Insectes soc. 49 (2002) 209–215
0020-1812/02/030209-07

Insectes Sociaux

Research article

Sex pheromone identified after Solid Phase Microextraction from tergal glands of female alates in *Cornitermes bequaerti* (Isoptera, Nasutitermitinae)

C. Bordereau [1], E. M. Cancello [2], E. Sémon [3], A. Courrent [1] and B. Quennedey [1]

[1] *Université de Bourgogne, C.N.R.S, U.M.R. 5548 "Développement, Communication chimique", 6 Bd Gabriel, 21000 Dijon, France, e-mail: christian.bordereau@u-bourgogne.fr*
[2] *Museu de Zoologia da Universidade de São Paulo, CP 42594, CEP 04299-970, São Paulo, Brazil*
[3] *I.N.R.A. Laboratoire de Recherches sur les Arômes, 17 rue Sully, 21034 Dijon cedex, France*

Received 16 July 2001; revised 4 February 2002; accepted 7 March 2002.

Summary. For the first time, a termite sex pheromone secreted by tergal glands has been isolated and identified. In the mandibulate nasute termite *Cornitermes bequaerti*, pairing of swarming imagoes is mediated by a sex pheromone secreted by females from their tergal glands. These well developed sexual glands located in front of tergites 8, 9, 10 are essentially composed of class 1 and 2 cells, but also of some glandular units of class 3 cells. The major volatile compound of tergal secretion was isolated by solid phase microextraction (SPME) and identified by GC-MS as (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol. Sex attraction bioassays with synthetic (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol showed that this alcohol was the main component of the sex pheromone in *C. bequaerti*. The cellular origin and the key role of (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol in the biology of termites are discussed.

Key words: Sex pheromone, termites, Nasutitermitinae, tergal glands, (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol, SPME.

Introduction

Termites generally reproduce during dispersal flights of alates that leave the mother colony and initiate new colonies from bisexual pairs. Depending on each species, pairing is mediated by females or/and males and different glands are involved in sex pheromone secretion.

In *Kalotermes flavicollis* (Kalotermitidae), sex pheromones are secreted by sternal and tergal glands of both sexes (Wall, 1971). In *Zootermopsis nevadensis* (Termopsidae), Pasteels (1972) discovered male and female sex-specific pheromones secreted by the sternal gland (Stuart, 1975). In *Trinervitermes bettonianus* (Termitidae, Nasutitermitinae), only female alates secrete sex pheromone from both the sternal and tergal glands (Leuthold and Lüscher, 1974; Leuthold, 1977). In several species of *Reticulitermes* (Rhinotermitidae), sex pheromone is only secreted by the sternal gland of female imagoes (Stuart, 1969; Buchli, 1960; Clément, 1982), as in *Pseudacanthotermes spiniger* (Termitidae, Macrotermitinae) that has a hypertrophied sternal gland (Bordereau et al., 1991). In *Syntermes dirus* (Termitidae, Nasutitermitinae), sex attraction is performed by female alates with their tergal glands (Barth, 1955). *In Macrotermes annandalei* and *M. barneyi* (Termitidae, Macrotermitinae), Peppuy et al. (1998) have shown that the sex pheromone is secreted by the posterior sternal glands of females. In *Hodotermes mossambicus* (Hodotermitidae), sex pheromone is secreted by male alates from their hypertrophied sternal gland (Leuthold, 1977). In these examples, the distinction between the actual sex attraction at distance and attraction during tandem behaviour is not always clear. Postflight behaviour and pairing in termites surely is less uniform than previously thought and clearly deserves more attention to understand the evolutionary tendencies of the phenomenon.

Besides the behavioural aspects, very little is known about the chemical nature of sex pheromones in termites. N-tetradecyl-propionate was claimed to be a sex pheromone in *Reticulitermes flavipes* by Clément et al. (1989) but this substance required extra-physiological concentrations for eliciting sex attraction. Mac Dowell and Oloo (1984) showed that neocembrene was the trail-following pheromone in *Trinervitermes bettonianus* and suggested it was probably the sex pheromone but no sex attraction bioassays were performed. (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol was found to be the sex pheromone of *P. spiniger* (Bordereau et al. 1991), *R. santonensis* (Laduguie et al. 1994) and probably *R. lucifugus*

grassei (Wobst et al. 1999). Termite sex pheromones are not easy to study as dispersal flights generally occur only once a year for few minutes and because very tiny quantities of pheromones are secreted. Moreover, it is generally very difficult to purify the mixtures of glandular secretions extracted by solvents. Noirot (1969) underlined our poor knowledge about the structure of termite tergal glands, the nature and the role of their secretions. Ampion (1980) presented a detailed and comparative structural study about tergal glands in Isoptera, but no data have been published on the nature of tergal secretions. In our study, we have used the solid phase microextraction (SPME) to isolate the component(s) specific to the gland secreting the sex pheromone in *Cornitermes bequaerti* (Termitidae, Nasutitermitinae*)*, and the GC-MS to identify the first termite tergal sex pheromone.

Materials and methods

Study site and study species

In Brazil, our study was carried out in Botucatu in the state of São Paulo, at about 200 km west of São Paulo city. This hilly region, situated 800 meters above the sea level, is an ancient forested area now mostly covered with pastures, ants (*Atta*, *Acromyrmex*) and termites (*Cornitermes bequaerti*, *C. cumulans*, *Syntermes praecellens*, *S. grandis*, *S. nanus* and many species of full nasute Nasutitermitinae).

C. bequaerti is a mandibulate neotropical Nasutitermitinae very abundant in Brazil where it is often found in deforested areas in sympatry with *C. cumulans*. It builds earthen nests with small epigeous constructions. The habitacle, of 60–80 cm in diameter, is entirely subterranean and contains several tens of thousands of individuals. This species feeds on dry grass and may cause severe damage to pastures. Dispersal flights generally occur in September-October at sunset.

Alates were collected on the campus of Botucatu, SP (Universidade Estadual Paulista Julio de Mequita Filho, UNESP) in 1999. Behavioural observations were performed in Brazil, while chemical analyses and scanning electron microscopy were undertaken in São Paulo (MZUSP) and in Dijon (France). Transmission electron microscopy was made in France.

Pheromone extracts

Liquid phase extraction (LPE) with organic solvents and solid phase microextraction (SPME) were used. For LPE, sternal and tergal glands of male and female alates were prepared by immersion in pentane or hexane for 12 h at 4 °C. Glandular extracts were performed by dissecting the glands from cold anesthesized individuals under a stereomicroscope with microscissors and forceps. For SPME, cold anesthesized dealate females were artificially induced to expose their glands by stretching the abdominal segments covering sternal or tergal glands. A Supelco 65 µm-Polydimethylsiloxane/Divinylbenzene fiber for SPME was gently rubbed against the tergal glands of 25 dealate females, and immediately desorbed in the injection port of a gas chromatograph for 3 min for GC-MS analysis. Controls were prepared by rubbing non glandular abdominal surfaces with the same type of fiber.

Sex attraction bioassays

Sex attraction bioassays were performed by introducing 2 males in a 15 cm Petri dish containing folded pieces of Whatman n° 1 filter paper (1 cm^2) treated with 20 µl pentanic extracts each. The solvent was evaporated immediately before the bioassay. Procedures for the control were the same, except that the filter paper was treated with pentane only. Two males were introduced at the same time and at the same distance from the pieces of paper. Two males were used because a single male is deprived of tactile communication and shows rather unstable behaviour. During 300 seconds the time spent by one or two males licking or palpating the different pieces of paper was measured. After each bioassay, Petri dishes were cleaned with ethanol and pentane, and the termites and pieces of paper replaced. For all bioassays, the extracts were tested at a concentration of one individual equivalent. Results obtained from 10 replicates were statistically analyzed by the Mann and Whitney U test.

Gas chromatography and gas chromatography-mass spectrometry analyses

GC analyses were carried out with a Chrompack CP-9002 (Chrompack®, USA) instrument fitted with a split-splitless injector and a flame-ionisation detector. A DBTM-Wax (30 m X0.32 mm i.d., 0.5 µm film thickness) fused silica capillary column was used for analyses with temperature programming from 40 °C to 240 °C, at 2 °C/min. Helium was used as the carrier gas at a velocity of 37.5 cm/s. The temperature of injector and detector was set to 250 °C and 270 °C respectively. Evaluation of the data was done automatically by a PC-software (Maestro, Chrompack).

GC-MS analyses were carried out with a Nermag R10–10C quadrupole mass spectrometer directly coupled to a Hewlett Packard 5890 gas chromatograph fitted with a splitless-split injector. The analytical column used was a fused silica capillary column (30 m, 0.32 mm i.d.) coated with DB™–5 (J&W Scientific, 1 µm film thickness). Helium was used as the carrier gas at a velocity of 37 cm/s. The GC temperature was programmed from 40 °C to 220 °C at 3 °C/min. Electron impact (EI) mass spectra were obtained with an electron energy of 70 eV by scanning the instrument from 25 to 300 a.m.u. in 0.8 sec.

Structural and ultrastructural observations

Histology was made on material fixed with Duboscq-Brasil's mixture. Samples were embedded in paraffin, cut in 7 µm sections and stained with Heidenhain'azan.

For scanning electron microscopy, tergites 8, 9 and 10 were cleaned by immersing in acetone for 1 hr, dried and covered with gold or gold-palladium before observation under microscope Philips E.S.E.M. XL30 at the Center of Microscopy applied to Biology (CMAB, Dijon, France) or under a LEO-400 microscope at MZUSP in Brazil.

For transmission electron microscopy, the specimens were fixed for 24 hr with glutaraldehyde-paraformaldehyde according to Friend and Farquhar (1967), post-fixed in 1% osmium tetroxide for 1 hr, and embedded in epon-araldite mixture. Ultra-thin sections were stained with uranyl acetate and lead citrate according to Reynolds (1963). Samples were observed with a Hitachi H600 microscope.

Results

Alates and post-flight behaviour

In *C. bequaerti*, alates show a clear sexual dimorphism, with females 25 to 50% heavier than males, and only females possess abdominal tergal glands. These glands are located in front of tergites 8-9-10. The sternal gland present in both sexes shows an unusual sclerotized and pigmented surface, it is slightly larger in females than in males.

After flight and landing, both sexes spontaneously shed their wings. While standing still the female assumes a calling posture, exposing its 3 huge tergal glands (Fig. 1). Once attracted, the male touches the female, placing its prothoracic legs on the abdominal pleural membranes of the female. A

nuptial promenade in tandem is rapidly started with the female as a leader, and the male licking the tergal secretions, walking only with meso and meta-thoracic legs. After choosing an appropriate site for nesting, both sexes dig the first subterranean chamber in which they mate. It could not be observed if a trail pheromone was released by the female during the tandem running. When the pair looses contact, the female immediately stops and starts again the calling behaviour by exposing its tergal glands for several tens of seconds. If no male is attracted, the female walks few centimeters, then stops for calling. Calling behaviour was never observed in *C. bequaerti* males; only females secrete sex pheromone.

Origin of sex pheromone

Results are summarized in table 1. They show that the bioassays are not biased when using two males as these two individuals never joined together for a long time on control papers in the Petri dish. The males were strongly attracted by female tergal gland extracts. In all cases, the males started running rapidly toward the tergal gland extracts and met each other on the extract. Then they stayed on the extract showing a state of great excitement while licking and palpating the piece of paper and each other alternately. On the other hand, the males were only slightly attracted by extracts of female sternal gland, and never showed any excitement behaviour in contact with these extracts. When given a choice between extracts of sternal and tergal female glands, the male always chose the tergal gland extract. Thus, the female sex pheromone of *C. bequaerti* is secreted by tergal glands.

Identification of female sex pheromone

After SPME of the surface of tergal glands of 25 dealate females, 9 main peaks were observed in GC. (Fig. 7a). The most volatile compound was quantitatively important, and could be identified by its retention time and its mass spectrum as (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol (Fig. 7b). The other compounds were unsaturated and saturated, linear and branched hydrocarbons from C_{25} to C_{30}. By comparing the compounds present on the non glandular tergal cuticle (tergites 2-3-4) and those present on glandular areas (tergites 8-9-10), it could be observed that dodecatrienol was specific to the tergal glandular area. The cuticular hydrocarbons, except 2 unsaturated C_{25} hydrocarbons were common to glandular and non glandular abdominal integument.

Biological activity of synthetic dodecatrienol

Results are summarized in Table 1. Synthetic dodecatrienol clearly induced attraction of *C. bequaerti* males at 1 ng concentrations and further elicited excitement behaviour at 10 ng concentrations. When offered a choice between 10 ng dodecatrienol and extracts of tergal glands (glands 8–10), males stayed longer on glandular extract. In contrast, when offered a choice between 100 ng dodecatrienol and extracts of tergal glands 8–10, they clearly preferred synthetic dodecatrienol. From these results, it could be concluded that dodecatrienol is the main component of the female sex pheromone of *C. bequaerti*. Bioassays suggest that this compound is secreted at a concentration situated between 10 and 100 ng. By GC of extracts of sternal glands, its concentration was estimated at 20–30 ng per female.

Structure of tergal glands

Tergal glands are located at the anterior part of tergites 8 – 10 and are highly developed. Glandular areas are 1.5×10^6 µm², 1.2×10^6 µm², 0.8×10^6 µm² for tergites 8–10, respectively. They are normally covered with the posterior margin of the preceding tergite but are exposed at calling posture (Fig. 1).

When observed in scanning electron microscopy, the cuticular surface of female tergal glands shows a fine scaled structure different from the more smooth adjacent non glan-

Table 1. Sex attraction bioassays : Choice tests for male dealates between pieces of filter paper impregnated with pentane (control), with extracts of sternal gland, tergal glands (8–10) at the concentration of one female equivalent or with synthetic (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol (DTE-OH) at concentrations 1ng, 10 ng and 100 ng. The bioassay consists in measuring the time spent by one or two males licking or palpating the different pieces of filter paper. Duration of the bioassay = 300 seconds. Results expressed in the table are mean time in seconds with standard deviation. N = 10 replicates. (S) = time significantly different from that of control or tergal glands bioassay (Mann and Whitney U test, $p < 0.01$)

Bioassay	Time (sec)					
	Control	Tergal glands	Sternal glands	DTE-OH 1 ng	DTE-OH 10 ng	DTE-OH 100 ng
1	1.5 ± 1.6	–	–	–	–	–
2	3.1 ± 1.9	248.4 ± 31.9 (S)	–	–	–	–
3	5.1 ± 3.0	–	37.1 ± 10.4 (S)	–	–	–
4	–	228.4 ± 82.8	16.7 ± 10.2 (S)	–	–	–
5	6.5 ± 4.3	–	–	188.6 ± 29.1 (S)	–	–
6	3.4 ± 2.4	–	–	–	260.1 ± 42.5 (S)	–
7	–	216.8 ± 37.3	–	–	78.9 ± 41.3 (S)	–
8	–	58.6 ± 19.5	–	–	–	221.7 ± 60.6 (S)

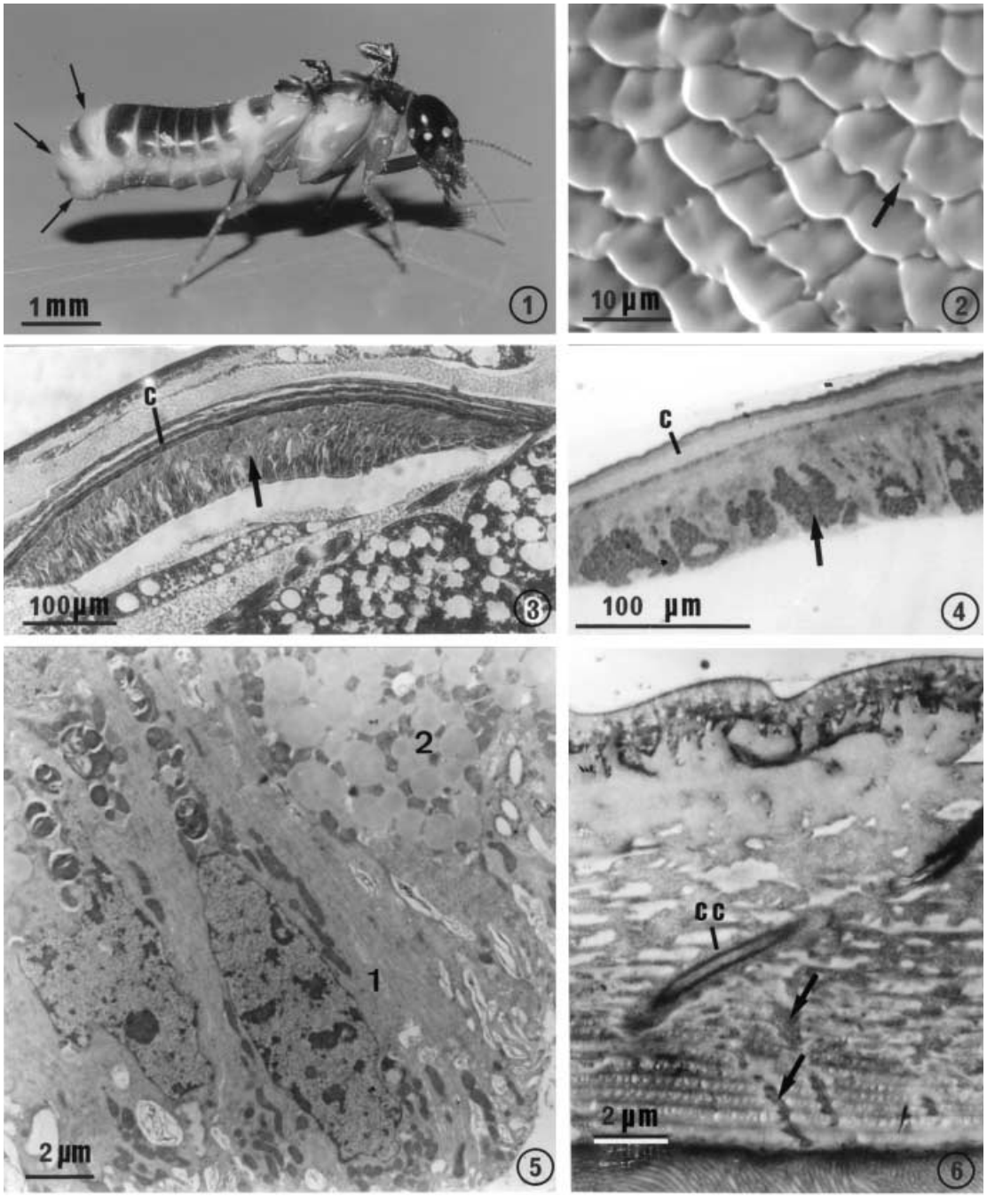

Figure 1. Female dealate of *Cornitermes bequaerti* in a calling posture. Tergal glands 8, 9, 10 are well exposed during releasing of the sex pheromone. X = 7

Figure 2. Surface of tergal gland of tergite 8th observed with scanning electron microscope, showing microscaled structure and narrow openings (arrow) of class 3 cells. X = 1,600

Figure 3. Histological section of tergal gland of tergite 8th coloured with azan. The gland is covered by the posterior margin of the 7th tergite. The glandular epithelium (arrow) is essentially composed of class 1 cells with basal nuclei and highly developed apical microvilli, and class 2 cells with central nuclei and granulous cytoplasm. Class 3 cells are rare. The cuticle (c) covering the gland is composed of partially stabilized material (meso-cuticle), and shows an aspect different from the normal cuticle (see detail Fig. 6). X = 160

Figure 4. Semi thin section coloured with methylene blue of tergal gland of tergite 8th showing numerous granulous areas (arrow) corresponding to secretory class 2 cells. X = 300

Figure 5. Ultra-thin section of tergal gland of tergite 8th showing class 1 cells (1) and secretory vesicles associated with mitochondria of class 2 cells (2). X = 5,500

Figure 6. Ultra-thin section of glandular cuticle. The loose texture of this special cuticle allows the passage of the secretion of class 1 and 2 cells (arrows), whereas secretion of class 3 cells is released through canals (cc). X = 6,500

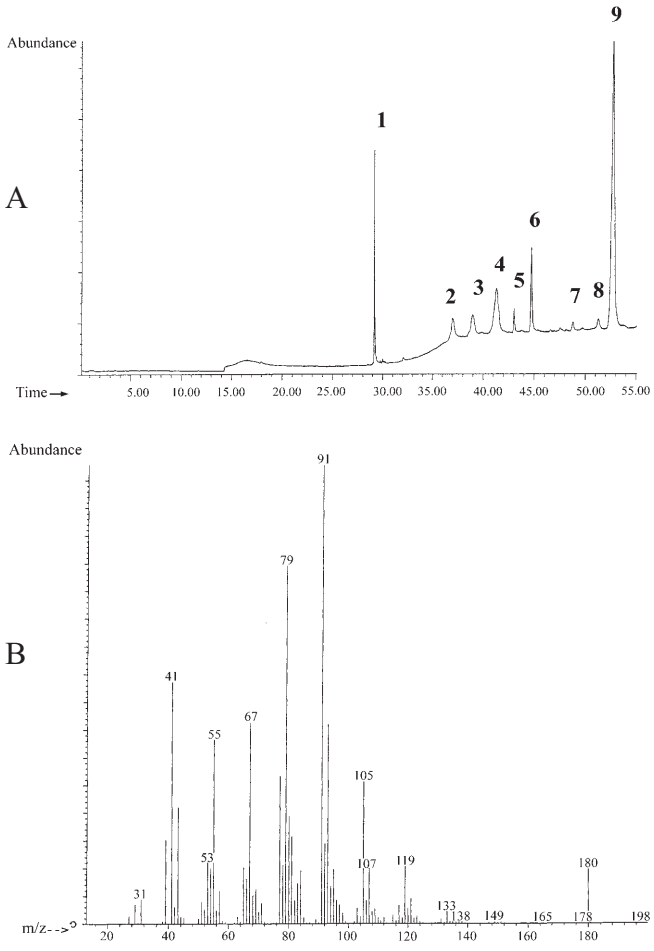

Figure 7. a Gas chromatogram of an extract of the tergal gland surface after Solid Phase Microextraction. The volatile component (1) is specific to the glandular area and corresponds to (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol. Sex attraction bioassays with synthetic standard have shown that this alcohol is the main component of the female sex pheromone of *Cornitermes bequaerti*. Peaks 2 to 9 are cuticular hydrocarbons, respectively: 2: unsaturated linear C25, 3: unsaturated linear C25, 4: mix of saturated and unsaturated linear C25 and C26, 5: saturated linear C25, 6: saturated and branched C27, 7: saturated and branched C28, saturated 29, 8: unsaturated and branched C30. Except hydrocarbons 2 and 3, all other hydrocarbons are common to glandular and non glandular areas. **b** EI (70 eV) mass spectrum of peak 1 corresponding to (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol

dular cuticle (Fig. 2). Each scale corresponds to an underlying epidermal cell, more or less hexagonal in form and measures 10–12 µm in width. Narrow openings (0.4–0.5 µm in diameter) are visible between scales, the density of these openings varies across the gland.

The glandular epithelium is about 70–80 µm in height and covered with a cuticle less sclerotized than that of the respective tergite. On histological sections stained with azan, this glandular cuticle appears to be composed of a thin outer layer coloured in amber comprizing the epicuticle and a partially sclerotized layer (2–2.5 µm), an intermediary blue-yellow granulous layer (4.5–5.5 µm) and an inner red layer of mesocuticle (4–5 µm) (Fig. 3).

On semi-thin sections stained with methylene blue, numerous ovoid and granulaceous areas, easily recognizable in the cytoplasm, probably correspond to class 2 secretory cells (Fig. 4).

At the ultrastructural level, the glandular epithelium appears essentially composed of class 1 and 2 cells according to Noirot and Quennedey's terminology 1974; 1991). Class 3 cells with reservoirs and cuticular ducts are more rarely observed. Class 1 cells appear as elongated and narrow cells (40–50 µm in height, 3–5 µm in diameter) that join both the basement membrane and the cuticle, bearing highly developed microvilli at the apex (10–15 µm in length) and contain many dense granules within their cytoplasm. Their nuclei are generally located at the basis of the cells. Class 2 cells are ovoid in shape (30–35/10 µm) and have no contact with the basal membrane and cuticle. Their cytoplasm is filled with abundant small clear droplets, associated with very numerous small mitochondria (Fig. 5). Most often, their nuclei are located in the center of the cells. Their secretion necessarily migrates through or between class 1 cells before being released from the gland. Glandular units of class 3 cells are scarce and composed of a glandular cell with a receiving canal and a canal cell surrounding a thin conducting canal which goes through the cuticle and opens at the surface of the cuticle (Fig. 6).

Discussion

This study shows that in *Cornitermes bequaerti* pairing is mediated by female dealates that secrete a sex pheromone from 3 huge abdominal tergal glands. The same situation occurs in another mandibulate Nasutitermitinae, *Syntermes dirus* (Barth, 1955). In contrast, in other species, both tergal and sternal glands are involved in postflight behaviour. For example, in the full nasute *Trinervitermes bettonianus*, a long distance sex attraction is assumed by tergal glands while a short distance sex attraction is mediated by the sternal gland. This sternal gland also secretes a trail-following pheromone during the nuptial promenade, allowing the pair to reunite if an accidental disconnection occurs (Leuthold, 1977). In species without tergal glands, the sternal gland is generally hypertrophied and secretes both sex pheromone and trail-following pheromone, as is the case in *Pseudacanthotermes spiniger* and *P. militaris* (Bordereau et al., 1991; 1993).

For the first time, a sex pheromone of Nasutitermitinae secreted by tergal glands has been identified. This pheromone elicits both attraction at distance and during the tandem behaviour. By using SPME, the major component specific to the surface of the sexual glands could be easily detected, identified and tested on males. Surprisingly, the tergal sex pheromone is (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol, the same molecule identified as sex pheromone in *Reticulitermes santonensis* (Rhinotermitidae) (Laduguie et al., 1994) and *P. spiniger* (Termitidae Macrotermitinae) (Bordereau et al. 1991), and secreted by the sternal gland in both species. These two species do not have tergal glands. In *R. santonensis*, the sternal gland shows two cellular lobes, the anterior

lobe is composed of class 1 and 2 cells, the posterior lobe of class 3 cells (Quennedey 1971, 1977); in *P. spiniger*, the sternal gland of the female alate only possesses class 1 and 2 cells. Thus, (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol is probably secreted by class-1 and 2 cells. Class 2 cells are especially remarkable, their cytoplasm entirely filled with clear droplets closely associated with numerous small mitochondriae, suggesting active metabolism. They could be the main site of synthesis of this sex pheromone.

Results show again that (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol plays a major role in the biology of termites. This alcohol is present in wood decayed by the fungus *Gloephyllum trabeum* and eaten by some Rhinotermitidae (Matsumura et al., 1969). It is also the major component of the trail-following pheromone and the sex pheromone of several species of Rhinotermitidae and Termitidae (Pasteels and Bordereau, 1998). The dual function of this molecule is resulting from different amounts of pheromone secreted by workers and alates and from the different sensitivity of the respective castes to this alcohol (Bordereau et al., 1993). (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol without doubt plays a crucial role in strategies of chemical communication in termites that appear much less based on specificity than in ants, although even in these Hymenopteran social insects, anonymous signals also exist for recruitment such as 3-Ethyl-2,5-dimethylpyrazine for species of several myrmicine genera (Hölldobler et al., 2001). In termites, an extreme pheromonal parsimony is observed, the same molecule being secreted by different glands, different species and for different functions. Thus, (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol is an anonymous signal (Hölldobler and Carlin, 1987) for a great number of species of termites with different bio-ecological characteristics. However, (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol is not an anonymous signal for all Isoptera. In the "lower" termites, such as Mastotermitidae, Hodotermitidae, Zootermopsidae and Kalotermitidae it has not been found until now and pseudergates or workers of these termites are not sensitive to this molecule. Even in Termitidae where (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol is especially active, other molecules have been identified for trail-following or sex pheromones. Thus, in several Asiatic and African *Macrotermes*, the trail-following pheromone is the monoene (3*Z*)-dodecen-1-ol (Peppuy et al., 2001a, b) whereas it is the diene (3*Z*, 6*Z*)-dodecadien-1-ol in other fungus-growing termites (Peppuy, 1999).

The contradiction between the structural complexity of sternal glands of termites suggesting complex and diverse pheromonal secretions, and the apparent chemical uniformity and simplicity has been often underlined (Quennedey, 1977; Pasteels and Bordereau, 1998). This is illustrated by our results showing the secretion of identical compounds by different glands. However, only the major volatile components of trail-following or sex pheromones have been identified whereas species-specific or caste-specific behavioural responses could be elicited by minor components (Howard et al., 1976; Kaib et al., 1982; Affolter and Leuthold, 2000; Reinhard and Kaib, 2001; Peppuy et al., 2001b). This could perhaps partly explain the ultrastructural complexity of secretory glands. Moreover only compounds involved in trail-following or sex attraction have been investigated for sternal secretions. Other chemical components volatile or not, and especially those secreted by class 3 cells, could be implicated in other related phenomena of sex behaviour, such as chemical stabilization or inactivation of pheromonal secretions, secretion of ligands for transport of pheromone through the cuticle, cohesion of the tandem during nuptial promenade, stimulation of mating after digging of the copularium, or even in resistance to diseases (Traniello and Leuthold, 2000). All these substances could intervene in the functional complexity of sexual glands of termites. Without doubt, the SPME will be a useful technique, in the future, for the analysis of this chemical complexity that may mediate a variety of behaviors in termites.

Acknowledgements

We are grateful to Dr. Luiz Carlos Forti for providing laboratory facilities in Botucatu, and to the "Netto Denko Japanese Company" for the generous donation of (3*Z,* 6*Z,* 8*E*)-dodecatrien-1-ol standard. We also thank Prof. Charles Noirot, Dr. André Quennedey and Dr. Alexandre Pires Aguiar (MZUSP) for review of the manuscript.

References

Affolter, J. and R.H. Leuthold, 2000. Quantitative and qualitative aspects of trail pheromones in *Macrotermes subhyalinus* (Isoptera, Termitidae). *Insectes soc*. *47*: 256–262.

Ampion, M., 1980. Les glandes tergales des imagos de termites: étude comparative et signification évolutive. Thesis. University of Dijon, 107 pp.

Barth, R., 1955. Über die tergitalen Druesenfelder von *Syntermes dirus* (Isoptera*). Rev. Brasil. Biol*., *15*: 257–263.

Birch, A.J., W.V. Brown, J.E.T. Corrie and B.P. Moore, 1972. Neocembrene-A, a termite trail pheromone. *J. Chem. Soc. 1*: 2653–2658.

Bordereau, C., A. Robert, O. Bonnard and J.-L. Le Quéré, 1991. (3*Z,* 6*Z,* 8*E*)-3,6,8-Dodecatrien-1-ol: sex pheromone in a higher fungus-growing termite, *Pseudacanthotermes spiniger* (Isoptera, Macrotermitinae). *J. Chem. Ecol*. *17*: 2177–2191.

Bordereau, C., A. Robert, N. Laduguie, O. Bonnard and J.-L. Le Quéré, 1993. Détection du (3*Z,* 6*Z,* 8*E*)-3,6,8-Dodecatrien-1-ol par les ouvriers et les essaimants de deux espèces de termites champignonnistes: *Pseudacanthotermes spiniger* et *P. militaris* (Isoptera, Macrotermitinae). *Actes Coll. Insectes Soc. 8*: 145–149.

Buchli, H., 1960. Les tropismes lors de la pariade des imagos de *Reticulitermes lucifugus. Vie et Milieu*, *11*: 308–315.

Clément, J.L., 1982. Phéromones d'attraction sexuelle des termites européens du genre *Reticulitermes*. Mécanismes comportementaux et isolements spécifiques. *Biol. Behav*. *7*: 55–68.

Clément, J.L., H. Llyod, P. Nagnan and M.S. Blum, 1989. N-Tetradecyl propionate: Identification as a sex pheromone of the eastern subterranean termite *Reticulitermes flavipes*. *Sociobiology 15*: 19–24.

Friend, D.S. and M.E. Farquard, 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol. 35*: 357–376.

Hölldobler, B. and N.F. Carlin, 1987. Anonymity and specificity in the chemical communication signals of social insects. *J. Comp. Physiol*. *A*, *161*: 567–581.

Hölldobler, B., E.D. Morgan, N.J. Oldham and J. Liebig, 2001. Recruitment pheromone in the harvester ant genus *Pogonomyrmex. J. Insect Physiol., 47*: 369–374.

Howard, R., F. Matsumura and H.C. Coppel, 1976. Trail following pheromone of Rhinotermitidae : approaches to their authentication and specificity. *J. Chem. Ecol*. *2*: 147–166.

Kaib, M., O. Bruinsma and R.H. Leuthold, 1982. Trail-following in termites: evidence for a multicomponent system. *J. Chem. Ecol*. *8*: 1193–1205.

Laduguie N., A. Robert, O. Bonnard, F. Vieau, J.L. Le Quéré, E. Sémon and C. Bordereau, 1994. Isolation and identification of (3*Z*, 6*Z*, 8*E*)-3,6,8-Dodecatrien-1-ol in *Reticulitermes santonensis* Feytaud (Isoptera, Rhinotermitidae): roles in worker trail-following and in alate sex-attraction behavior. *J. Insect Physiol*. *40*: 781–787.

Leuthold, R.H., 1977. Post-flight communication in two termite species, *Trinervitermes bettonianus* and *Hodotermes mossambicus*. In *Proc. 8th Int. Congr. IUSSI, Wageningen*, pp. 62–64.

Leuthold, R.H. and M. Lüscher, 1974. An unusual caste polymorphism of the sternal gland and its trail pheromone production in the termite *Trinervitermes bettonianus*. *Insectes soc*. 21: 335–341.

Matsumura, F., A. Tai and H.C. Coppel, 1969. Termite trail-following substance, isolation and purification from *Reticulitermes virginicus* and fungus infected wood. *J. Econ. Entomol*. *62*: 599–603.

McDowell, P.G. and G.W. Oloo, 1984. Isolation, identification, and biological activity of trail following pheromone of termite *Trinervitermes bettonianus* (Sjöstedt) (Termitidae: Nasutitermitinae). *J. Chem. Ecol*. *10*: 835–851.

Noirot, Ch., 1969. Glands and secretions. In: *Biology of Termites,* vol. 1. (K. Krishna and F.M. Weesner, Eds.) Academic Press, New York. pp. 89–123.

Noirot, C. and A. Quennedey, 1974. Fine structure of insect epidermal glands. *Ann. 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.

Pasteels, J.M., 1972. Sex-specific pheromones in a termite. *Experientia 28*: 105–106.

Pasteels, J.M. and C. Bordereau, 1998. Releaser pheromones in termites. In: *Pheromone Communication in Social Insects* (R.K. Vander Meer, M.D. Breed, M.L. Winston and K.E. Espelie, Eds.), Westview Press, Boulder. pp. 193–215.

Peppuy, A., A. Robert and C. Bordereau, 1998. Comportement sexuel et phéromones chez deux espèces sympatriques de *Macrotermes* asiatiques, *M. annandalei et M. barneyi* (Isoptera, Macrotermitinae). Mise en évidence de nouvelles glandes sexuelles. *Actes Coll. Insectes Soc., 12*: 143–150.

Peppuy, A. 1999. Termites du Nord Vietnam, communication chimique et isolement spécifique. Thèse Université Pierre et Marie Curie (Paris VI), 146 pp. PhD., thesis.

Peppuy, A., A. Robert, E. Sémon, C. Giniès, M. Lettere, O. Bonnard and C. Bordereau, 2001a. (*Z*)-dodec-3-en-1-ol, a novel termite trail pheromone identified after solid phase microextraction from *Macrotermes annandalei*. *J. Insect Physiol*. *47*: 445–453.

Peppuy, A. A. Robert, E. Sémon, O. Bonnard, Ngo Truong Son and C. Bordereau. 2001b. Species specificity of trail pheromones of fungus-growing termites from northern Vietnam. *Insectes soc*. *48*: 245–250.

Quennedey, A., 1971. Les glandes exocrines des termites. Organisation de la glande sternale des Rhinotermitidae. Etude ultrastructurale préliminaire. *C.R. Acad. Sci.* Paris *273*: 376–379.

Quennedey, A., 1977. An ultrastructural study of the polymorphic sternal gland in *Reticulitermes santonensis* (Isoptera, Rhinotermitidae); another way of looking at the true termite trail-pheromone. In *Proc. 8th Int. Congr. IUSSI, Wageningen*, pp. 48–49.

Reinhard, J. and M. Kaib, 2001. Trail communication during foraging and recruitment in the subterranean termite *Reticulitermes santonensis* De Feytaud (Isoptera, Rhinotermitidae). *J. Insect Behav. 14*: 157–171.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol. 17*: 208–213.

Stuart, A.M., 1969. Social behavior and communication. In: *Biology of Termites* vol. 1. (K. Krishna and F.M. Weesner, Eds.) Academic Press, New York. pp. 193–232.

Stuart, A.M., 1975. Some aspects of pheromone involvement in the post-flight behavior of the termites *Zootermopsis angusticollis* (Hagen) and *Reticulitermes flavipes* (Kollar). In: *Pheromones and Defensive Secretions in Social Insects* (Ch. Noirot, P.E. Howse and G. Le Masne, Eds.) Université de Dijon, pp. 219–223.

Traniello, J.F.A. and R.H. Leuthold, 2000. The behavior and ecology of foraging in termites. In: *Termites: Evolution, Sociality, Symbiosis, Ecology* (T. Abe, S. Higashi and D.E. Bignell, Eds.), Kluwer, Dordrecht. pp. 141–168.

Wall, M., 1971. Geschlechtbiologie der Termite *Kalotermes flavicollis* (Fabr.) (Isoptera). *Acta Tropica, 28*: 17–60.

Wobst, B., J.P. Farine, C. Ginies, E. Sémon, A. Robert, O. Bonnard, S. Connétable and C. Bordereau, 1999. (3*Z*, 6*Z*, 8*E*)-3,6,8-Dodecatrien-1-ol, a major component of the trail-following pheromone in two sympatric termite species *Reticulitermes lucifugus grassei* Clément and *R. santonensis* Feytaud. *J. Chem. Ecol*. *25*: 1305–1318.