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Sex-pairing pheromone of *Ancistrotermes dimorphus* (Isoptera: Macrotermitinae)

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ABSTRACT

Ancistrotermes dimorphus is a common Macrotermitinae representative, facultative inquiline by its lifestyle, occurring in South-East China. Sex pheromone is used for couple formation and maintenance, and it is produced by and released from the female sternal gland and is highly attractive to males. Based on our combined behavioral, chemical and electrophysiological analyses, we identified (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol as the female sex pheromone of *A. dimorphus* as it evoked the tandem behavior at short distance, and the active quantities ranged from 0.01 ng to 10 ng. Interestingly, GC–MS analyses of SPME extracts showed another compound specific to the female sternal gland, (3*Z*)-dodec-3-en-1-ol, which showed a clear GC-EAD response. However, this compound has no behavioral function in natural concentrations (0.1 ng), while higher amounts (1 ng) inhibit the attraction achieved by (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol. The function of (3*Z*)-dodec-3-en-1-ol is not fully understood, but might be linked to recognition from sympatric species using the same major compound, enhancing the long-distance attraction, or informing about presence of other colonies using the compound as a trail-following pheromone. The sternal gland secretion of *Ancistrotermes* females contains additional candidate compounds, namely (3*E*,6*Z*)-dodeca-3,6-dien-1-ol and (6*Z*)-dodec-6-en-1-ol, which are not perceived by males' antennae in biologically relevant amounts.

1. Introduction

Fungus-growing termites (Termitidae: Macrotermitinae) are the principal decomposers of plant organic matter in tropical areas of South Asia (Eggleton et al., 1997). Similarly to other termites, Macrotermitinae show manifold positive effects by making the nutrients from dead organic matter accessible for plants, but negative effects dominate in human-made environments, such as cities, crop fields and plantations (Evans et al., 2011, 2013). The most common termites in warm low-land sites in South-East China belong to the genera *Macrotermes* and *Odontotermes*, which build large epigeal or subterranean nests linked to foraging sites by numerous tunnels and galleries (Zhu, 2000; Jia, 2012). *Ancistrotermes dimorphus* is also a fungus grower, a facultative inquiline species, i.e. it can build a nest independently of other termites or it may benefit from inhabiting nests of other species (Jia, 2012). In the latter case, the host's nests always belong to other Macrotermitinae, i.e. *Macrotermes annandalei, Macrotermes barneyi* and *Odontotermes formosanus*, in whose nest-walls *A. dimorphus* builds colonies including own fungal gardens (Jia, 2012). After the dispersal flight, *A. dimorphus* females land and attract males by their sex pheromone, dig into the substrate in tandem and establish their own colony (Jia, 2012, personal observation).

The chemical nature of termite pheromones is very conservative at broader taxonomic scale (Bordereau and Pasteels, 2011; Sillam-Dussès, 2010). The same compound may be both a sex pheromone and a trail pheromone, depending on the concentration and context in which it is released and the caste involved, e.g. 4,6-dimethyldodecanal in *Zootermopsis* spp. (Bordereau et al., 2010), etc. The compounds the Macrotermitinae released as sex pheromones are (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and/or (3*Z*)-dodec-3-en-1-ol (Bordereau et al., 1991; Robert et al., 2004; Wen et al., 2012). The latter two compounds

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represent the major and the minor components of *O. formosanus* sex pheromone in the ratio 15:1. Moreover, the minor component, (3*Z*)-dodec-3-en-1-ol, shows a synergistic effect to the major one, and improves the long-range attraction of males towards females (Wen et al., 2012). In other cases, the use of additional minor components ensures the species-specificity of the sex pheromone in related sympatric species (Bordereau et al., 2011).

This parsimony in chemical communication concerns also the glands producing the sex pheromones. The function is performed from 3 specialized abdominal glands: tergal, sternal or posterior sternal glands (Ampion and Quennedey, 1981; Peppuy et al., 2004; Quennedey et al., 2008). Macrotermitinae reveal a high diversity in the glands used since all three types of glands are known to occur in Macrotermitinae in contrary to most termite groups. However, *Ancistrotermes latinotus* is known to possess only a sternal gland (Ampion and Quennedey, 1981).

The knowledge of sex pheromones in termites remains very fragmentary. We decided to investigate the chemical signals used in the fungus-growing termite, *A. dimorphus*. To achieve this goal, we used a combination of advanced techniques such as solid-phase microextraction (SPME), gas chromatography coupled with mass spectrometry (GC–MS), gas chromatography coupled with electroantennographic detection (GC-EAD), and behavioral bioassays.

2. Materials and methods (see Table S1 about the sample arrangements)

2.1. Insects

Alates of *A. dimorphus* were collected from the host nests of *M. annandalei* (Silvestri, 1914) and *O. formosanus* (Shiraki, 1909) in woods of Shangsi County of Fangchengang City, Guangxi, China (22°09′03.90″N, 107°52′12.68″E and 22°07′57.79″N, 107°51′31.08″E) in July 2013. Alates were caught during the dispersal flight at 9 pm on rainy nights from May to July in 2013 and 2014. Males and females were separated immediately after collection and held in Petri dishes (ø 9 cm) lined with clean and damp filter paper.

2.2. Behavioral bioassays

Short-distance attraction bioassays were performed following previously described methods (Robert et al., 2004; Wen et al., 2012). Moist filter paper disks (1.0 cm in diameter) were used as sample dispensers in the bioassays. For each experiment, one male termite was placed in an opened Petri dish (ø 9.0 cm) for 3 min. Then, two pieces of conditioned filter paper were placed in symmetrical position along the edge of the Petri dish immediately after evaporation of hexane. 1.0 μL of a sample extract (in concentration series of 0.001 gland equivalent [eq]), 0.01 eq, and 0.1 eq) and pure hexane as solvent control, were applied on two moist filter papers disks. For localization of the female pheromone source, sample extracts were made with dissected tergites, non-glandular sternites and sternal glands (Wen et al., 2012), and the active sternal gland extracts were used in subsequent comparison experiments. (3*Z*,6*Z*)-Dodeca-3,6-dien-1-ol standards were introduced the same way in concentration series of 0.001, 0.1, 1 and 10 ng. Relevant amounts of the (3*Z*)-dodec-3-en-1-ol (0.1 and 1 ng) were added to the most active concentrations (0.01, 0.1 and 1 ng) of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol to study the effect of the other EAD active compound (see below).

The behavioral responses of the tested termite were recorded for 3 min using a digital camera (CONTOUR-IR digital, Belarus). The video files were analyzed for the duration of the first contact, the accumulated duration of contacts and the running distances during the experiments. The termites were traced in the video files for analysis of running routes using Image Pro Plus (Media Cybernetics, US) software. Each experiment was made from 5 to 12 times, 9 times on average. Data were analyzed using Turkey LSD multiple comparison. Significance level was set to $p < 0.01$.

2.3. Chemical standards

(3*Z*)-Dodec-3-en-1-ol and (3*Z*,6Z)-dodeca-3,6-dien-1-ol were synthesized following previously described procedures (Peppuy et al., 2001a; Robert et al., 2004), but we slightly modified the procedure for the second compound. Thus, the crude product of coupled dodecadiynol THP ether was evaporated under vacuum (50–500 Pa) for 30 min at 60 °C to remove any Lindlar inhibitive DMF and iodo-octyne. Then, the diynol THP ether was converted smoothly to the dienol THP ether through Lindlar catalyzed reduction. After removal of the THP group, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol was obtained in 71% yield. Both compounds were purified with silica column chromatography or 2% silver nitrite silica chromatography before use in bioassays. Because the cis-β,γ-unsaturated alcohol group was the most common base in C12 termite pheromones structures, and because this base contributed mainly to the electrophysiological responses in GC-EAD experiments (Wen et al., 2012, 2014), we ascertained that the two physiologically inactive minor C12 alcohol isomers did not contain a 3Z double bond in the structure. After further consideration of potential candidate structures according to the DMDS derivatization results, the structures were elucidated as dodec-6-en-1-ol isomers and dodeca-3,6-dien-1-ol isomers. These isomers were synthesized for identification (Fig. S1). A mixture of (6*Z*/*E*)-dodec-6-en-1-ol was prepared from a Wittig reaction (Fig. S1A) while a mixture of (3*Z*/*E*,6*Z*/*E*)-dodeca-3,6-dien-1-ol was also prepared from two step Wittig reaction (Fig. S1B). Isomerically pure (6*Z*)-dodec-6-en-1-ol was prepared by Lindlar catalyzed reduction of the coupled dodec-6-yn-1-ol THP ether (Fig. S1C), while pure (3*E*,6*Z*)-dodeca-3,6-dien-1-ol was prepared using a revised Doebner–Knoevenagel condensation method (Milite et al., 2012) and cis-reduction of the coupled alkynol (Fig. S1D).

2.4. Pheromone extraction and DMDS derivatization

For bioassays, sternal glands were dissected with a piece of the fifth sternite from mature females, and the fat body was removed with a filter paper strip. Ten glands were extracted with 100 μL bi-distilled hexane for 30 min and then diluted for bioassays. Abdominal tergites and non-glandular sternites were dissected and extracted the same way for localization of the pheromone source. All extracts were stored at −20 °C before use. For gas chromatography with a flame-ionization detector (GC-FID; 4 termites extracted in each sample), gas chromatography coupled with mass spectrometry (GC–MS; 8 termites extracted in each sample) and gas chromatography coupled with electroantennographic detection (GC-EAD; 4 termites extracted in each sample), the sex pheromone was also collected by rubbing the glandular surface of each female using a Solid Phase Micro Extraction (SPME) 65 μm polydimethylsiloxane/divinylbenzene fiber for 12 s as previously described (Bordereau et al., 2010; Sillam-Dussès et al., 2011; Wen et al., 2012). Abdominal cuticular surfaces of corresponding tergites were rubbed and extracted using the same SPME fiber for control. The same procedure was applied also to males. For DMDS derivatization used to confirm the double bond position of the pheromonal components, the crude extract of 20 female sternal glands in 100 μL of hexane was subjected to revised DMDS derivatization procedures (Millar and Haynes, 1998; Hanus et al., 2012). In order to avoid uncertain identification of irregular bi-adducts, the reac-

tion time was reduced to 10 h at 40 °C in a GC oven to ensure the presence of unreacted α,β- or β,γ-unsaturated molecules suitable for identification. The crude extract was concentrated to 2 μL and then injected completely into GC.

2.5. Chemical analysis

GC-FID analyses of the SPME extracts were carried out on a PE-480 instrument (Perkin Elmer, US). For comparison, 10s headspace SPME extracts from a 5 mg mixture of synthetic (3*Z*)-dodec-3-en-1-ol and (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (1:1) were desorbed in GC. An HP-INNOWAX capillary column (30 m × 250 μm × 0.25 μm) (Agilent Technology, US) was used with Nitrogen as carrier gas at a steady pressure of 17.8 psi supplied to column head. The injection port was set to splitless mode and heated to 250 °C. The oven ramp was 50 °C held for 1 min then 10 °C/min to 240 °C held for 10 min. The flame ionization detector (FID) temperature was 260 °C.

The GC–MS analyses were performed on an HP7890-5975C GC–MS system (Agilent Technologies, US). SPME extracts of 8 termites were desorbed in the splitless injection port at 250 °C. The same HP-INNOWAX column used in the GC analysis was used with 1 mL/min helium as the carrier gas. The oven ramp was set as 50 °C for 2 min and then 8 °C/min to 240 °C for 10 min. The transfer line was heated to 250 °C. A 70 eV EI ion source was used in the quadrupole mass spectrometer, and heated to 230 °C. The mass range scanned was *m*/*z* 25–300 at a rate of 2 × 4 scan/s. The abundance threshold for detection was set to 5. Data were analyzed using Chemstation software (Agilent Technologies, US).

2.6. Gas chromatography coupled with electro-antennographic detection (GC-EAD)

For GC-EAD, head capsule of a male was cut out with a razor. The tip of a glass grounding electrode was inserted into the brain through the incision. The antennal tips were immersed in the saline solution (NaCl 9.0 g/L, KCl 0.71 g/L, $CaCl_2$ 0.46 g/L) through the tip opening of the glass recording electrode. The recording electrode (Ag/AgCl) was connected to an IDAC4 data collector (Syntech, NL) through an EAG KOMBI-PROBE amplifier (Syntech, NL). An HP6890N GC instrument (Agilent Technologies, US) was used for separation of the blend components. The injection port was set to splitless mode and heated to 250 °C. An HP-FFAP column (30 m × 250 μm × 0.25 μm) (Agilent, US) was used with 2 mL/min Nitrogen as the carrier gas. The oven ramp was 80 °C for 1 min then 8 °C/min to 230 °C for 10 min. The elution was split by an OSS-2 splitter (SGE, AU) to the EAD and FID at a ratio of 4:1, respectively. The EAD transfer line and FID detector were both heated to 250 °C. The Pasteur pipette of EAD was swept with a clean and wet air flow (active charcoal filtered, 20 °C, RH 90%, 30 cm/s). The antennal preparation was positioned 2 mm away from the outlet of the Pasteur pipette. Data were analyzed with the GC-EAD 2011 software (Syntech, NL).

3. Results

3.1. Origin and biological activity of the female sex pheromone

Dispersal flight of *A. dimorphus* took place at 7 pm and lasted for several hours in a rainy summer night. Both sexes shed their wings right after landing, females quickly exposed their sternal gland by raising their abdomens, while males were running and scanning the space to locate the females. Tandems were formed quickly after encounter and females always took the lead on the search for places to hide in order to establish their colonies. During this tandem-running behavior, males used their mouthparts to stay connected to the females. The same behavior can be observed in the laboratory. Moreover, excess of males resulted in the formation of homosexual couples, which broke off shortly after introduction of additional females.

A. dimorphus males were clearly excited ($p < 0.01$) by the female sternal gland extracts (duration of the first contact 65.8 ± 26.8 s, $n = 5$) and not by any other part of the female body used as control extracts (duration of the first contact 5.9 ± 2.6 s $n = 10$). Immediately after the introduction of the cartridge with sternal gland extract, the males ran sprightly and scanned the space with their antennae (zigzag searching behavior) in order to detect the origin of the stimulation. After localization of the source which was a filter paper disk impregnated with the sternal gland extract (or the standard; see below), the males palpated the disks using their antennae for a considerable time. Then, they were observed to run in the vicinity of the samples and to repeat the palpating behavior whenever they encountered the sample again. The sternal gland was clearly the only source of the female sex pheromone.

3.2. Chemical nature of the pheromone components

A single compound, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol, was identified from the extracts of the female sternal glands, and no cuticular hydrocarbons were detected on the female body surface using GC-FID (Fig. 1). Males also secrete the same compound from their sternal glands, but in lower quantity (about half of the quantity in females) according to the peak areas (not shown).

GC-EAD with male and female sternal glands extracts and series of standards dilutions revealed two major responses on male antennae, one at 9.86 min which did not correspond to any obvious peak in male or female sternal glands, and another at 10.45 min which corresponded to (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (Fig. 2).

GC–MS analyses of SPME extracts revealed four peaks specific to the cuticle covering the female sternal gland in comparison with the GC–MS analysis of the SPME extract of female tergites (Fig. 3), while male produced two peaks (same retention times as peaks 1 and 2, respectively). The ratio of the four peaks was 100.00:0.67:0.42:0.33 (1:2:3:4). Mass spectra of peaks 1 and 2 were identical to those of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and (3*Z*)-dodec-3-en-1-ol, respectively. DMDS derivatives of the gland extracts revealed the presence of two minor isomers with an unsaturated bond at the 6 position in the C12 alcohols (Fig. 4). The comparison with the synthetic standards revealed the identity of these minor components as (6*Z*)-dodec-3-en-1-ol (peak 3) and (3*E*,6*Z*)-dodeca-3,6-dien-1-ol (peak 4). However, these compounds did not elicit any EAD responses and were thus not considered to participate in mate attraction.

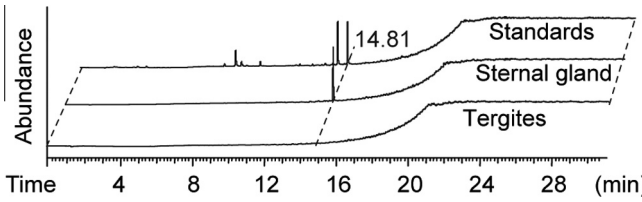

Fig. 1. Comparative analysis of the GC-FID profiles generated from SPME extracts of sternal gland cuticle, from SPME extracts of tergal cuticle of *Ancistrotermes dimorphus* females, or from headspace SPME extracts of synthetic (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and (3*Z*)-dodec-3-en-1-ol. The sternal gland extract showed one specific peak at 14.81 min, which is the retention time of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol. No other specific peak was detected in the extract, even at the retention time of (3*Z*)-dodec-3-en-1-ol.

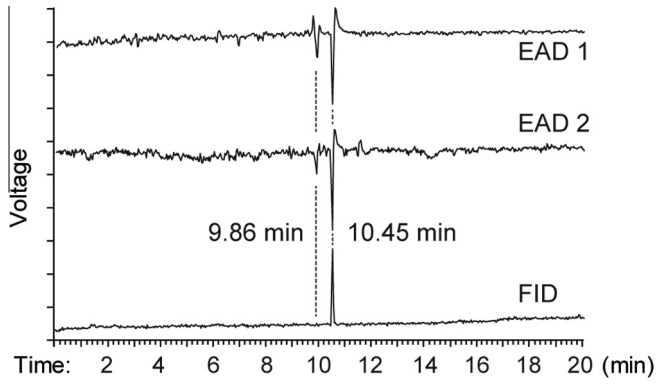

Fig. 2. GC-EAD analysis of sternal gland extracts from *Ancistrotermes dimorphus* females using male antennae (EAD scale = 0.2 mV/div, FID scale = 5 mV/div). Two reproducible EAD responses corresponding to the retention time of (3*Z*)-dodec-3-en-1-ol (9.86 min) and (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (10.45 min) were observed.

3.3. Behavioral responses of males to the pheromone

A. dimorphus males were clearly excited by all samples containing (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (alone or in the mixture with (3*Z*)-dodec-3-en-1-ol in the natural ratio), and revealed responses similar to the ones observed during female encounter (strong interest, antennation, palpating all sources of it). We analyzed a series of behavioral variables of tested males, such as the duration of the first contact with paper disks (Fig. 5A), the accumulated duration of contacts within 180 s after the first contact (Fig. 5B), the total distance walked since the first contact (Fig. 5C) and the running routes of the tested termite (Fig. 5D). The concentrations of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol showing the significant behavioral responses on dealate males ranged from 0.01 ng to 1 ng. Low quantities of (3*Z*)-dodec-3-en-1-ol (0.1 ng) alone neither elicit male attraction nor provide a positive synergistic effect to (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol, irrespective of the quantity used. On the

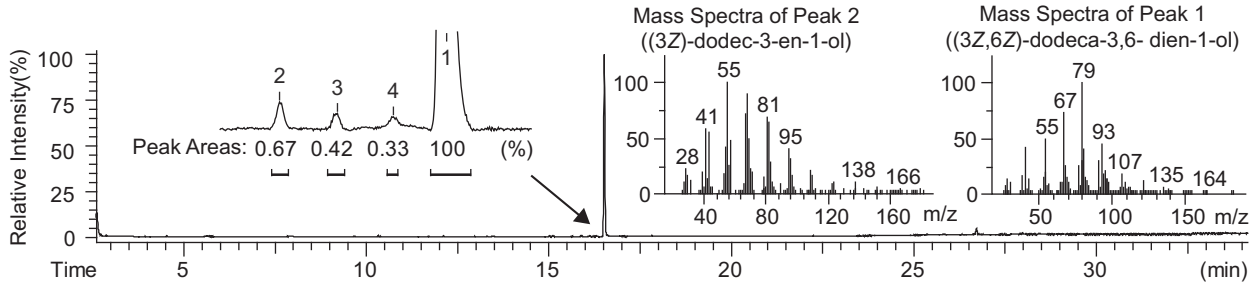

Fig. 3. GC–MS analysis after SPME of sternal gland extracts from *Ancistrotermes dimorphus* females showing 4 peaks (peaks 1 and 2 identified as (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and (3*Z*)-dodec-3-en-1-ol).

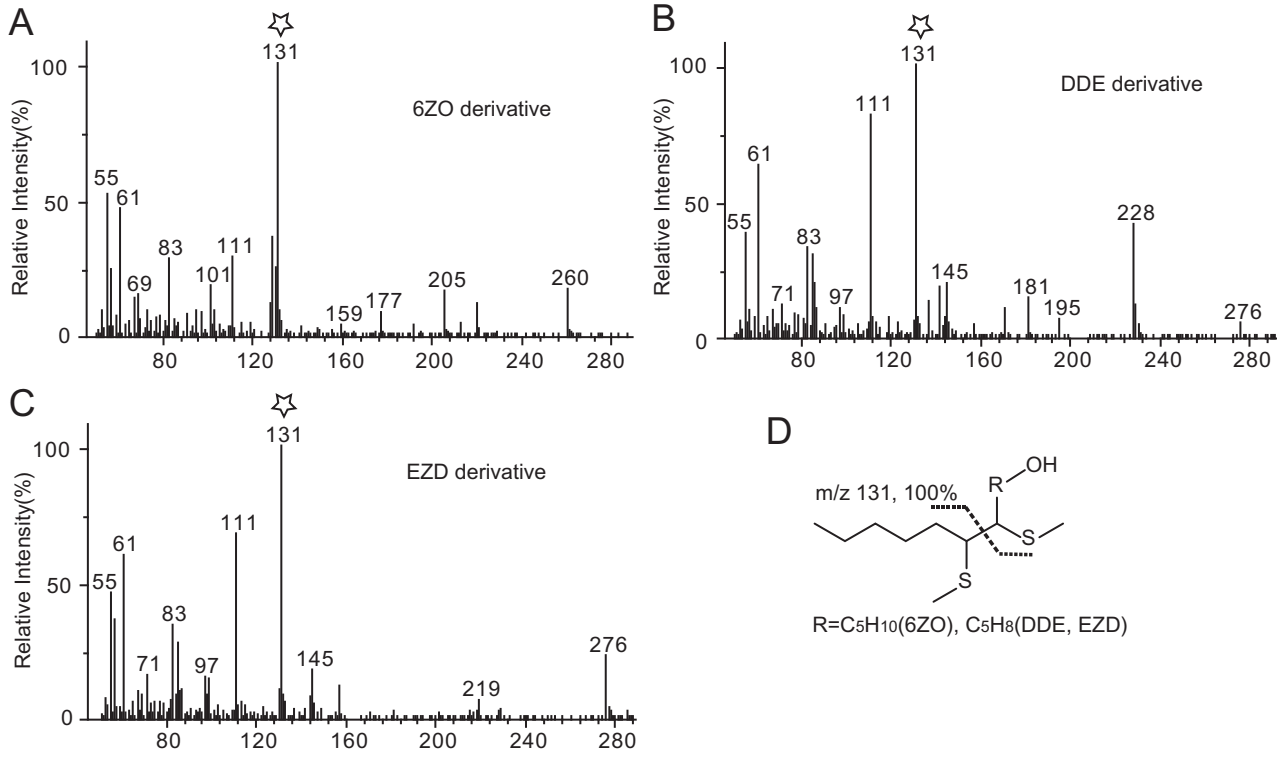

Fig. 4. Mass spectra of partial DMDS derivatives of an extract of 20 female sternal glands. The partial derivatives of the 3 components with a 6 double bond in the structure had the same mass spectra as synthetic (6*Z*)-dodec-6-en-1-ol (6ZO, mass spectrum of its partial derivate was A), (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (DDE, mass spectrum of its partial derivative was B) and (3*E*,6*Z*)-dodeca-3,6-dien-1-ol (EZD, mass spectrum of its partial derivative was C), respectively. Characteristic ion (pentacle marked) for derivatives of the 6 double bond was *m*/*z* 131, 100% in intensity (D).

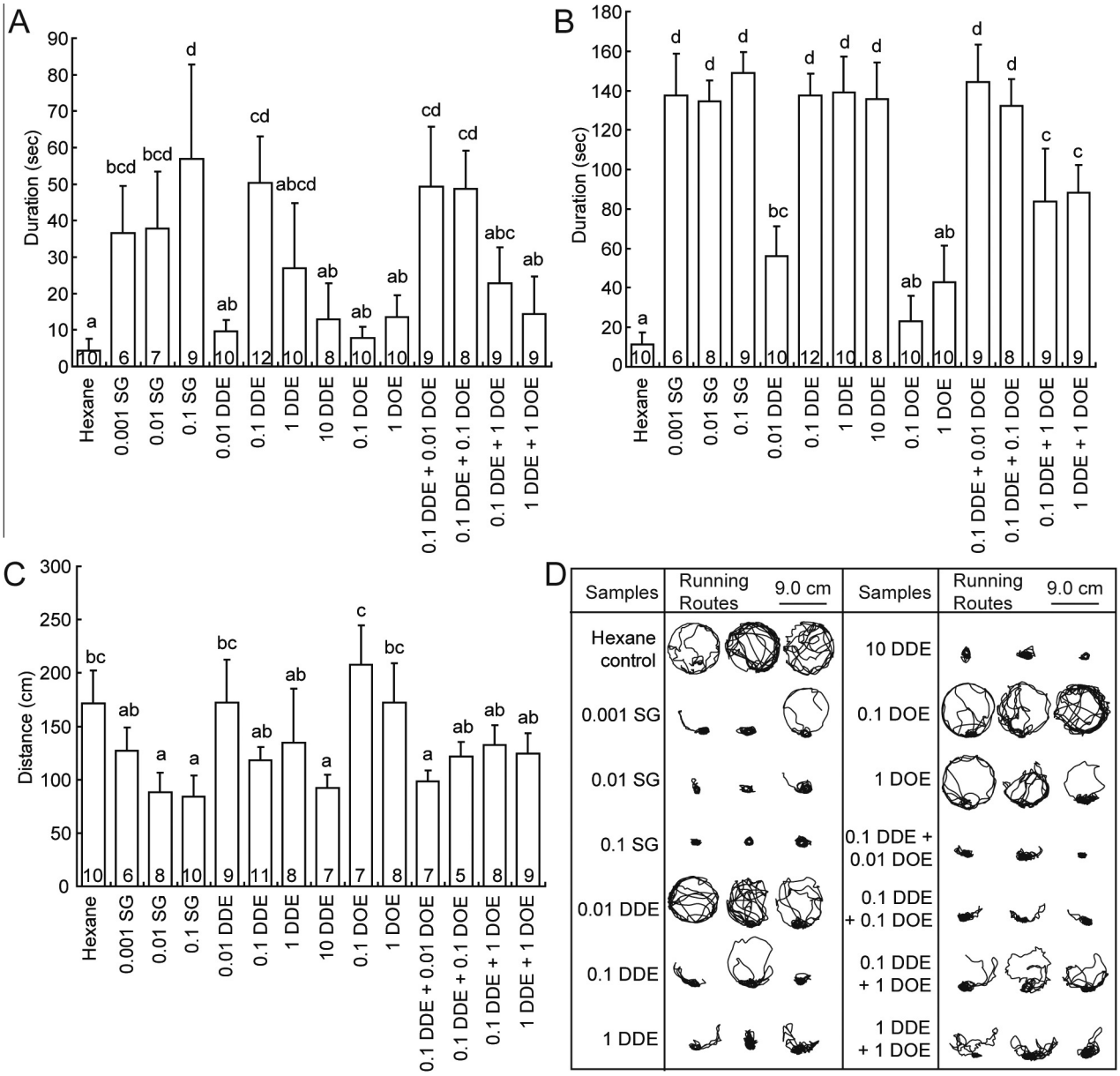

Fig. 5. Behavioral bioassays for courtship behavior of males in *Ancistrotermes dimorphus* using female sternal gland extracts (SG), (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (DDE), (3*Z*)-dodec-3-en-1-ol (DOE) or hexane as a control (unit of the quantities of synthetic chemicals is ng). For (A), (B), and (C), the number of replications is marked at the base of each column; the error bar indicates the standard deviation; columns marked with different small letters are significantly different ($p < 0.01$). (A) Duration of the first contact between one male and the filter paper disk. Males were significantly attracted to all female sternal gland extracts tested and to DDE at 0.1 ng but not to DOE. This latter compound seemed to inhibit at high quantity the activity of DDE when both are mixed. Males were over excited with high concentration of DDE, which led to a shorter duration of first contact. (B) Accumulated duration of contacts within 3 min since the male encountered the testing samples. DDE tested alone was always significantly attractive while DOE was never attractive. The mixture of both compounds showed a repulsive effect due to DOE when used in high quantity. (C) Accumulated running distance of males within the first 180 s since the encounter of samples. Males were clearly walking shorter distances when detecting high quantity of DDE. (D) Running routes of males in the presence of samples (three routes for each sample showed). Males ran randomly in the presence of hexane, DDE in low quantity, and DOE. Males could not locate precisely the mixture samples made of 0.1 ng to 1 ng DDE and of 1 ng DOE.

other hand, higher quantities of (3*Z*)-dodec-3-en-1-ol (1 ng) inhibit the attraction caused by (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol since males spent significantly shorter time around a cartridge impregnated with 1 ng of (3*Z*)-dodec-3-en-1-ol, irrespective of the quantity of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol. According to the responses to female sternal gland extracts, the quantity of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol was estimated as about 10 ng per female.

4. Discussions

The daily life of termites depends on a plethora of chemicals, providing basic orientation in their space. Among many other compounds released from the environment, termites produce their own signals, e.g. pheromones (sex, trail, and food-marking belong to well-known examples) or recognition cues (cuticular hydrocarbons) (Bagnères and Hanus, 2015). While environmental odors do not change the behavior of termites, pheromone perception usually results in abrupt change of behavior according to the message received. Sex pheromone communication is particularly a good example, as termite females produce strong male attractants from tergal, sternal or posterior sternal glands (Bordereau and Pasteels, 2011). The female sex pheromone of *A. dimorphus* is released from the sternal gland and the attractive effect can be explained by a single compound, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol. Moreover, we

identified three additional compounds released by the sternal gland, (3*Z*)-dodec-3-en-1-ol, (3*E*,6*Z*)-dodeca-3,6-dien-1-ol, and (6*Z*)-dodec-6-en-1-ol.

(3*Z*)-Dodec-3-en-1-ol is electroantennographically active, and it does not provoke attraction in males, but instead has a repellent effect. We cannot exclude another behavioral function, e.g. long-distance attraction similar to another fungus-growing termite, *O. formosanus* (Wen et al., 2012). The sympatric species, *Ancistrotermes pakistanicus*, uses (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol as its sex pheromone, and is also not sensitive to (3*Z*)-dodec-3-en-1-ol in sex attraction bioassay (Robert et al., 2004). This latter compound has not been tested with GC-EAD in *A. pakistanicus*, and so it is not possible to compare the behavioral effects of (3*Z*)-dodec-3-en-1-ol between *A. pakistanicus* and *A. dimorphus*. Another hypothesis consists in (3*Z*)-Dodec-3-en-1-ol being responsible for species-specificity of the signal in order to avoid formation of heterospecific couples, since this component is the sex pheromone of the sympatric species *O. formosanus* (Wen et al., 2012) and is secreted in high quantity equivalent to the quantity inhibiting the attraction in *A. dimorphus*. Minor components of sex pheromone blends are responsible for mate selection in *Cornitermes* spp., with (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol being always the major component (Bordereau et al., 2011). This compound is the only component of the sex pheromone in *Cornitermes bequaerti*, whereas it is associated with (*E*)-nerolidol in *Cornitermes cumulans*, and with (*E*)-nerolidol and (3*Z*)-dodec-3-en-1-ol in *Cornitermes silvestrii* (Bordereau et al., 2011). Interestingly, (3*Z*)-dodec-3-en-1-ol may also inform *A. dimorphus* couples about the presence of the host's nest, because the compound is the trail pheromone of the host species, *M. barneyi*, *M. annandalei* and *O. formosanus* (Peppuy et al., 2001b; Bordereau and Pasteels, 2011; Sillam-Dussès, 2011; Wen et al., 2014). In other words, the sensitivity of *A. dimorphus* to (3*Z*)-dodec-3-en-1-ol could help the imagoes to locate the host's nest, but not to get too close to host trails in order to prevent the encounter with workers and soldiers which could kill them. Inquiline way-of-life is based on conflict-avoiding strategies, and thus it would be adaptive if also sterile colony members could avoid the host termites based on perception of heterospecific trails, analogously to *Inquilinitermes microcerus* and its host *Constrictotermes cyphergaster* (Cristaldo et al., 2014). The scarcity of the cuticular hydrocarbons revealed by GC–MS could help to conceal the identity of *A. dimorphus*, and thus represent another adaptation to inquilinism.

Neither (3*E*,6*Z*)-dodeca-3,6-dien-1-ol nor (6*Z*)-dodec-6-en-1-ol elicited any antennal activity and thus we consider them by-products or intermediates of the biosynthetic pathways leading to (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and (3*Z*)-dodec-3-en-1-ol, respectively.

Our results highlight again the conservative nature of the chemical communication in termites. (3*Z*,6*Z*,8*E*)-Dodeca-3,6,8-trien-1-ol, the most common trail and sex pheromone of termites occurs in many taxa of Neoisoptera, and the same is true to a lesser extent for (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and (3*Z*)-dodec-3-en-1-ol (Bordereau and Pasteels, 2011; Sillam-Dussès, 2010). It is likely that the nature of the sex pheromones did not change much during the evolution because other factors allow termites to maintain the reproductive isolation, such as dispersal flight timing, biotope preferences or the effects of minor components of the pheromonal blends in addition to the major one if the same compound is used by related species (Bordereau et al., 2011). Surprisingly, the same compounds not only play a role of either trail or sex pheromones depending on quantity and context, but are often produced by different glands. While trail pheromones are always produced by the sternal gland, the sex pheromones are primarily produced by the tergal glands, which disappeared in many lineages and their function was adopted by sternal or posterior sternal glands (Ampion and Quennedey, 1981; Bordereau and Pasteels, 2011; Sillam-Dussès, 2011). So in fact both, the identity and the glandular source of the trail and sex pheromones, point at their common origin in termite ancestry.

Although termites have an important and beneficial effect on organic-matter transformation and strongly modify the soil environment through their mechanical activities, many species are serious pests in man-made biotopes (Evans et al., 2011). It is particularly true for many Macrotermitinae including *A. dimorphus*, which can cause severe damage to sugarcane, timber, and rubber trees (Jia, 2012). The identification of termite sex pheromones is thus essential to design pheromone trapping of dispersing alates, an environment friendly termite control method. The conservative nature of sex pheromones helps in this aspect, as the trapping system might be efficient in controlling many different pest species with the same active compound.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2015.11.006.

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