Incipient Colony Development and Biology of *Odontotermes formosanus* (Shiraki) and *O. hainanensis* (Light) (Isoptera: Termitidae)

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**ABSTRACT** *Odontotermes formosanus* (Shiraki) and *O. hainanensis* (Light) (Isoptera: Termitidae) are two dominant termite species that damage earthen dikes in China. This paper describes behavioral biology (mating, egg caring, hatching, and molting) and development of incipient colonies of these two species under laboratory conditions. Oviposition of *O. formosanus* and *O. hainanensis* began 8–11 d and 2–5 d after mating, respectively. The eggs of both species were laid in batches. Egg hatching of *O. formosanus* and *O. hainanensis* required assistance from parents or nestmates. Average hatching periods of these two species were 31 d and 27 d, respectively. The molting process of *O. formosanus* and *O. hainanensis* also required help from nestmates. Such assistance was more essential for *O. hainanensis* than for *O. formosanus*. Average developmental times from newly hatched *O. formosanus* to adult workers and to soldiers were 16.7 d and 23.8 d, respectively. For *O. hainanensis*, these times were 18.1 d and 19.6 d, respectively. The survival rate of the incipient colonies of *O. formosanus* at 6 mo after establishment was below 50%. Colony death probably was due to unsuccessful culture of the fungus, *Termitosphaeria duthiei*.

**KEY WORDS** Termitidae, developmental time, caste, culturing, behavior, incipient colony

**Introduction**

Termite tunnels can cause leaks in earthen dikes and dams that can lead to structural collapse (Li et al. 1999). Li & Huang (1991) reported on the relationship between dam collapses and termite tunneling, and several dam collapses in the 1980s and 1990s have been directly attributed to termite infestations (Tian et al. 2008). The main earthen dike-infesting termite species in China include *Odontotermes formosanus* (Shiraki), *O. hainanensis* (Light), *O. fontanellus* Kemner, and *Macrotermes barneyi* Light (Isoptera: Termitidae) (Li et

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al. 1999). Among them, *O. formosanus* and *O. hainanensis* are the most common, causing widespread dike damage in southern China and other southeast Asian countries like Vietnam (Li & Xu 2000) and Thailand (Sornnuwat et al. 2004). Based on the distribution patterns of swarming exits, Li et al. (1983) located *O. formosanus* master nests where queens lived. Careful excavation of several master nests showed that an estimated 15-year-old mature nest of *O. formosanus* could penetrate 1.5–2.0 m into a dike and seriously compromise its stability (Li et al. 1986). The management of dike-infesting termites in China often relies on applications of poisonous baits to kill colonies. The visible growth of the fungus, *Xylaria nigripes* (Klotzsch) Sacc. (Xylariaceae), is also used as an indicator of dead nest locations so that the weakened dike sections can be reinforced (Li et al. 1990).

Understanding the establishment, development, and behavioral biology of incipient termite colonies is important in development of management tactics. These studies depend on successful rearing techniques. For example, the development, caste differentiation, and colony structure of the devastating subterranean termite species *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) has been intensively studied for incipient colonies in culture (Huang & Chen 1983a,b, 1984, Waller & La Fage 1988, Li et al. 1995, Fei & Henderson 2003). Pan et al. (1990) and Qian et al. (2005) reported on the culturing methods for *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae) and *Cryptotermes domesticus* (Haviland) (Isoptera: Kalotermitidae), respectively. However, studies on incipient colonies of *O. formosanus* and *O. hainanensis* are rare. The only published study was conducted by Liu et al. (1995) on *O. formosanus*, where three laboratory culture methods for incipient colonies were evaluated. Environmental conditions for long-term rearing of incipient colonies of *O. formosanus* were also described in the study by Liu et al. (1995). No studies on incipient colonies of *O. hainanensis* have been published.

In this paper, we describe culturing methods for incipient colonies of *O. formosanus* and *O. hainanensis*. Observations of nesting and mating behaviors, oviposition and egg hatching, molting, and caste differentiation also are reported. Factors that can affect survival and behavioral activities are discussed. This work provides a scientific basis for developing new and effective prevention and/or remedial tactics against dike-infesting termites.

### Materials and Methods

**Source of alates.** *Odontotermes formosanus* was collected in April 1979 from the Shatian Reservoir in the suburbs of Guangzhou, Guangdong, China. *Odontotermes hainanensis* was collected in the summer of 1983 and 1984 from the Zhanjiang Guantian Reservoir, Hainan, China. The collections were made by locating and excavating nests during swarming seasons. Collected alates were placed in wooden boxes (350L × 240W × 300H mm) that contained two layers of moist tissue paper and were transferred to the laboratory immediately.

**Establishment of incipient colonies.** Humus-rich yellow clay collected from around earthen dikes was used as a culture medium. Water was added to the clay before putting it into rearing containers/arenas. Initially, two sizes of test tubes (9 tubes were 40D × 200L mm; 101 tubes were 25D × 75L mm) and glass-plate arenas were used for *O. formosanus* rearing. The yellow-clay culture
medium was put into each test tube to about 80% of its volume. Glass-plate arenas were used for better observation of mating behavior. Each arena consisted of two glass plates (150L × 120W mm) stacked together with 6-mm space between them that was filled with the yellow-clay culture medium. Rearing arenas were surrounded by fine moistened sand to maintain a moderate humidity. A single pair of dealates was introduced into each test tube or glass-plate arena. A total of 135 pairs (110 pairs in test tubes and 25 pairs in glass-plate arenas) were set up for the tests with *O. formosanus*. For continuous rearing and observation, 60 test-tube pairs were transferred with their tubes to larger containers one week after establishment. For this, 25 pairs were put individually into glass cylinders (100D × 200L mm), 25 pairs were put individually into small glass boxes (270L × 220W × 150H mm), and 10 pairs were put together into one large glass box (1000L × 1000W × 600H mm) where individual pairs could move freely. Termite pairs that were initiated in the glass-plate arenas did not require transfer.

*Odontotermes hainanensis* was cultured in Petri dishes (180D × 28H mm) or glass-plate arenas as described above with moist tissue paper and a piece of water-filled sponge to maintain moisture. A total of 138 pairs were set up for this species.

**Rearing conditions and food sources.** The incipient colonies of *O. formosanus* were cultured in darkness. The average temperatures were as follows: 25.5°C before oviposition, 25.7°C from egg-laying to hatching of the first batch, and 27.3°C during the development period of workers/soldiers. The incipient colonies of *O. hainanensis* were cultured at a room temperature range of 25–32°C. The culture medium was kept at a moderate humidity (clay could formed into a ball when squeezed, but without excess water). Water was added every 8–10 d to keep the sand and the tissue paper moistened. Food sources for both species included *Eucalyptus* bark, fresh fungal gardens of *Termitosphaeria duthiei* (Berk.) Ciferri, pine pollen, cellulose powder, pine wood strips, corn cobs, and bagasse, provided simultaneously. Food was provided as workers appeared in the colonies and started to build mud tubes outward to forage.

**Data collection.** Observations of termite behaviors, oviposition, and colony activities were made four times a day prior to oviposition, twice a day during the peak of oviposition, and once a day toward the end of the oviposition period. All observations were made using a stereo microscope (5×) from the underside of royal chambers.

**Results and Discussion**

*Odontotermes formosanus* - nesting behavior. After dealate pairs were placed into the culture containers, they typically moved quickly on the surface of the clay medium. Upon finding an acceptable spot, they dug and built a sealed chamber. The majority of the pairs took about 30 min to finish building the chamber, which they sealed by piling up soil particles into small cone-shaped mounds at the entrance. In the glass-plate arenas, the 6-mm spacing formed man-made cavities that the pairs settled into directly and sealed with soil in 3–4 d.

*Odontotermes formosanus* - mating behavior. During the first 2–3 d in the chambers, the pairs rarely moved. Then they started to mate, which
generally took place from 9:00 pm to 10:00 pm. The mating process began with mutual grooming or with the pair chasing each other in a circle for about 2 min. During copulation, both sexes stretched out all six legs and raised their abdomens so that they touched at the tips. Copulations lasted about 1–2 min with slight contractions and vibrations being observed. At the end of copulation, the male groomed the female’s reproductive aperture and/or other body parts and chased the female for about 1 min before separating.

**Odontotermes formosanus - oviposition.** Before the onset of oviposition, the male tended to groom the female’s abdomen or head frequently, while the female stayed still with her head down, her thorax lifted slightly, and her swollen abdomen raised. Next, the female’s body started to contract and vibrate continuously. The female then extended her thorax and slowly bent the tip of her abdomen with strong up and down contractions and vibrations to discharge the eggs gradually. Some females moved around dragging eggs that had not yet been fully discharged. Eggs adhered to the tip of an abdomen for up to 2 h, and they usually fell off upon touching the wall of the culture containers and were not detached by males or workers. After the eggs were fully separated from the females, males tended to groom the tip of the females’ abdomens repeatedly. Then the males gathered the eggs and piled them up.

Based on observations of 21 colonies maintained in the glass-plate arenas, females started to lay eggs 8–11 d after mating. The first oviposition period lasted 62–65 d with the average length of 64 d. Females produced 60–103 eggs with an average of 84.3 eggs per female. The maximum number of eggs laid per female per day varied between 10 and 31. However, the average daily number of eggs laid within a colony ranged from 0.92 to 1.58 eggs per female, with a mean of 1.32 eggs per female per day. The oviposition process for incipient colonies of *O. formosanus* was slow and at a non-uniform pace, as eggs were laid in several batches. The first batch of eggs was laid within the first 9 d of the oviposition period with a yield of 28–46 eggs per female (average 36 eggs). After about a 7-day rest period, a second batch of eggs was laid at a slower pace. The number of eggs laid increased rapidly after soldiers appeared within a colony.

**Odontotermes formosanus - egg caring and hatching assistance.** The egg stage of *O. formosanus* lasted 32–36 d. The hatching period (the time period from the first egg hatch to the last egg hatch) lasted 30–32 d with average of 31 d. Kings, queens, and/or nestmates continuously tended egg piles and provided assistance in the hatching of the eggs. Several days before hatching, the parents groomed the eggs frequently and moved them away from the egg piles. During this time, the eggs enlarged gradually, the eggshells became thinner, and their color changed from beige to light whitish-yellow then becoming more transparent. When an egg was near hatching, the eggshell first split at one end. The other end of the egg was held by the mouthparts of a king, a queen, or a worker, who used their maxillary palps to constantly comb the egg with a motion directed from the split end to the non-split end. After a while, a milky-white newly hatched larva could be seen emerging. The parents or workers then began to groom the larva until its head and appendages extended from the egg cuticle. In the process, they would eat or remove the eggshell that might be still attached to the abdomen of the larva.

The total number of newly hatched termite larvae per colony ranged from 46 to 70, with an average of 56.8 new larvae. The maximum number of eggs hatching
per day per colony varied between 5 and 9. However, the average daily number of eggs hatching per colony ranged from 1.4 to 2.1, with the mean of 1.7 new termites per day. The young larvae limited their activity inside the nests and were frequently groomed by the king and queen. The king and queen also cleared un-hatched eggs and fed the larvae when they were too young to feed on their own.

*Odontotermes formosanus* - molting and differentiation of workers and soldiers. Like egg hatching, molting also required assistance from parents or nestmates. The first, second, and third molts were helped by kings, queens, or workers; while the fourth and fifth molts were assisted mainly by workers. The early instars had great difficulty in molting without the help from their parents or workers, while the older larvae (4th and 5th instar) were better able to remove their old cuticles; however, the process was much easier with help from workers.

Larvae differed little in appearance during development. The duration from egg hatching to differentiation into adult workers was 14–19 d, with an average of 16.7 d. The adult workers started to leave nests to build mud tubes outward and feed on their own 13–16 d after differentiation. The developmental time from newly hatched larvae to soldiers was 20–26 d with average of 23.8 d. There also was a pre-soldier stage, in which individuals had white heads, before termites fully differentiated into soldiers. The pre-soldier stage lasted 8.5 d on average.

*Odontotermes formosanus* - survival of incipient colonies and colony development. After 6 mo of establishment, the survival rate of incipient colonies was 36% in the glass cylinders and 40% in the small glass boxes, based on a survey of 25 colonies each. A colony was considered dead when either the king or queen was found dead, as queens cannot produce eggs continuously or establish a colony after the king has died. However, the ‘dead’ colonies could be re-established by replacing the dead king or queen. Glass cylinders (100D × 100H mm) with two layers of filter paper on the bottom were used for re-establishment. Before the new reproductive pair was transferred, some yellow clay was put on the filter paper, a small cavity was made in the clay to receive the pair, and food (all types mentioned above) was provided. The behavior of these new pairs in digging holes, building chambers, copulating, and laying viable eggs was normal. The workers in re-established colonies also built mud tubes to forage. However, as expected, the survival rate of the re-established colonies was lower (22.2% after 68 d) than that of the undisturbed incipient colonies.

The number of individuals and their castes in six randomly selected colonies that had been cultured for 90 d were examined (Table 1). Among the colonies, four had a single pair of reproductives, one had one king and two queens, and one had two pairs of reproductives. For the four colonies with a single pair of reproductives, eggs were the most abundant life stage, ranging from 49 to 71 (average of 57.0) (Table 1). The number of workers ranged from 29 to 45 (average of 35.8) in the single-pair colonies. There were an average of 7.5 soldiers and 8.0 newly hatched larvae in each colony. The proportion of soldiers ranged from 12.5% to 17.3% of the colony population. In addition, more individuals and eggs were present in the two colonies with two queens than in those colonies with a single pair of reproductives (Table 1).
The kings and queens from incipient colonies fed little until about 15 d after the appearance of workers when they were ready to forage. Foraging workers would tear pieces of Eucalyptus bark (the preferred food) into small pieces and bring them back to the nest where they piled them up for food storage and for growing *Termitosphaeria duthiei*. Pine pollen and fresh fungal garden of *T. duthiei* also were favorite foods for incipient colonies. When workers encountered a small piece of fungal culture in mud tubes, they immediately protected it with mud and took it back to the nest (chamber). Colony survivorship seemed closely related to establishing fungal gardens of *T. duthiei*. For many colonies, a great number of mud sheets and mud tubes appeared inside the rearing containers before mid-October (about 6 mo after establishment), when the food was consumed quickly. Fewer new mud sheets or mud tubes were formed after mid-October and the number of termites and eggs in the colonies dropped dramatically, which was often followed by the death of the whole colony. The exact reason for this sudden colony collapse is unclear. According to Liu (1995), an incipient colony of *O. formosanus* could only survive and develop over a long period of time with a successful *T. duthiei* culture. After examining the nests of the 50 dead colonies in the glass cylinders and small glass boxes, we found indeed that fresh *T. duthiei* gardens had not successfully established in these nests. Additionally, only one out of ten colonies established in the large glass box survived for more than 6 mo and had live *T. duthiei* in its nest. Thus, the sudden death of incipient colonies appeared to be related directly to the unsuccessful inoculation of *T. duthiei*.

**Odontotermes hainanensis** - mating behavior. Unlike *O. formosanus*, mating of *O. hainanensis* took place before nesting behavior. After alate pairs were placed into the culture containers, the male typically chased the female. If the male and female lost contact, the female would stop moving and raise and vibrate her abdomen frequently. The male would move rapidly in search of the female and touch her abdomen with his mouthparts. After they reconnected, the two continued to stay in tandem and eventually began to copulate. During the chase and copulation, the termites dropped their wings, and afterwards they sought an appropriate place to nest.

**Odontotermes hainanensis** - nesting behavior. When an appropriate nesting location was found, the male and female dug a hole together for about
15 min. The excavated soil was often used to block the entrance by forming a small cone-shaped mound with a typical size of approximately $30 \times 20 \text{ mm}$ (bottom diameter \times height). The dimensions of an initial nesting chamber were about $13L \times 10W \times 7H \text{ mm}$ on average either in glass-plate arenas or in Petri dishes. When building the initial nesting chamber, the male and the female sometimes abandoned the first hole and dug another one elsewhere.

**Odontotermes hainanensis** - oviposition. The eggs of *O. hainanensis* were oval and light beige. Oviposition started 2–5 d after parents built nests. First, the female moved around in search of the right place to lay eggs. During egg laying, she stayed still with her six legs stretched out and her abdomen slightly contracted. After the eggs were laid, the male held them with his mouthparts and frequently groomed them. Often, when the male put the eggs down, the female would pick them up and groom them too. Without the help of the male, the eggs would often adhere to the outside of the genital orifice of the female. After detaching the eggs, the male often groomed the female genital orifice before carrying the eggs with his mouthparts and piling them up.

The oviposition rate of seven colonies of *O. hainanensis* for a 45-day period is presented in Table 2. An average of 92.7 eggs was observed with the range of 76–136 eggs per colony. During the first 8 d of the oviposition period, the colonies produced an average of 28.6 eggs, which was significantly more eggs produced than for later periods. For the next 8 d an average of only 11.4 eggs were produced. However, from the 17th d to the 24th d, the average oviposition rate increased to 19.3 eggs. After that, the average production decreased slightly through the final 21 d of the oviposition period (Table 2). The fluctuation in egg laying suggests that the ovum inside the ovary of the queens in incipient colonies of *O. hainanensis* matures in batches.

**Odontotermes hainanensis** - egg care and hatching assistance. Once the eggs were laid, the male and the female often held and groomed them with their mouthparts. Then they piled the eggs up at a protected spot in the nesting chamber. If the egg pile was disturbed, the parents would pick up the scattered eggs and pile them up again. After being groomed by the parents, the surfaces of the eggs became wet and shiny.

### Table 2. Egg production of seven incipient colonies of *Odontotermes hainanensis* cultured for 45 d.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Days after pairing</th>
<th>Total number of eggs</th>
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<tr>
<td></td>
<td>1–8</td>
<td>9–16</td>
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<tr>
<td>1</td>
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<tr>
<td>7</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Average</td>
<td>28.6</td>
<td>11.4</td>
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</table>
The hatching period lasted 25–28 d with an average of 27 d. The male and the female separated the eggs from an egg pile prior to hatching. Before hatching, the eggs greatly increased in size making the eggshells much thinner, whiter, and more transparent. Appendages (antennae and legs) were visible through the eggshell. Then a slit appeared on one side of a hatching egg. During this stage, the male and the female held the eggs in their mouthparts frequently. Once the eggshell split open, the parents would immediately hold the egg in their mouthparts, and then set it down. This movement exposed parts of the larva by further splitting the eggshell. Then, either the male or the female would hold the protruding body parts in their mouthparts, while the other parent would pull the eggshell in the opposite direction so that the whole body of the larva could be freed from the eggshell successfully. The eggshell was then consumed by the male and the female. Our observation revealed that the hatching process required assistance of others in order to be successful. Newly hatched, transparent, and soft larvae tended to lie on their sides with their legs and antennae not yet extended. The male and the female then frequently groomed the larva until its appendages extended and were able to move. This process took about 15 min. If workers were present in a colony, they would assist the male and the female in the egg caring and hatching processes. As the number of workers in a nest increased, these tasks were done entirely by workers.

**Odontotermes hainanensis** - molting and differentiation of workers and soldiers. Like egg hatching, molting of larvae also required the assistance of adult termites. Without assistance, larvae regardless of age would die during molting. When molting, the larva’s cuticle first turned grey and the abdomen tip swelled like a vesicle. Before molting, the larva would lie on its side waiting for the male, female, or another nestmate to pick it up using their mouthparts and set it down repeatedly until the cuticle split at the head. After that, the parents/workers kept grooming slowly to free the head, thorax, and abdomen from the old cuticle. Once the old cuticle was shed down to the abdomen tip, the helpers tore it off and consumed it. The whole molting process took about 25–35 min, including 5–15 min of grooming for the newly molted larva to fully extend its appendages and move freely again. Early stage molting (1st to 3rd instar) took longer than the later stage molting (4th and 5th instar). The color of newly molted immatures was transparent white.

The larval stage of workers lasted 15–20 d with an average of 18.1 d. Solider formation required an average of 13.3 d (range 9–16 d) from a newly hatched larva to a pre-soldier. The developmental time from pre-soldiers to soldiers was 4–9 d (average 6.3 d). Thus, workers and soldiers of *O. hainanensis* appeared in a colony at about the same time, which was different from *O. formosanus*. Caste composition and colony survivorship were not recorded for this species.

Our observations indicated that the development processes for incipient colonies of *O. formosanus* and *O. hainanensis* were similar, but the developmental rate of *O. formosanus* was slower than that of *O. hainanensis*. Both species exhibited complex and elaborate social behaviors, including parental care, which is characteristic of the family Termitidae, one of the most evolved and diverse families in the Order Isoptera (Ohkuma et al. 2004). Their kings, queens, and workers always offered assistance to eggs and immatures in hatching and molting processes. Additional observation showed that *O. hainanensis* showed no
colony discrimination as eggs from other colonies received the same good care. Such elaborate social ability might be an important reason that *O. formosanus* and *O. hainanensis* can develop huge colonies and become destructive pests. The knowledge of incipient colony development provides sound scientific basis for the management of these two dike-infesting termite species.

References Cited


