Nymph	Pseudergate
January	3	11	9	2189	5485	0.1	0.1	28.5	71.3
February	16	324	1514	5950	18306	1.2	5.8	22.8	70.2
March	1	0	0	0	1106	0.0	0.0	0.0	100.0
April	9	0	206	707	13969	0.0	1.4	4.8	93.9
May	16	1787	9879	3931	19602	5.1	28.1	11.2	55.7
June	37	5548	10124	8727	50858	7.4	13.5	11.6	67.6
July	11	961	1166	2690	12536	5.5	6.7	15.5	72.2
August	16	1104	351	2185	25192	3.8	1.2	7.6	87.4
September	10	175	95	3543	3098	2.5	1.4	51.3	44.8
October	7	171	386	2112	7492	1.7	3.8	20.8	73.7
November	6	0	0	1470	8986	0.0	0.0	14.1	85.9
December	2	1	79	680	461	0.1	6.5	55.7	37.8

Relationship between disperser production in field colonies and environmental factors

The rate of alate production inside the colonies was significantly correlated with the monthly means of air temperature, atmospheric pressure, relative humidity, and precipitation; however, no significant correlation was found between the rate of alate production and monthly mean wind velocity (Fig. 5.4). The increase in the rate of alate production was correlated with increasing temperature, relative humidity, and precipitation (Fig. 5.4). Atmospheric pressure was correlated with a decrease in the rate of alate production (Fig. 5.4). No significantly correlations were found between the rate of pre-alate nymph production and any of the environmental factors.

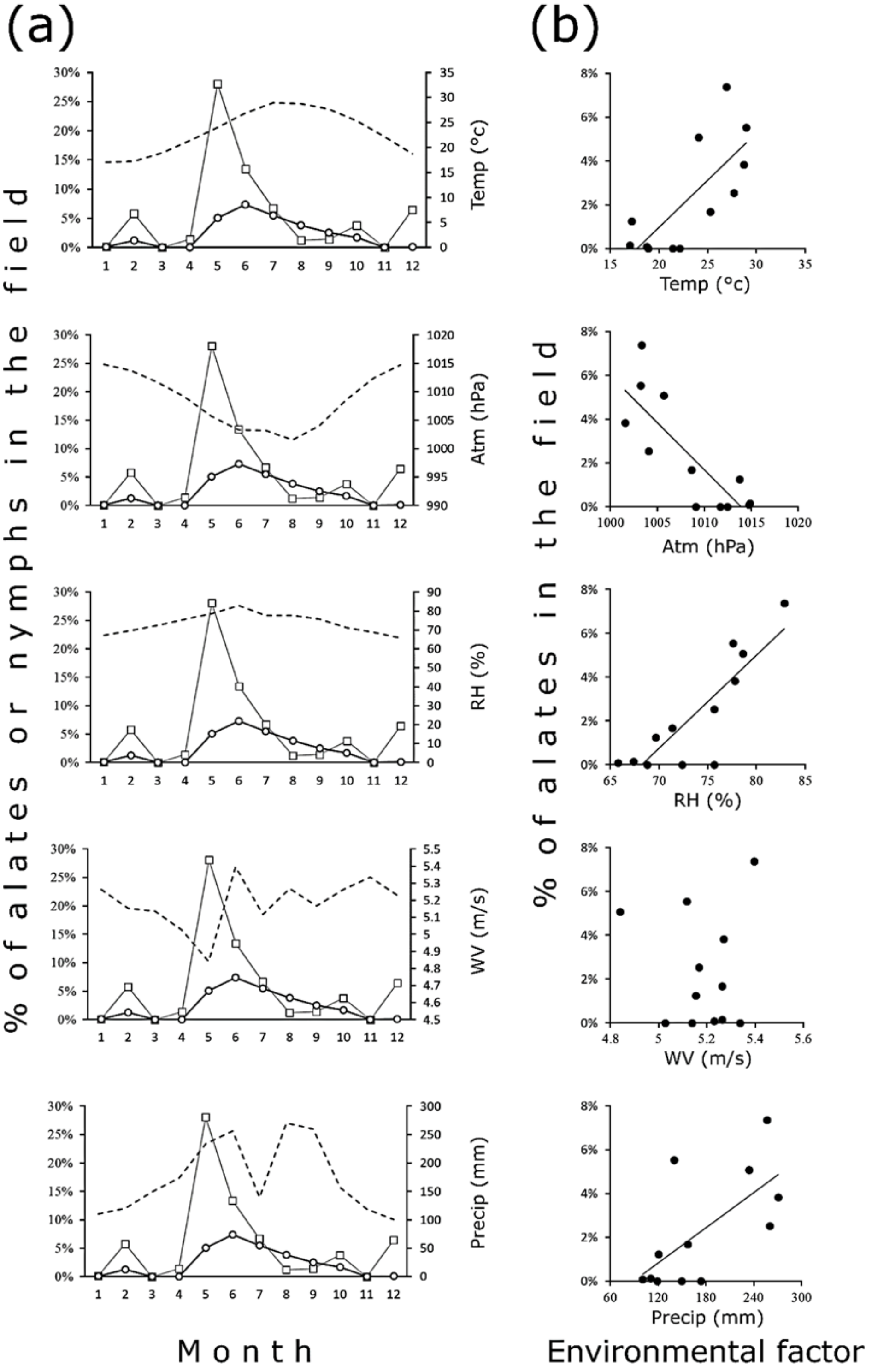


Fig. 5.4 Relationship between disperser production of the colony and monthly environmental conditions: (a) seasonal change in the ratio of dispersers (solid black lines = alates, solid gray lines = pre-alate nymph) in the colony and environmental factors (broken lines); (b) correlations between the ratio of alates in the field colonies and the environmental factors.

Relationship between daily flight activity and environmental factors

Multivariate GLM with negative binominal distributions showed that daily flight activity was significantly correlated with air temperature, atmospheric pressure, relative humidity, and precipitation (Table 5.5). The increase in daily flight activity by alates was correlated with increasing air temperature and relative humidity (Table 5.5). In comparison, the decline in daily flight activity was correlated with increasing atmospheric pressure and precipitation (Table 5.5). Wind velocity had no significant relationship with daily flight activity.

Table 5.5 Summary of generalized linear models (with Negative Binomial distributions) on the daily abundance of trapped alates during May 23 to November 4, 1983.

Item	Estimate	SE	*Z* -value	*P* -value
Intercept	99.422	29.365	3.386	< 0.001
Daily temperature	0.582	0.053	11.063	< 0.001
Daily atmospheric pressure	-0.126	0.028	-4.487	< 0.001
Daily relative humidity	0.176	0.023	7.641	< 0.001
Daily wind velocity	-0.101	0.073	-1.384	0.166
Daily precipitation	-0.027	0.007	-3.722	< 0.001

Data analysis of the factors affecting monthly flight activity

Multivariate GLM with Poisson distributions showed that monthly flight activity was significantly correlated with the density of alates in the field colonies and air temperature. However, the estimated coefficient of temperature was much smaller than that of the density of dispersing alates in field colonies (Table 5.6).

Table 5.6 Summary of the generalized linear models (with Poisson distributions) on the monthly abundance of trapped alates.

Item	Estimate	SE	*Z*-value	*P*-value
Intercept	40.232	133.336	0.302	0.763
Monthly ratio of alates inside colonies	73.958	8.562	8.607	<0.001
Monthly mean temperature	0.335	0.084	3.974	<0.001
Monthly mean relative Humidity	-0.030	0.051	-0.578	0.564
Monthly mean atmospheric pressure	-0.048	0.131	-0.366	0.714
Monthly mean wind velocity	0.607	0.579	1.047	0.295
Monthly total precipitation	0.002	0.002	1.422	0.155

5.3.2 The phenology of dispersal flight in experimental colonies

Phenology and correlation analysis of alate emergence among experimental colonies

Colonies A, C, D, and E were characterized by peak alate emergence around July 20th, whereas peak emergence from Colony B was observed around July 1st (Fig. 5.5). It was observed that there were only a few days on which the number of alates emerging from the colonies of *Neotermes sugioi* was 2% or more of the total number of alates emerging during the entire observation period (Fig. 5.6). However, an overlap among colonies on these major flight days was recorded several times during the experimental period. On a few flight days, it was also observed that synchrony between colonies during the non-flight times. In a single 24-h observational period from the morning of July 30th to that of the following day, it was detected that overlapping peak flight times only between colonies C and E. Specifically, alate emergence occurred at 01:00–05:00 in colony A, 23:00–03:00 in colony B, 22:00–06:00 in colony C, 22:00–05:00 in colony D, and 01:00–03:00 in colony E, with peaks occurring at 03:00–04:00 in colony A, 01:00–02:00 in colony B, and 02:00–03:00 in colonies C and E. In contrast to the other four colonies, I observed no peak in the emergence of alates from colony D (Fig. 5.7).

The results of correlation analyses revealed statistically significant positive correlations between all colony pairs, with an average correlation coefficient of 0.498 ± 0.132 (Average ± SD; minimum = 0.258, maximum = 0.630; Table 5.7). The correlation between colony pairs with respect to monthly emergence was found to change according to season (Table 5.8), with a higher correlation being detected for most combinations in July and November. Compared with these months, although some combinations of colonies showed higher correlation, the correlation coefficient values decreased in June, August, September, and October (Table 5.8).

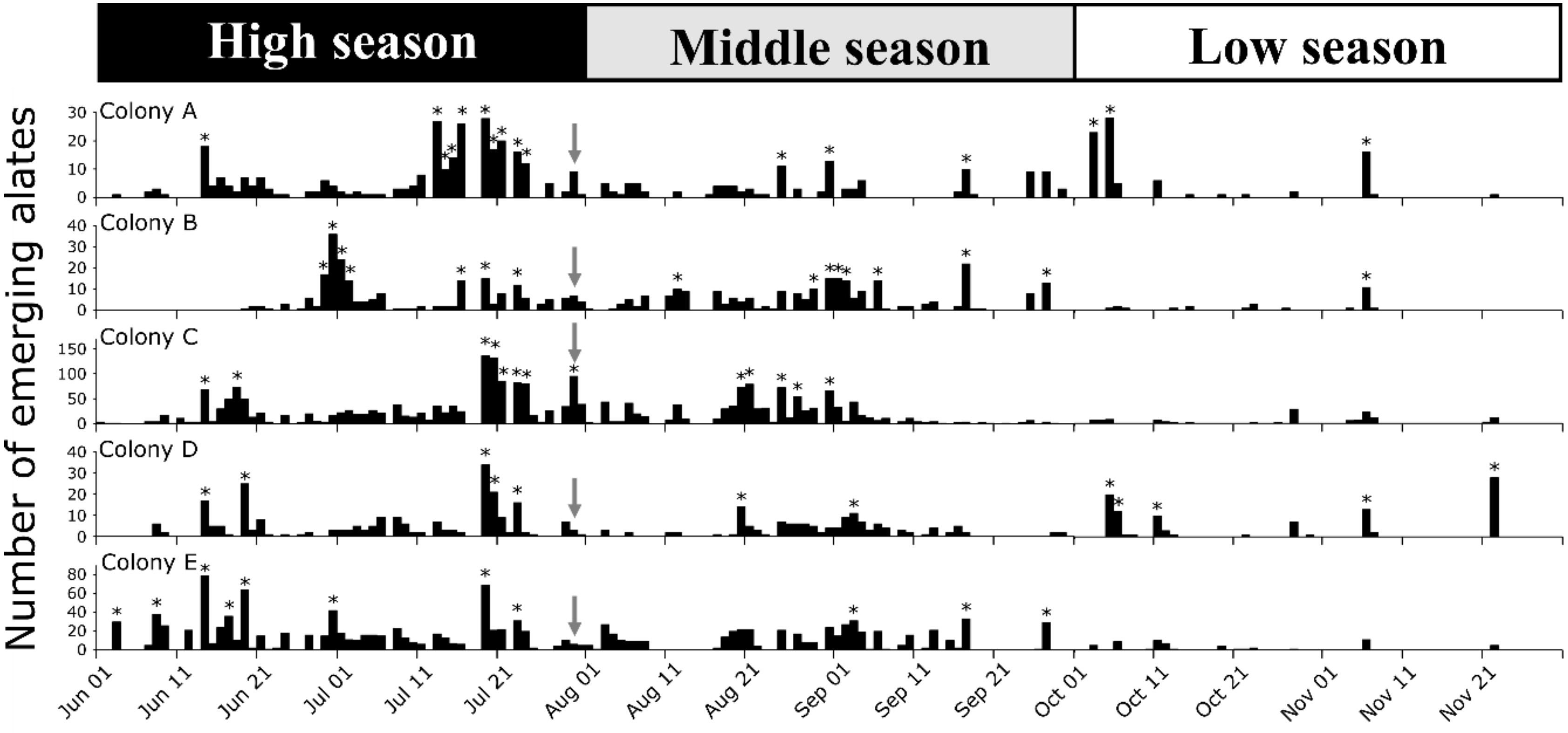


Fig. 5.5 Phenology of the alates emerging from five experimental colonies in June and July. The gray arrow indicates July 30th, on which a single-day fixed-time survey was conducted (see also Fig. 5.7). *: days on which the alates emerging from a colony represented 2% or more of the total number of alates emerging during the observation periods.

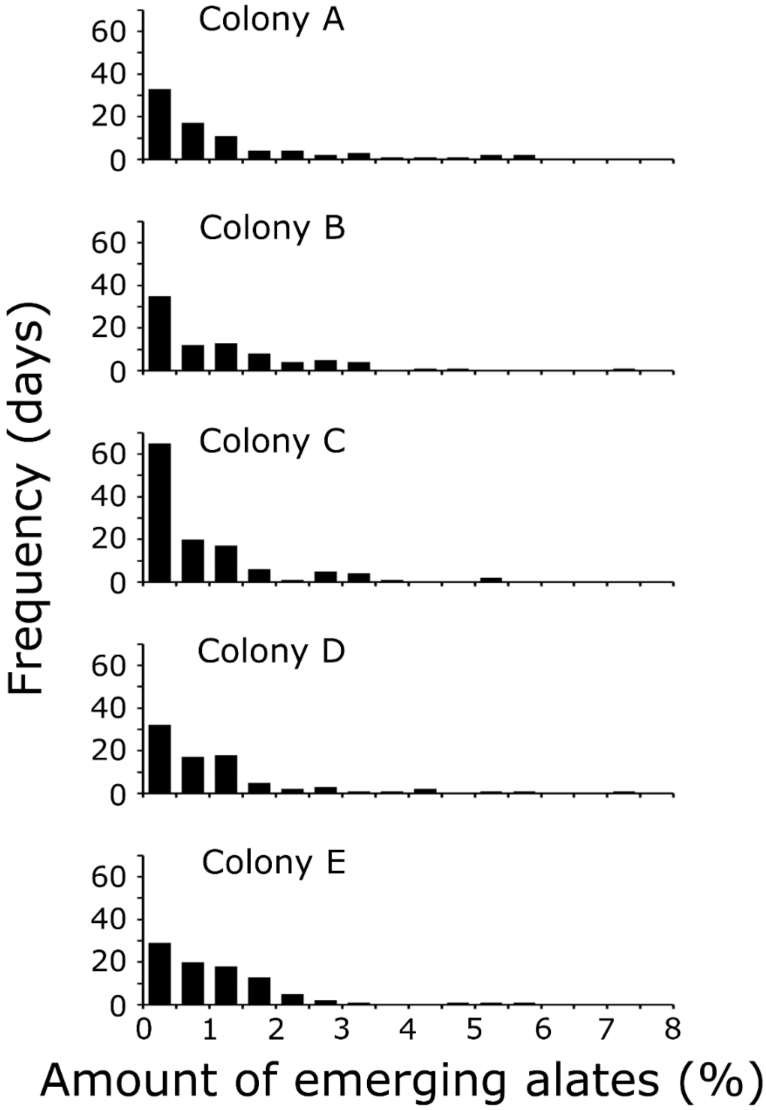


Fig. 5.6 Frequency distribution of the rate of the daily emergence of alates from laboratory colonies of *Neotermes sugioi* in 1985. During the investigation period of 183 days from June 1st to November 30th, alate emergence was observed on 81, 84, 121, 84, and 91 days (times) in colonies A, B, C, D, and E, respectively. The number of days on which the number of alates emerging from a colony represented 2% or more of the total number of alates in the observation period was 16, 16, 13, 12, and 11 for colonies A, B, C, D, and E, respectively.

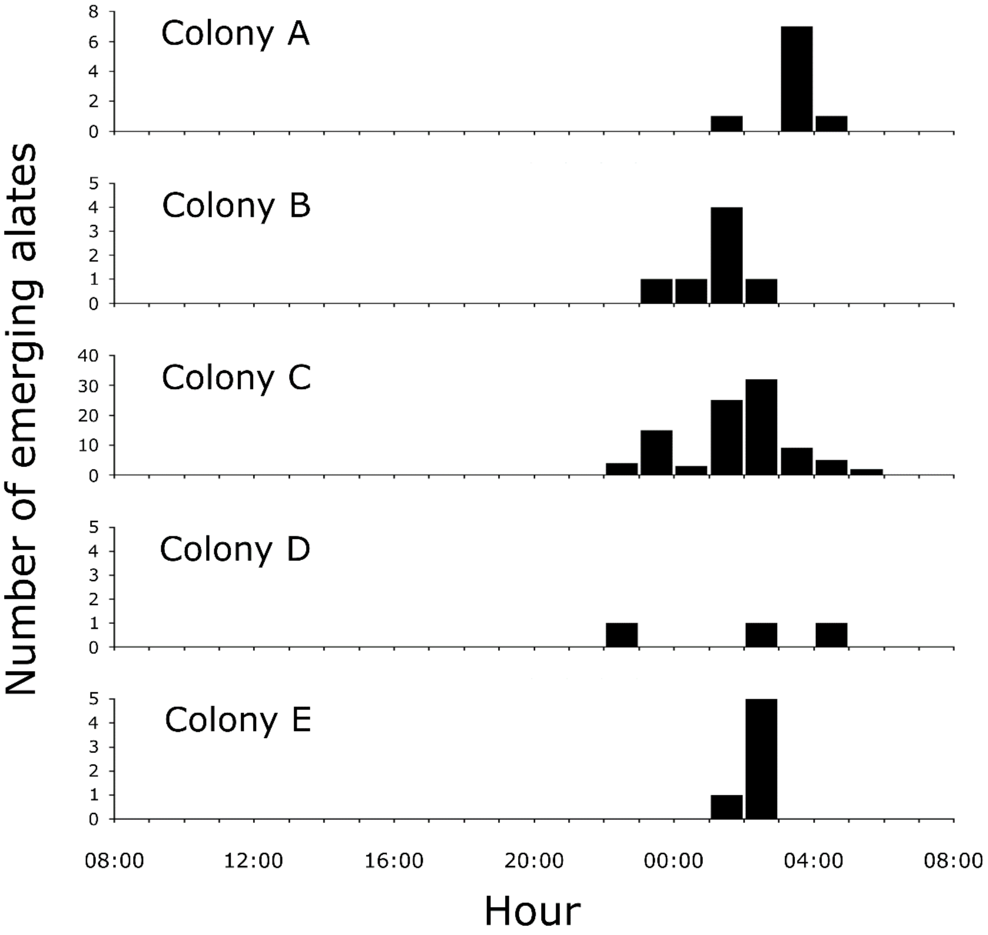


Fig. 5.7 Flight duration of alates during a 24-h period on July 30th. I conducted a single-day fixed-time survey to ascertain the timing of disperser emergence from each colony of *Neotermes sugioi*. I checked the trap at hourly intervals for 24 h, collecting alates from 08:00 in the morning until 08:00 the following morning.

Table 5.7 Correlation matrix of the number of alates emerging from colonies over a long-term period.

V.S.	Col. A	Col. B	Col. C	Col. D	Col. E
Colony A					
Colony B	0.353*				
Colony C	0.595*	0.369*			
Colony D	0.569*	0.258*	0.620*		
Colony E	0.501*	0.464*	0.620*	0.630*	

*: Significance determined by sequential Bonferroni correction ($p < 0.05$).

Table 5.8 Correlation coefficients for the number of alates emerging from paired colonies of *Neotermes sugioi* over short-term periods.

Month	Correlation coefficient for each colony combination										Average [SD]
	AB	AC	AD	AE	BC	BD	BE	CD	CE	DE	
Jun	0.421	0.719*	0.750*	0.725*	0.300	0.354	0.191	0.663*	0.666*	0.671*	0.546 [0.207]
Jul	0.551*	0.782*	0.636*	0.668*	0.680*	0.534*	0.582*	0.688*	0.713*	0.881*	0.671 [0.106]
Aug	0.398	0.675*	0.317	0.719*	0.655*	0.418	0.389	0.752*	0.723*	0.410	0.546 [0.172]
Sep	0.549*	0.234	0.251	0.392	0.738*	0.621*	0.800*	0.658*	0.609*	0.729*	0.558 [0.201]
Oct	0.314	0.566*	0.503	0.527*	0.261	0.342	0.187	0.427	0.488	0.411	0.403 [0.124]
Nov	0.655*	0.702*	0.997*	0.817*	0.652*	0.616*	0.401	0.701*	0.583*	0.829*	0.695 [0.161]

*: Significance determined by sequential Bonferroni correction ($p < 0.05$).

Colony composition

A population survey conducted following the observation period revealed that the colonies contained between 869 and 6,683 individuals, including the already emerged alates (Table 5.9). The proportion of produced alates (total number of emerged and pre-emergence alates) relative to colony size, including alates emerging during the observation period, was 0.412 ± 0.183 (Average ± SD; minimum = 0.213, maximum = 0.632). No statistically significant relationships (Spearman's ρ test) were found between the rank of the correlation coefficient for colony combinations in long-term alate emergence and the rank of inter-colony differences in any of the survey items (number of pseudergates: $\rho = -0.321$, $p = 0.366$, $n = 10$; number of produced alates: $\rho = -0.297$, $p = 0.405$, $n = 10$; total number of colony members: $\rho = -0.115$, $p = 0.751$, $n = 10$; proportion of produced alates among all individuals: $\rho = -0.115$, $p = 0.751$, $n = 10$).

Table 5.9 Caste composition of the experimental colonies.

Colony	Number of individuals in a branch nest							Number of emerged alates (F:M)	Total number of colony-members	Proportion of produced alates among all individuals
	Reproductive	Larva	Pseudergate	Early-nymph	Pre-alate nymph	Alate	Soldier			
A	2 (Q-mNeo)	178	1187	535	1	47	86	492 (202:292)*	2528	0.213
B	2 (Q-mNeo)	0	188	144	3	21	20	491 (195:296)*	869	0.589
C	2 (Q-K)	628	1583	1126	11	176	616	2541 (876:1665)*	6683	0.407
D	2 (Q-K)	60	1157	380	8	153	36	475 (190:285)*	2271	0.277
E	2 (Q-mNeo)	0	471	278	4	48	66	1360 (537:823)*	2229	0.632

Q-mNeo: the colony is headed by an adult queen and a male neotenic (replacement reproductive)
Q-K: the colony is headed by an adult queen and an adult king
*: sex ratio differs significantly from a male:female ratio of 1:1, as determined by a binominal test under sequential Bonferroni correction ($p < 0.05$).

Relationship between alate flight and meteorological factors

The overlap of temperature and relative humidity distribution in the HS-HFA, HS-LFA, LS-HFA, and LS-LFA categories is shown in Fig. 5.8. In high season, active flight was caused under conditions of 23.1–28.3 C° and 68–94% humidity, with the core zone experiencing 26.0–29.0 C° and 75–90% humidity. In low season, active flight occurred at 22.5–26.5 C° and 72–90% humidity.

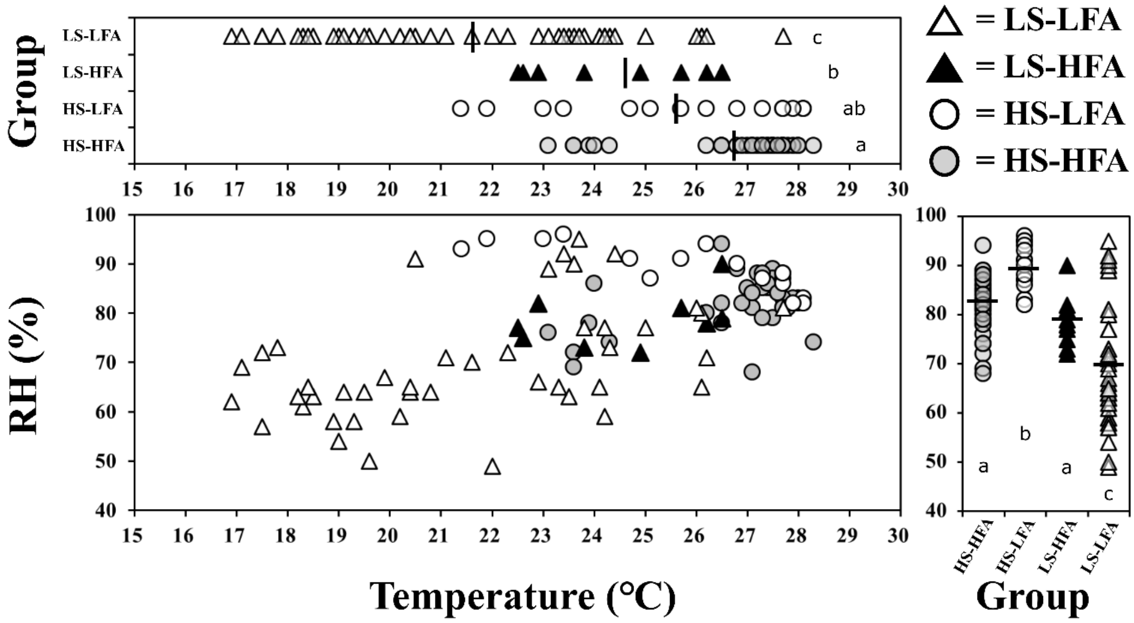


Fig. 5.8 Alate flight activity and distribution meteorological factors. Abbreviation (also see main text): HS-HFA, high season-high flight activity; HS-LFA, high season-low flight activity; LS-HFA, low season-high flight activity; LS-LFA, low season-low flight activity. Black bars shows the average of each item. Different letters (a, b, and c) indicate significant differences among each item at $p = 0.05$, as determined by pairwise comparisons using the Mann–Whitney U-test with the Holm method after Kruskal–Wallis analysis.

Sex ratio

In all colonies, the sex ratio of emerging alates over the entire observational period was significantly biased toward males (Table 5.9), with a ratio of 0.611 ± 0.025 (Average ± SD; minimum = 0.593, maximum = 0.655). It was detected that no significant correlation between time and sex ratios in any of the colonies, based Kendall rank correlation analysis with sequential Bonferroni correction (Fig. 5.9). Analysis of the seasonal changes in the sex ratio of all alates emerging from the five colonies revealed that the male-biased alate emergence was fairly constant from July to the end of the observational period, whereas female-biased alate emergence was observed during early June and mid-October (Fig. 5.9). Specifically, the emergence of female alates in early June was higher than that of males in four of the five colonies (Fig. 5.9).

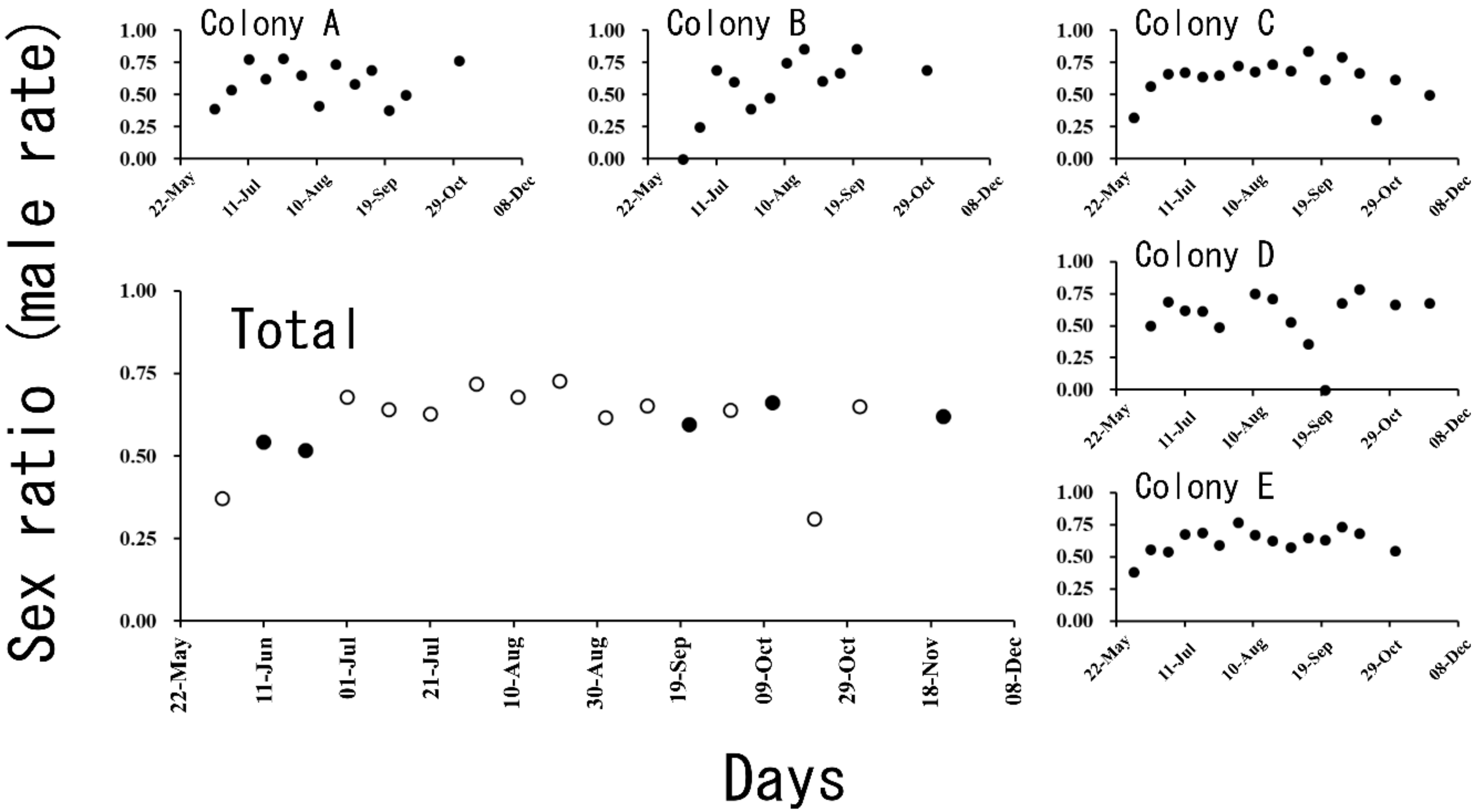


Fig. 5.9 Sex ratio of *Neotermes sugioi* alates emerging during the observation period. White circles in the "Total" graph indicate a significant deviation of the sex ratio from equal numbers of sexes, as determined by a binomial test with sequential Bonferroni correction.

5.4 Discussion

5.4.1 The phenology of dispersal flight in the field colonies

The phenology of dispersal flight in *Neotermes sugioi* reflects that described for the dispersal of alates of other single wood nesters in previous studies (Medeiros et al. 1999; Martius 2003; Huang et al. 2004b; Cabrera and Scheffrahn 2005; Huang et al. 2007; Bourguignon 2009). In our study, the dispersal flight period of *N. koshunensis* lasted several months (i.e., from May to October). A gradual increase and a gradual decrease in flight activity was observed through the season (Fig. 5.1), with just 6.6% of all alates being captured on the days when most individuals were trapped. In comparison, separate-piece nesters exhibit major flight days across a given season, implying the synchronized release of alates by local colonies, which might enhance outbreeding (Jones 1981). Furthermore, in the current study, flight continued for as long as 6–8 h each day (Fig. 5.2). Thus, the synchronous release of similar volumes of alates, as recorded for multiple-wood nesters, was not likely. The flight activity of individual colonies was not assessed; thus, it was cannot state whether the observed continuous flight arises due to differences in the timing of the intensive release of alates by each colony or whether each colony releases only a few alates continuously. Further studies are required to examine the pattern of alate release by each colony.

Previous studies investigating sexual differences in the development of termites did not report protogyny (the emergence of adult females earlier than males), whereas protandry (the emergence of adult males earlier than females) has been reported for some termite species (Luykx 1986; Matsuura 2006; Vargo and Husseneder 2011). My light trapping study in 1983 showed that more females emerged before males (Fig. 5.1); thus, protogyny might exist in this taxon. However, further evidence is required, because the sexual differences in phototactic responses might have influenced these results (Cheng et al. 2016). It was also measured how the disperser production phase in *N. sugioi* shifted over time by analyzing the ratio of pre-alate nymphs to alates in colonies in the field (Fig. 5.3). The observed trend in the flight activity of *N. sugioi* based on light traps reflected my direct counts of dispersers in the field-collected colonies (Figs. 5.1 and 5.3).

This study supported previous studies (reviewed by Nutting 1969) in that certain termite species appear to produce dispersers in relation to the annual biological clock or meteorological cues, which is reflected by the seasonal production and flight of alates (possibly during the optimal period for successful reproduction by the alates of a given species). However, a noticeable number of *N. sugioi* colonies also retained alates and pre-alate nymphs in winter, which is outside of the dispersal flight period (Fig. 5.1). A previous study also recorded the presence of alates in four out of 10 colonies during

January and February (Maki and Abe 1986). This phenomenon might arise because some alates and pre-alate nymphs produced during the summer might remain in the colony after the dispersal flight season for use in the subsequent season (i.e., the following year). As another possible explanation, the environmental conditions preceding flight might enhance the development of alates inside colonies; however, flight did not occur by the lack of daily environmental conditions that trigger the release alates. Consequently, these alates were kept in the colony until the next swarming season. Alternatively, the production of alates in winter might represent an alternative strategy of the colony to potential adverse conditions, different to the typical seasonal disperser production by the colony. For instance, a decline in food resources led to the production of alates in *Cryptotermes secundus* (Kalotermitidae). The frequency of colonies with a decline in food resources in *N. sugioi* was unknown; however, this process occurs in all of the colonies of single-piece nesters because their colonies never move to other trees. Thus, A similar mechanism might explain the off-season disperser production of *N. koshunensis*. Future studies should investigate the status of colonies in relation to the production of disperser in this species.

This study suggested that alate production inside the colony is positively correlated with temperature, relative humidity, and precipitation (Fig. 5.4). This result was supported by previous studies, which showed that the development of termite individuals is enhanced at higher temperatures and relative humidity (Nutting 1969). The negative relationship between alate production and atmospheric pressure might be pseudo-correlated, because atmospheric pressure was negatively correlated with temperature and relative humidity (Fig. 5.4). The daily number of trapped alates in 1983 was positively correlated with temperature and relative humidity (Table 5.5); however, the main effect of these environmental factors might enhance the flight activity of this termite by enhancing alate production inside the colony (not a direct trigger of the colony's release of alate) (Figs. 5.3 and 5.4). Alternatively, air temperature had a significantly positive effect, even in the model incorporating the density of alates in the colony along with environmental factors (Table 5.6). Thus, air temperature might increase the dispersal flight of alates from colonies. Moreover, precipitation might also be associated with the onset of flight by alates present in the colonies, because it has the opposite effect on alate production inside the colony with flight activity in the field (Fig. 5.4 and Table 5.5). The negative relationship between the daily number of trapped alates and atmospheric pressure seemed to be pseudo-correlated. The environmental factors that enhance or lessen alate release from colonies need to be quantified through controlled experiments under laboratory conditions.

5.4.2 The phenology of dispersal flight in experimental colonies

In this study, it was detected that synchrony of alate emergence among colonies of the termite *N. sugioi* with respect to both major flight days and on non-flight days (Fig 5.5), as reflected by the positive correlation in alate emergence between all colony pairs or most colony combinations over both the short and long terms (Table 5.1, Table 5.7). However, given the low correlations observed in some months and inconsistencies in the peaks of alate emergence among colonies on a single day, synchronization in this species appears to be less well defined than that of subterranean termites characterized by pulsed dispersal flights (Nutting 1969; Kusaka and Matsuura 2017). Moreover, the synchronicity of alate emergence among colonies was found to appears to be unrelated to the stage of colony development (colony growth or dispersion) (Table 5.9), although in this regard, environmental conditions such as temperature need to be taken into consideration (Nutting 1969).

It has previously been reported that there is a positive correlation between environmental factors (temperature and relative humidity) and the phenology of the alate flight of *N. sugioi* in the field (Sugio et al. 2018a). However, the main effect of these environmental factors was to enhance alate production within the colony, whereas the influence on flight activity was interpreted as being a sub-effect. In the present study, it was found that temperatures greater than 22°C and a humidity greater than 70% are necessary to promote high flight activity during both the high and low flight seasons (Fig.5.8). Since alates need several days to few weeks to complete ecdysis and maturation (Nutting 1969), our results suggest that both environmental factors function as trigger for flight control. Although both temperature and humidity influence the occurrence of high flight activity, their effect depends on the season. High temperature in particular promotes high flight activity in both low and high seasons, whereas although high humidity promotes high flight activity in the low season, it has the tendency to suppress emergence in the high season. Although, it will be necessary to quantify the environmental factors that enhance or curtail alate release from colonies through controlled experiments under laboratory conditions, our observations at least provide some evidence that environmental conditions of 26.0–29.0°C and 75%–90% humidity are conducive to the flight activity of *N. sugioi*.

During the early flight season, I found that the sex ratio of emerging *N. sugioi* alates in was female biased (Fig. 5.9). Although the males and females of this species have the same body size (Yashiro et al. 2019), during the flight season males might carry less fat than females (West 2009; Chouvenc et al. 2017). Moreover, this protogyny is indicative of an inbreeding avoidance mechanism (Chouvenc et al. 2017), although it could simply reflect a time lag between dispersal and establishment of the different sexes within the colony (Kusaka and Matsuura 2017; Mizumoto et al. 2017). However, studies based on larger sample sizes would be necessary to better characterize this bias. Nevertheless, throughout the entire flight season, a male-biased alate emergence was frequently detected among all experimental colonies (Table 5.9 and Fig. 5.9), which contrasts with the female-biased dispersal flights throughout the flight season that have been reported in the field (Fig. 5.1). As proximity factors, this phenomenon might be explained, at least partially, by the sex-related difference in phototactic response (Cheng et al. 2016) or sex-biased production of alates at the colony and population levels. However, differences in field and laboratory environmental condition would need to be taken into consideration in any future assessments of these factors (Nutting 1969).

Indirect mechanisms for inbreeding avoidance refer to those exhibited up to the formation of de-alates, with the overall effect of decreasing the probability of encounter of individuals of different sex from the same colony. Specifically, these mechanisms can be categorized into the following four types (Aguilera-Olivares et al. 2015): (1) sex-biased production of alates at the colony level (produce one sex in preference to the other), (2) sex-biased emergence timing (one sex emerges from a given colony earlier than the other does), (3) dispersal distance (the chance of encountering a nestmate decreases as the distance flown increases), and (4) sex-biased dispersal (one sex disperses to longer distances than the other).On the basis of my observations, it would appear that *N. sugioi* utilizes at least two mechanisms (i.e., 1 and 2); however, this conclusion will need to be verified in further studies, owing to the small number of samples used in the present study and certain methodological limitations.

In conclusion, the synchronicity of alate emergence among colonies of termites characterized by a continuous period of dispersal flight is less complete and more variable than that of termites with a pulsed dispersal flight. This characteristic alate emergence may indirectly serve to minimize the potential for inbreeding.

5.5 Chapter summary

Neotermes sugioi do not build belowground tunnels and aboveground shelter tubes, so the infection of the trees requires founding a new colony by alates released from infested trees. However, there is little information on the dispersal flight. Here, I report the seasonality of disperser production and the phenology of dispersal flight of *N. sugioi* in the field colonies, and the alates from five isolated colonies under semi-field conditions.

A light-trap survey showed that *N. sugioi* exhibited an extended dispersal flight period from late April to early November, peaking in June, with a female-biased sex ratio. Between 1983 and 2012, the collection of 134 whole colonies of *N. sugioi* from the surrounding area confirmed the presence of alates and pre-alate nymphs within the colonies over seven months, reflecting the extended flight season of this termite species, probably in association with the extended dispersal flight season. The daily number of trapped alates in 1983 was positively correlated with temperature and relative humidity; however, alate production inside the colony was also positively correlated with temperature, relative humidity, and precipitation. Thus, these environmental factors might promote the flight activity of this termite by enhancing alate production inside the colony. Furthermore, temperature also had a significantly positive effect in the model incorporating the density of alates in the colony, along with environmental factors; thus, temperature might facilitate the release of alate from colonies.

A survey of experimental colonies showed that evidence for the synchronicity of alate emergence among colonies of *N. sugioi* based on observations of synchronous patterns on major flight and non-flight days, which showed positive correlations in alate emergence between all colony pairs. However, due to the occurrence of months with small correlation coefficients, and inconsistent alate emergence peaks among colonies on a single day, *N. sugioi* showed less synchronization than subterranean termites characterized by pulsed dispersal flights. Environmental conditions of temperatures and humidities greater than 22°C and 70% were found to be necessary for high flight activity during both high and low flight seasons. In all colonies, the sex ratio of emerging alates during the observation period was significantly biased toward males. Given that adjustment of synchronous and asynchronous alate release among neighboring colonies could reduce the risk of sibling pair mating and inbreeding, the characteristic alate emergence in *N. sugioi* may indirectly minimize the potential for inbreeding.

Chapter 6
Conclusions

Termites are not only known as the major pests of architectural timber and wooden structures such as houses and buildings, but also, they cause damage to living plants such as agricultural crops, trees in forests, and urban and garden trees worldwide. In Japan, some authors have reported termite damage to fruits, urban trees, and garden trees and predict the spread of this damage; however, because of a lack of research, the basic ecology of termites nesting in living trees is not fully understood.

Termite damage to living trees can be broadly divided into two patterns based on their life history: (1) subterranean termites attack tree roots from underground using below-ground tunnels and above-ground shelter tubes extended from colonies, and (2) drywood termite (nested within a limited part of a tree's branches and trunk) alates invade the branches of living trees and expand their nesting gallery toward the roots while damaging the inside of the tree. In Japan, there have been reports of attacks on trees by subterranean termite species, but there have been few reports of damage to trees by drywood termites. The Ryukyu drywood termite *Neotermes sugioi* is a termite that might be a pest for living trees; however, accumulation of basic ecological information is insufficient. Therefore, the purpose of this study was to investigate in detail the damage of this termite to live trees and its ecological characteristics.

In Chapter 2, the results on the population and wood size of *N. sugioi* colonies support and reinforce the results of previous studies on this termite. A colony size of 1,000– 4,000 individuals (2058.2 ± 1695.0 [mean ± SD]) was common and the average length of colony branches was less than 100 cm. Queens and kings were found in the same or nearby nest areas, and more predominantly in the central or root side of nest wood areas. The relative position of reproductives was mainly in the center of the nest or toward the root-side area of the nest in many colonies. Most reproductive pairs were found in the same block area of the colony. Given the range of nest-gallery system in this termite, it will not be difficult to estimate the location of the reproductive center of termite colonies in the field. Therefore, the removal of an entire branch infested with termites by pruning and application of a patch/paint to the pruning scars may be a simple method to manage this species. Moreover, Incipient colonies (i.e., colonies at an early stage of development) were found on 11.3% of branches of *Leucaena leucocephala* that did not show any obvious signs of infestation. The regular pruning of dead branches to enhance the appearance of trees in parks might help to remove small (early-stage) nests of the termites.

A study of the economically valuable tree, Taiwan cherry trees (*Cerasus campanulata*), were conducted to clarify the damage to trees caused by *N. sugioi*, and the ecological characteristics of such damage, in Chapter 3. Damage by *N. sugioi* was confirmed in 21 of the 36 sites surveyed (58.3%) and in 76 of the 1,076 trees surveyed (7.1%). However, damage by Formosan subterranean termites (*Coptotermes formosanus*), a house pest, was only observed at six sites (16.7%), and 10 trees (0.9%), indicating that most damage was caused by *N. sugioi*. Furthermore, the probability of the presence of damaged trees close to other damaged trees was significantly higher than the probability of the presence of damaged trees close to undamaged trees, implying that destructive colonies spread from damaged trees to other trees nearby. Therefore, care for trees in the initial stages of termite invasion seems to be the best method for preventing the spread *N. sugioi* damage to other trees in the same area.

Based on the results of the present study, *N. sugioi* can be called a major pest of Taiwan cherry trees in Okinawa. Furthermore, because damage has been recognized in other representative trees in Okinawa, a more detailed survey of the basic ecology of this species is required. *N. sugioi* is distributed only in the Ryukyu Archipelago, but its detailed distribution was poorly studied. In Chapter 4, the distribution range with special references to soil characteristics of this species in Okinawa Island have been clarified. Termite nests were found at 115 of the 257 sites investigated. The results suggest that 26°25′00″N is the northern limit for this species. However, new zone lacking this species was found in the southern end of the island. The comparison of the termite distribution and the soil distribution suggested that the presence/absence of this species might be related with the soil distribution of Shimajiri mahji and Jahgaru. In addition, there are a total of 29 tree species with the termite nests.

Because this species does not build belowground tunnels and aboveground shelter tubes, so the infection of the trees requires founding a new colony by alates released from infested trees. Therefore, knowledge of dispersed flight is important when considering the control of this species. Then, Chapter 5 described the phenology of this species' dispersal flight. A light-trap survey showed that *N. sugioi* exhibited an extended dispersal flight period from late April to early November, peaking in June, with a female-biased sex ratio. The daily number of trapped alates was positively correlated with temperature and relative humidity; however, alate production inside the colony was also positively correlated with temperature, relative humidity, and precipitation. Thus, these environmental factors might promote the flight activity of this termite by enhancing alate production inside the colony. Furthermore, temperature also had a significantly positive effect in the model incorporating the density of alates in the colony, along with environmental factors; thus, temperature might facilitate the release of alate from colonies.

A survey of experimental colonies showed that evidence for the synchronicity of alate emergence among colonies of *N. sugioi* based on observations of synchronous patterns on major flight and non-flight days, which showed positive correlations in alate emergence between all colony pairs. However, due to the occurrence of months with small correlation coefficients, and inconsistent alate emergence peaks among colonies on a single day, *N. sugioi* showed less synchronization than subterranean termites characterized by pulsed dispersal flights. Environmental conditions of temperatures and humidities greater than 22°C and 70% were found to be necessary for high flight activity during both high and low flight seasons. During the early flight season, it was found that the sex ratio of emerging *N. sugioi* alates in was female biased. Nevertheless, throughout the entire flight season, a male-biased alate emergence w frequently detected among all experimental colonies, which contrasts with the female-biased dispersal flights throughout the flight season that have been reported in the field. As proximity factors, this phenomenon might be explained, at least partially, by the sex-related difference in phototactic response or sex-biased production of alates at the colony and population levels. However, differences in field and laboratory environmental condition would need to be taken into consideration in any future assessments of these factors. Given that adjustment of synchronous and asynchronous alate release among neighboring colonies could reduce the risk of sibling pair mating and inbreeding, the characteristic alate emergence in *N. sugioi* may indirectly minimize the potential for inbreeding.

As a result of this study, a simple method of controlling this species can be proposed. For example, the colonies of *N. sugioi* nesting on tree branches can be detected by periodic inspection of pest control professionals, which probably do not require sophisticated techniques. Removing the small nests that are difficult to detect is probably feasible, along with regular pruning of dead branches. Frequent health checks and management of trees would eliminate termite nests, thereby contributing to keep the value of urban landscapes.

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