Structure of Spermatheca, Sperm Dynamics, and Associated Bacteria in Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

Author(s): Ashok Raina, Charles Murphy, Chistopher Florane, Kelley Williams, Yong Ihl Park, and Bruce Ingber
Source: Annals of the Entomological Society of America, 100(3):418-424.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0013-8746(2007)100[418:SOSSDA]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1603/0013-8746%282007%29100%5B418%3ASOSSDA%5D2.0.CO%3B2

Structure of Spermatheca, Sperm Dynamics, and Associated Bacteria in Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

ASHOK RAINA,[1,2] CHARLES MURPHY,[3] CHISTOPHER FLORANE,[1] KELLEY WILLIAMS,[4] YONG IHL PARK,[1] AND BRUCE INGBER[5]

Ann. Entomol. Soc. Am. 100(3): 418–424 (2007)

ABSTRACT Primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), complete their first reproductive cycle in ≈60 d after nest formation. During this period, the pairs mate several times. The spherical, aflagellate sperm, after transfer by the male, are stored in the female's spermatheca. Sperm numbers in the spermatheca increase significantly between day 20 and 40, and thereafter they show a steep decline, indicating that the pairs may not be mating after day 40. The spermatheca is bean shaped with an extremely narrow duct. The thick wall of the spermatheca consists mainly of type 3 cells made up of secretory and duct cells. Cuticle-lined ducts are interspersed throughout these cells. Finger-like extensions of the cuticle-lined interior wall project into the spermathecal lumen. The secretory cells presumably provide nutrition for the sperm during their long storage. Eleven anaerobic and six aerobic species of bacteria were cultured and identified from the spermatheca. The role of these bacteria is unknown.

KEY WORDS primary reproductives, mating, oviposition, ultrastructure, bacteria

Colony formation in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), a major urban pest, is initiated by a primary reproductive pair soon after swarming. After losing their wings, the alates form tandem pairs, search for a suitable nesting site and form a nuptial chamber. Under laboratory conditions, the pairs were reported to mate after 1 d (Raina et al. 2003). The tail-to-tail mating lasted an average of 28 s, and the pairs were observed to mate repeatedly, with as many as three matings in a 24-h period. Egg laying started 2 d after the first mating. Approximately 40 eggs were laid during the first oviposition cycle that lasted for >2 mo. Significantly fewer progeny were produced if the males were removed after the first mating (Raina et al. 2003). Sperm transferred to the female during mating is stored in the spermatheca and used to fertilize eggs just before oviposition.

Although much information has been published on the structure of spermatheca in social insects (Weesner 1969, Wheeler and Krutzsch 1994, Pabalan et al. 1996, Martins and Serrão 2002, Costa-Leonardo and Patricio 2005), very little is known about its ultrastructure. In addition, no details are available about this structure in *C. formosanus.* Costa-Leonardo and Patricio (2005) studied the structure of spermatheca in specimens of five termite families, including *Reticulitermes virginicus* (Banks) and *Coptotermes gestroi* (Wasmann) in the Rhinotermitidae. The spermatheca in most termite species has been described as a finger-like structure with a recurved tip. With no spermathecal duct present, the spermatheca opens directly into the roof of the genital chamber (Weesner 1969, Costa-Leonardo and Patricio 2005). Mature sperm in termites, in particular the Rhinotermitidae, are spherical without the characteristic tail and have a very simple structure (Grassé 1949, Baccetti 1972, Baccetti et al. 1974, Jamieson 1987). However, limited information about the numbers of sperm was reported only for *Reticulitermes hesperus* (Banks) (Weesner 1956).

Here, we describe the detailed structure of the spermatheca in primary reproductive females of *C. formosanus* and report changes in the number of sperm as they relate to other parameters during the first oviposition cycle. We also report the presence of several types of bacteria found in association with sperm in the spermatheca.

Materials and Methods

Insects. Alates of *C. formosanus* were collected in light traps after swarming during May 2003 in New Orleans, LA. Naturally dealated individuals

Mention of a trademark or a propriety product does not constitute an endorsement of the product by USDA to the exclusion of other products that may also be suitable.

[1] Formosan Subterranean Termite Research Unit, USDA–ARS, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

[2] Corresponding author, e-mail: araina@srrc.ars.usda.gov.

[3] Soybean Genomics and Improvement Laboratory, USDA–ARS, Beltsville, MD 20705.

[4] Commodity Utilization Research Unit, USDA–ARS, New Orleans, LA 70124.

[5] Cotton Structure Quality Research Unit, USDA–ARS, New Orleans, LA 70124.

were sexed, and 100 single pairs in total were set up in petri dishes containing agar–sawdust mixture as reported previously (Raina et al. 2003).

Fecundity and Sperm Numbers. Five reproductive pairs were randomly selected at 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, and 80 d after the initial pairing. After counting the number of eggs laid, each female was dissected to determine the number of mature oocytes. The spermatheca was removed in phosphate-buffered saline (PBS; 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4). Its size (maximum width and length) was measured using a stereomicroscope with a micrometer, and then it was transferred to 100 μl of PBS in a 200-μl Eppendorf tube. The spermatheca was broken up using a Sonic Dismembrator 60 (Fisher, Pittsburgh, PA), and the sperm were counted in a sperm-counting chamber (Hausser Scientific, Horsham, PA). Comparisons for area of the spermatheca and sperm numbers were performed using one-way analysis of variance (ANOVA) and Dunnett's posttest by using the observations recorded on day 2 as control (GraphPad Prism version 4.02, GraphPad Software Inc., San Diego, CA).

Electronmicroscopy. Spermathecae were dissected from 20-d-old mated females and immediately fixed in Karnovsky fixative for 1 d. The tissues were washed in three to four changes of 0.05 M phosphate buffer, postfixed in buffered 2% osmium tetroxide for 2 h, dehydrated in a graded ethanol series, and infiltrated with and embedded in Spurr's low-viscosity medium. Sections (1 μm in thickness) were stained with methylene blue and examined under the light microscope. Ultrathin sections were cut on a Reichert/AO Ultracut (Leica Microsystems, Deerfield, IL) microtome with a diamond knife. The sections were stained with 2.5% uranyl acetate for 30 min, followed by 3% lead citrate for 5 min. Sections were viewed in a Hitachi H-7000 (Hitachi, Tokyo, Japan) transmission electron microscope.

Bacterial Characterization. Three excised spermathecae were placed in 3 ml of BIOLOG (Biolog Inc., Haywood, CA) anaerobic inoculating fluid and homogenized using two methods: a Glass-Col tissue grinder (Kontes, Terra Haute, IN) and a Sonic Dismembrator (Fisher). The homogenate was diluted 1:1000 (to obtain distinct colonies for the purpose of identification) and plated on BIOLOG Universal Anaerobic (BUA) prepoured agar plates. For anaerobes, the inoculated BUA plates were placed in anaerobe pouches and incubated at 30°C. Homogenization, dilution, and plating were completed within 5 min to preserve anaerobic conditions. For the aerobes, spermathecae were homogenized as described above using BIOLOG inoculating fluid and BIOLOG Universal Medium (BUG) plus 5% sheep's blood. Both anaerobic and aerobic plates were checked at 24-h intervals for growth. Isolated pure colonies were placed in a sterile solution of 40% glycerol in deionized water for storage at −81°C until identifications were made. All anaerobic cultures were analyzed for the following characteristics; cell morphology, colony color, colony margin, colony elevation, gram stain, KOH reaction, catalase reaction, oxidase reaction, and hemolysis. All gram-negative aerobic rods were analyzed for triple sugar iron reaction to identify any enteric bacteria. All isolates were identified using a suspension of pure culture in BIOLOG inoculating fluid to a transmittance percentage required by BIOLOG specifications; gram-negative nonenteric 52% T, gram-negative enteric 61% T, gram-positive rods 20% T, gram-positive spore forming rods 28% T, anaerobes 65% T. The culture suspensions were used to inoculate BIOLOG microplates and allowed to incubate at 30°C for 24 h. Readings were taken on the microplates by using the MICROLOG reader with reference databases (BIOLOG version 4.01C, Biolog Inc. 2002), for each colony morphology. All tests were replicated three times.

Table 1. Measurements of fecundity and sperm numbers in female primary reproductives of *C. formosanus* during the first oviposition cycle

Age (d)	Eggs laid[a]	Mature oocytes	Spermatheca (width by length, μm)	No. sperm[b]
2	0.0	4.4	116 by 272	3,250
4	1.8	5.6	124 by 324	4,950
6	8.0	5.4	136 by 332*	4,000
8	13.2	4.2	128 by 316	5,600
10	15.3	3.6	132 by 300	4,120
20	24.8	3.0	128 by 304	14,700*
30	31.0	2.8	128 by 288	11,900*
40	35.0	2.2	124 by 304	10,600*
50	43.2	1.6	124 by 292	3,400
60	42.4	2.8	120 by 328	1,640
70	43.8	1.2	120 by 296	2,120
80	43.2	0.6	104 by 320	1,390

* Statistically significant ($P < 0.01$) compared with day 2.
[a] Cumulative average of five females paired individually with males.
[b] Sperm counts are averages of two hemacytometer fields from each of the five females.

Results

Reproductive Parameters. Most of the mortality (11%) among the reproductive pairs occurred during the first week after setting up the pairs. Five more pairs died by the end of the experiment. Egg laying in most cases commenced on the third day. Maximal eggs (per female per day) were laid between day 4 and 6 (Table 1). Total cumulative number of eggs laid peaked on day 50 and leveled thereafter. As the females aged, the number of mature oocytes decreased until it was <1 in 80-d-old females. The spermatheca was the smallest (0.0315 mm^2) in a 2-d-old female. Overall, there was a significant change in size of the spermatheca over 80 d ($P = 0.0177$). Although it increased in size, the change was only significant for 6-d-old females (0.0452 mm^2, $P < 0.01$). In 80-d-old females, the spermatheca remained long, but became narrower (Table 1). The average number of sperm in the spermatheca of 2-d-old females was 3,250 and remained low up until day 10. There was a highly significant difference in sperm number over the course of 80 d ($P < 0.001$). Between days 10 and 20, sperm number increased significantly ($P < 0.01$) and remained high until after day 40, when

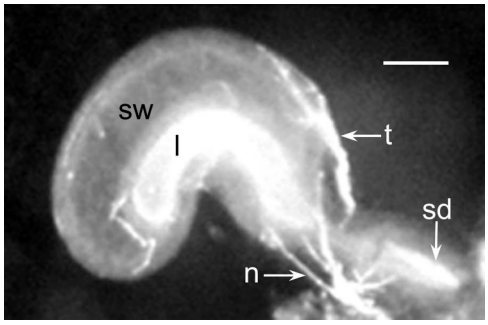

Fig. 1. Whole mount of the spermatheca from a 20-d-old mated *C. formosanus* primary reproductive female. Lumen (l), nerves (n), spermathecal duct (sd), wall of the spermatheca (sw), trachea (t). Scale bar = 50 μm.

sperm number dramatically decreased. No significant difference existed between day 2 observations and days 50–80. Only 1,390 sperm were observed in an 80-d-old female.

Structure of Spermatheca. The bean-shaped spermatheca in 20-d-old mated females were ≈130 by 300 μm (Fig. 1). The spermatheca opens into the dorsal area of the genital chamber through a short cuticle-lined duct. The spermatheca is amply supplied with fine trachea and innervated by nerves from the terminal abdominal ganglion. In a longitudinal section, the spermatheca is seen to have a thick cellular wall surrounding a lumen that continues proximally into a narrow duct enclosed by thick cuticle (Fig. 2). The lumen is divided into a large distal and a smaller proximal chamber by a constriction in the wall. The inner wall of the spermatheca has a thin cuticular layer from which finger like extensions project into the lumen. The lumen contains the sperm together with numerous bacteria.

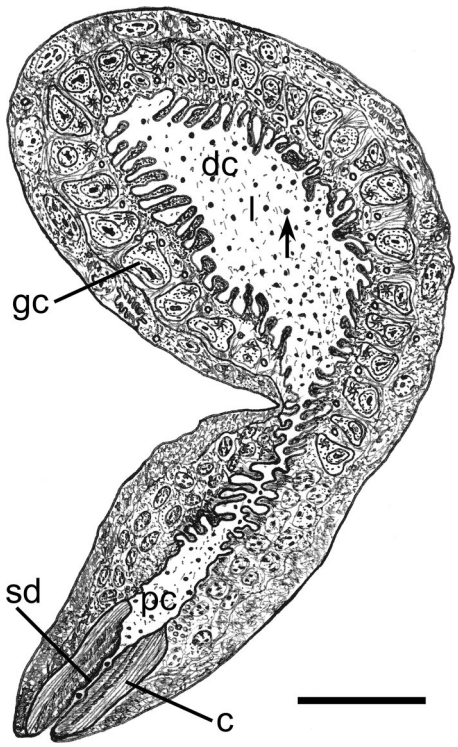

Fig. 2. Drawing from a longitudinal section of the spermatheca. The spermathecal wall consists of a prominent layer of glandular cells (gc). The lumen (l) shows the distal (dc) and proximal (pc) chambers with the latter leading into the spermathecal duct (sd) enclosed by thick cuticle (c). Scale bar = 50 μm.

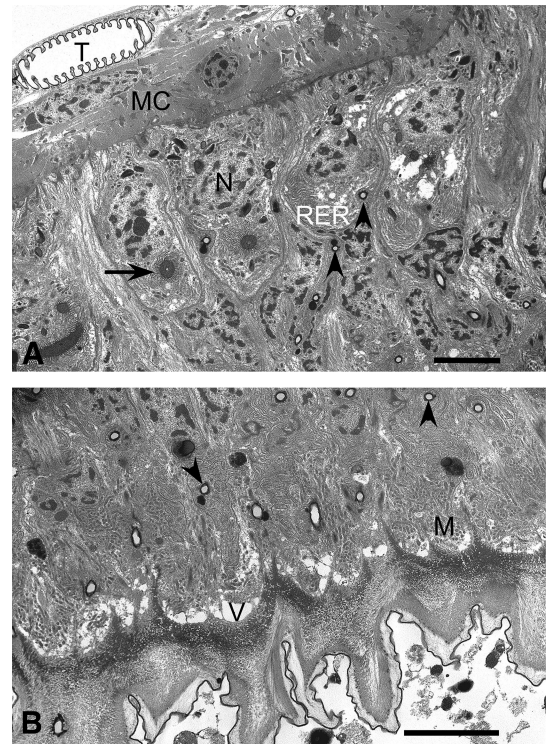

Fig. 3. (A and B) Thin sections through the wall of the spermatheca. Outer portion of the wall (A) showing a layer of muscle cells (mc) embedded with trachea (t) covering the layer of glandular cells with large apical nuclei (n), a whirl of microvilli surrounding the tubular receiving canal (arrow) and stacks of rough endoplasmic reticulum (rer). There are also many ducts (arrowheads) lined with cuticle. The inner portion of the wall (B) has a layer of columnar cells interspersed with ducts (arrowheads). The cells have apical nuclei (n) numerous mitochondria (m). A layer of large vesicles (v) separates these cells from the inner layer of endocuticular fibers with projections extending into the lumen. Scale bars = 5 μm.

At the ultrastructural level, the spermathecal wall is thick (40–45 μm) and has four distinct regions. On the outside is a thin layer of muscle cells interspersed with trachea (Fig. 3A). Below this lies a single layer of glandular cells with large apical nuclei. The basal portion of these cells has a whirl of microvilli surrounding a circular body containing dense granular material with a clear central core. The cells also contain stacks of rough endoplasmic reticulum and mitochondria.

Interspersed between these secretory cells are duct cells. Below the glandular area and toward the lumen lies another layer of cells (Fig. 3B). These cells have highly convoluted cell walls and contain large numbers of mitochondria, particularly in the basal region. Between these cells are symmetrically placed ducts (0.4 μm in diameter), the walls of which are lined with cuticle. Beneath these cells is a layer of large vacuoles. The cuticle lining the lumen forms finger-like projections that extend into the cavity of the spermatheca. The endocuticular lamina within these projections is composed of arced microfibrils of chitin. In a mated female, the lumen is full of spherical sperm (Fig. 4A) and large numbers of bacteria. The proximal smaller chamber leads into a narrow duct (0.15 μm in diameter) surrounded by a thick cuticular sheath that may have as many as 16 layers (Fig. 4B). The cells surrounding the cuticular sheath do not have characteristics of glandular cells, and there are no ducts in this region. The wall of the spermathecal duct, although very narrow, must be flexible enough to permit the passage of sperm into the spermatheca, after mating, and their passage outward (Fig. 5A), during fertilization of the eggs.

The sperm are almost spherical, ≈2 μm in diameter, and lack a flagellum (Fig. 5B). The sperm seem to be a nuclear mass surrounded by a thin layer of cytoplasm with a short knob-like projection on one side. Sometimes three to four translucent areas show up in the body of the nucleus.

Bacteria Cultured from Spermatheca. Spermatheca dissected from females and examined under the microscope always revealed large numbers of bacteria together with sperm. When cultured, bacterial colony counts for both anaerobic and aerobic serial dilutions ranged from 1 to 1,000 colony-forming units per ml. No counts were made on the plated spermatheca. Based on their characteristics and properties, a total of 11 anaerobic and six aerobic bacterial species were identified. One enteric organism was present and identified as *Serratia marcescens* Bizio. The anaerobic isolates were facultative anaerobes with *Actinomyces*, *Gemella*, and *Propionibacterium* species being predominant. The aerobic organisms identified were less diverse than the anaerobic population. Their population was dominated by two species each from two genera: *Aureobacterium* and *Tsukamurella.*

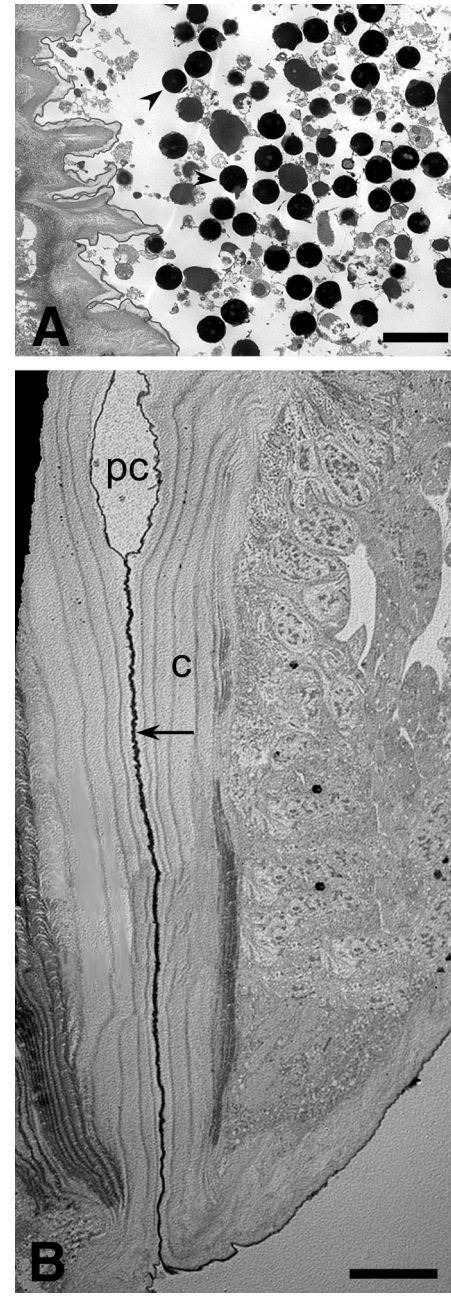

Fig. 4. (A) Lumen of the spermatheca is filled with sperm (arrowheads) and large numbers of bacteria. (B) The proximal chamber (pc) is connected to the outside by a narrow duct (arrow) sheathed in thick cuticle (c). Scale bars = 5 μm (A) and 10 μm (B).

Discussion

Incipient colonies of *C. formosanus*, during the first 2 yr of their life are known to go through three to four oviposition cycles with intervening periods of reproductive inactivity (King 1971, Higa 1981, Raina et al. 2003). Under laboratory conditions, the first one of these cycles is completed in ≈2 mo. The mean number of eggs laid during this period, in our study, was slightly higher than reported previously. Although, based on the presence of sperm in the spermatheca, it had been suggested that the first mating occurs within 24 h (Higa 1981), a detailed record of matings was provided by Raina et al. (2003). In the absence of any external genitalia in the male, the tail-to-tail mating lasted an average of 28 s. Because of the lack of a flagellum the sperm in most termite species is nonmotile (Grassé 1949, Weesner 1969). The sperm most likely is deposited in the genital chamber and transferred to the spermatheca through muscular contractions of the latter. Weesner (1969) suggested that in

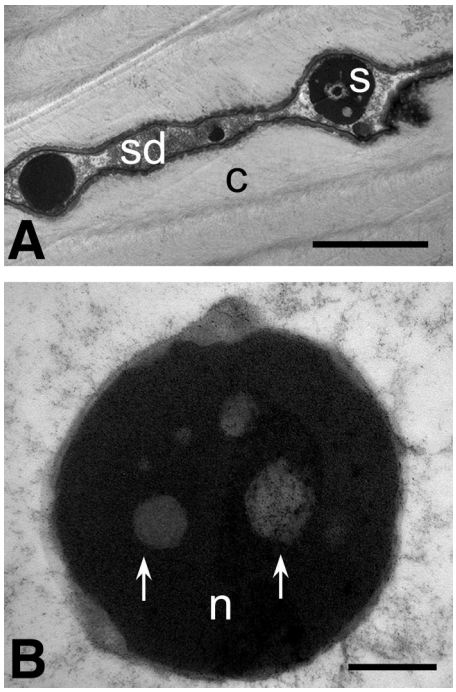

Fig. 5. Spermathecal duct (A). Sperm (s) passing down the spermathecal duct (sd). The latter is enclosed by thick cuticle (c). A single mature sperm (B). The dense nucleus (n) has few translucent areas (arrows). Scale bars = 4 μm (A) and 0.5 μm (B).

the Rhinotermitidae, there is a strongly chitinized arch in the roof of the genital chamber called the spermathecal furrow. The presence of such a furrow would seem to aid in the transfer of sperm from the genital chamber to the spermatheca. Weesner (1956) also suggested that the movement of nonflagellate and immotile sperm in *R. hesperus* may be caused by regular contractions of longitudinal muscle fibers. During the first 10 d, there were relatively low numbers of sperm in the spermatheca of mated females, even though it is expected that the females may have mated several times (Raina et al. 2003). A significant increase in the number of sperm on day 20 (could actually be between day 10 and 20), although supporting the possibility of additional matings, also may indicate that it took that long to physically move a majority of the sperm from the genital chamber to the spermatheca. Again a significant drop in the number of sperm on day 50, and their continued low numbers thereafter, indicates that the pairs may have stopped mating after day 40 and thereafter use the sperm already present in the spermatheca to fertilize the remaining eggs. After the first oviposition cycle is completed, the pairs go into a mode of sexual inactivity when even the reproductive organs are regressed (A.R., unpublished data). Weesner (1956) reported that in *R. hesperus* females after mating, there were 9,561 sperm in both areas (genital chamber and spermatheca) with 5,523 in the spermatheca alone. She further reported that even after 6 mo, with the male removed after the first month, there were 1,496 sperm.

The spermatheca in termites has been described as finger-like with a recurved end (Weesner 1969, Costa-Leonardo and Patricio 2005). In *C. formosanus*, the spermatheca is bean-shaped with a thick wall enclosed by a simple epithelium on the outside. Internally, the lumen of the spermatheca is lined with a thin layer of cuticle, indicating its ectodermal origin. Wheeler and Krutzsch (1994) reported a similar structural organization of the spermatheca in the ant *Crematogaster opuntiae* Buren. In *C. formosanus*, the cuticular layer forms finger-like projections that contain endocuticular fibers. A layer of muscle cells immediately next to the epithelium may be involved in facilitating the movement of the otherwise immotile sperm from the genital chamber into the spermatheca and later to regulate their outflow during fertilization.

The most prominent structures in the spermathecal wall are the glandular cells that resemble in some ways the class 3 secretory cells of insect epidermal glands, described by Noirot and Quennedey (1974, 1991). Suzzoni (1972) described similar secretory cells in the spermatheca of a beetle *Phosphuga atrata* L. In this beetle, the cuticular ductule arising from a secretory cell is surrounded by a ductule-carrying cell and opens into the lumen of the spermatheca. Abundance of rough endoplasmic reticulum in these cells indicates that they may be involved in the synthesis of polypeptides and other substances that are released into the lumen. In addition to the glandular layer, there is another layer of cells that is very rich in mitochondria, particularly near the base. A similar structure was reported by Fritz and Turner (2002) from the spermatheca in the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Structurally these cells resemble the canal cells described by Šobotník et al. (2003) in epidermal glands of the termite *Prorhinotermes simplex* (Hagen). It is possible that these cells also excrete their products into the lumen. The layer of vesicles could possibly contain the lipoidal material also released into the lumen. Cuticle lined ducts are interspersed in the entire spermathecal wall. In their study of the structure of spermatheca from five families of Isoptera, Costa-Leonardo and Patricio (2005) suggested that the spermatheca in the Rhinotermitidae is constituted only by the secretory portion. The exact nature of the secretions of the glandular cells is unknown, but it is speculated to provide a nutrient medium for the sperm (Costa-Leonardo and Patricio 2005) during their prolonged storage (Weesner 1969).

Typically, termite sperm lack flagella and are immobile (Grassé 1949, Weesner 1969, Baccetti 1972). Jamieson (1987) described the spermatozoa in the Rhinotermitidae as completely occupied by a compact spherical nucleus that contains some translucent areas and lacks most organelles normally attributed to sperm. According to Jamieson, such a sperm seem to be a terminal simplification. Baccetti (1972) and Baccetti et al. (1974) described the sperm in *Reticulitermes lucifugus* (Rossi) as a severely involuted cell consisting primarily of the nucleus, with two spheroi-

dal mitochondria and two truncated axoneme apices located in a small cytoplasmic rim. There was no acrosome at the posterior pole indicating a final step or consequence in the evolution of this structure. As observed in the current study, the sperm in *C. formosanus*, like most other rhinotermitids, consist primarily of the nucleus and are dependent on the muscular action of their bearer to move them around.

Detection of large numbers of bacteria in the lumen of the spermatheca is a novel finding. Although there are many published reports on bacterial microbiota of termites (e.g., Breznak 2000), we found no reports of bacteria in the spermatheca. Noda et al. (2005) reported abundant endosymbiotic bacteria belonging to the order Bacteriodales from the gut flagellate *Pseudotrichonympha grassi* Koidzumi of *C. formosanus.* The exact function of these cells, estimated to be $\approx 8.6 \times 10^7$ in each termite, remains largely unknown. Adams and Boopathy (2005) isolated four enteric bacteria, including *S. marcescens* from the gut of *C. formosanus.* They speculated that these bacteria may scavenge oxygen and create conditions for other anaerobic microorganisms that are essential for the digestion of cellulose. Presence of *Lactobacillus* in the spermatheca may be beneficial, just as lactic acid bacteria are considered important for the ecological balance in termite gut. Bauer et al. (2000) postulated that lactic acid bacteria act as an antagonist against colonization of the gut by opportunistic bacteria. Sacchi et al. (2000) reported transovarial transmission of symbiotic bacteria present in the bacteriocytes in ovarian fat body of *Mastotermes darwiniensis* Froggatt.

It has been stated earlier that the nutrients produced by the secretory cells and released into the lumen of the spermatheca may help in long-term storage of sperm. The bacteria found in the spermatheca may provide either additional nutrients or protection against other harmful entities that could flourish in such a rich medium. However, the exact role of these bacteria needs to be further investigated.

Acknowledgments

We thank Alan Lax for thoughtful discussions during the execution of these studies, and Drs. André Quennedey (Université de Bourgogne, Dijon, France), Raj Boopathy (Nicholls State University, Thibodaux, LA), Michael Haverty (University of California, Berkeley, CA), and an anonymous reviewer for critically reviewing the manuscript.

References Cited

Adams, L., and R. Boopathy. 2005. Isolation and characterization of enteric bacteria from the hindgut of Formosan termite. Bioresour. Tech. 96: 1592–1598.

Baccetti, B. 1972. Insect sperm cells. Adv. Insect Physiol. 9: 316–397.

Baccetti, B., R. Dallal, F. Rosati, F. Giusti, F. Bernini, and G. Selmi. 1974. The spermatozoon of Arthropoda. XXVI. The spermatozoon of Isoptera, Embioptera and Dermaptera. J. Microsc. (Paris) 21: 159–172.

Bauer, S., A. Tholen, J. Overmann, and A. Brune. 2000. Characterization of abundance of lactic acid bacteria in the hindgut of wood- and soil-feeding termites by molecular and culture dependent techniques. Arch. Microbiol. 173: 126–137.

Bilog Inc. 2002. Microlog user's manual, release 4.2. Biolog Inc., Hayward, CA.

Breznak, J. A. 2000. Ecology of prokaryotic microbes in the guts of the wood- and litter-feeding termites, pp. 209–231. *In* T. Abe, D. E. Bignell, and M. Higashi [eds.], Termites: evolution, sociality, symbioses, ecology. Kluwer, Dordrecht, The Netherlands.

Costa-Leonardo, A. M., and G. B. Patricio. 2005. Structure of the spermatheca in five families of Isoptera. Sociobiology 45: 659–670.

Fritz, A. H., and F. R. Turner. 2002. A light and electron microscopical study of the spermatheca and ventral receptacle of *Anastrepha suspensa* (Diptera: Tephritidae) and implications in female influence of sperm storage. Arthropod Struct. Dev. 30: 293–313.

Grassé, P.-P. 1949. Ordre des Isoptères ou termites, pp. 408–544. *In* P.-P. Grassé [ed.], Traité de zoologie, vol. 9. Masson, Paris, France.

Higa, S. Y. 1981. Flight, colony foundation, and development of the gonads of the primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus.* Ph.D. dissertation, University of Hawaii, Honolulu, HI.

Jamieson, B.G.M. 1987. The ultrastructure and phylogeny of insect spermatozoa. Cambridge University Press, Cambridge, United Kingdom.

King, Jr., E. G. 1971. Biology of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, with primary emphasis on young colony development. Ph.D. dissertation, Louisiana State University, Baton Rouge, LA.

Martins, G. F., and J. E. Serrão. 2002. A comparative study of the spermatheca in bees (Hymenoptera: Apoidea). Sociobiology 40: 711–720.

Noda, S., T. Iida, O. Kitade, H. Nakajima, T. Kudo, and M. Ohkuma. 2005. Endosymbiotic Bacteriodales bacteria of the flagellated protist *Pseudotrichonympha grassi* in the gut of the termite *Coptotermes formosanus.* Appl. Environ. Microbiol. 71: 8811–8817.

Noirot, C., and A. Quennedey. 1974. Fine structure of insect epidermal glands. Annu. Rev. Entomol. 19: 61–80.

Noirot, C., and A. Quennedey. 1991. Glands, gland cells, glandular units: some comments on terminology and classification. Ann. Soc. Entomol. Fr. 27: 123–128.

Pabalan, N., K. G. Davey, and L. Packer. 1996. Comparative morphology of spermathecae in solitary and primitively eusocial bees (Hymenoptera: Apoidea). Can. J. Zool. 74: 802–808.

Raina, A. K., Y. I. Park, and C. Florane. 2003. Behavior and reproductive biology of the primary reproductives of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiology 41: 37–48.

Sacchi, L., C. A. Nalepa, M. Lenz, C. Bandi, S. Corona, A. Grigolo, and E. Bigliardi. 2000. Transovarial transmission of symbiotic bacteria in *Mastotermes darwiniensis* (Isoptera: Mastotermitidae): ultrastructural aspects and phylogenetic implications. Ann. Entomol. Soc. Am. 93: 1308–1313.

Šobotník, J., F. Weyda, and R. Hanus. 2003. Ultrastructure of epidermal glands in neotenic reproductives of the termite *Prorhinotermes simplex* (Isoptera: Rhinotermitidae). Arthropod Struct. Dev. 32: 201–208.

Suzzoni, J-P. 1972. Ultrastructure de la glande de la spermathèque chez *Phosphuga atrata* L. (Coleoptera: Silphidae). Z. Zellforsch. 128: 426–437.

Weesner, F. M. 1956. The biology of colony foundation in *Reticulitermes hesperus.* Univ. Calif. Publ. Zool. 61: 253–314.

Weesner, F. M. 1969. The reproductive system, pp. 125–160. *In* K. Krishna and F. M. Weesner [eds.], Biology of termites, vol. 1. Academic, New York.

Wheeler, D. E., and P. H. Krutzsch. 1994. Ultrastructure of the spermatheca and its associated gland in the ant *Crematogaster opuntiae* (Hymenoptera: Formicidae). Zoomorphology 114: 203–212.

Received 16 October 2006; accepted 22 December 2006.