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Male-Produced Aggregation Pheromone Blend in *Platypus koryoensis*

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The ambrosia beetle, *Platypus koryoensis*, is a vector of Korean oak wilt disease, which causes massive mortality of oak trees (mainly *Quercus mongolica*) in Korea. So that a semiochemical-based control method could be developed, its aggregation pheromone was investigated. Whole body extract and body part extracts of male and female *P. koryoensis* were analysized using gas chromatography−flame ionization detector (GC-FID) and gas chromatography−mass spectrometry (GC-MS). All samples of male extracts contained nerol, neral, geraniol, and geranial. Those compounds were detected from female whole body extract as minor constituents and not detected from any female body part extracts. In addition to those compounds, citronellol was detected from the extract of boring dust produced by an unmated male. However, none of the five compounds were detected from the extract of boring dust produced by mated males and females or in artificial sawdust obtained from a beetle-infected *Q. mongolica* log. Male and female antennae of *P. koryoensis* responded to all five compounds in an electroantennography test. The blend of five components was tested in the field and attractive for male and female *P. koryoensis*. This result suggested that the blend of citronellol, nerol, neral, geraniol, and geranial served as an aggregation pheromone to *P. koryoensis*.

KEYWORDS: *Platypus koryoensis*; aggregation pheromone; citronellol; nerol; neral; geranial; geraniol; EAG; field assay

INTRODUCTION

Mass mortality of oak trees was reported at Sungnam city, Gyeonggi province, Korea, in 2004, and has spread to several areas of the Korean peninsula (*1*). It seemed to be occurring by a possible pathogenic fungus *Raffaelea* sp. spread by an ambrosia beetle, *Platypus koryoensis* (Coleoptera: Platypodidae) (*1*). *P. koryoensis* massively attacked healthy or stressed living *Quercus mongolica*. The numbers of *Q. mongolica* damaged or killed by oak wilt disease has increased annually since its first report (*2*). The damages caused by *Platypus* spp. have been reported worldwide. Pinhole borers *Platypus caviceps*, *Platypus apicalis*, and *Platypus gracilis* have damaged and killed living southern beech (*Nothofagus* spp.) and/or Kamawi (*Weinmannia racemosa*) in New Zealand (*3*−*5*). The relationship of pinhole bores, the fungal pathogen *Sporothrix*, and their host beech is well established (*3*). *Platypus subgranosus* was responsible for the death of myrtle beech (*N. cunninghamii*) in Tasmania, Australia (*6*). In southern Europe, *Platypus cylindrus* attacked cork oak (*Quercus suber*), and *Phytophthora cinnamoni* is known as a pathogen (*7*−*9*). *Platypus mutatus* was a primary pest of poplars, especially *Populus deltoides* in South America (*10*). Alfaro et al. (*11*) warned of the threat of *P. mutatus* to world poplar resources since its introduction to Italy in 2002. *Platypus quercivorus*, a vector of the pathogenic fungus *Raffealea quercivora*, was responsible for Japanese oak wilt disease on *Quercus crispula* and *Quercus serrata* (*12*).

In the genus *Platypus*, the existence of an aggregation pheromone or attractant produced by males has been described in *P. flavicornis* (*13*), *P. apicalis*, and *P. gracilis* (*14*), *P. cylindrus* (*15*), and *P. quercivorus* (*16*). Audino et al. (*17*) reported male *P. mutatus* (= *sulcatus*) seemed to produce a sex pheromone. *P. koryoensis* massively attacked its host plant, *Q. mongolica*. This and previous papers have suggested the existence of an aggregation pheromone of *P. koryoensis*. We report here the identification and field test of sex-specific pheromone components produced by adult male *P. koryoensis*.

MATERIALS AND METHODS

Insects. Logs (ca. 50 cm long) of *Q. monglica* infested by *P. koryoenesis* were collected from an oak forest in Paju, Gyeonggi province, Korea (37° 44′ N, 126° 54′ E) in spring (March−May) of 2007 and 2008. The cut ends of the logs were sealed with paraffin to keep moisture. They were put in a plastic box containing wet bog moss (*18*). The box was covered with net to catch the beetles emerging from the gallery. The plastic boxes were placed in a room maintained under

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a 16 h light/8 h dark photoregime at 25 °C. Adults emerged from their galleries were collected daily, sexed according to the method of Hong et al. (*1*), and used in the experiments.

Chemicals and Syntheses. Citral (purity > 96%, mixture of cis and trans), nerol (purity = 97%), and (±)-β-citronellol (purity = 95%) were purchased from Aldrich (Milwaukee, MI). Geraniol (purity = 95%) was purchased from Fluka (Buchs, Switzerland). Geranial (purity = 98%) and neral (purity = 98%) were prepared by following the method of Piancatellia and Leonelli (*19*). Briefly, geraniol or nerol (5.0 g, 32.5 mmol), aqueous pH 7.0 buffer solution (8 mL, Fluka), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) (490 mg, 3.45 mmol, TCI), and iodobenzene diacetate (11.49 g, 35.7 mmol, TCI) were dissolved in 28 mL of acetonitrile. The reaction mixture was stirred at 0 °C for 2 h. The solution was diluted with diethyl ether (100 mL). The organic layer was washed with saturated $Na_2S_2O_3$ twice and then brine and dried. After filtration and concentration, the residue was purified by silica gel chromatography and distillation. The structures of geranial and neral were confirmed by comparison of MS fragmentation pattern and by co-injection with authentic citral. The yields of synthesized geranial and neral were 81.8 and 77.9%.

Gas Chromatography and GC−Mass Spectrometry. GC analysis was performed on an Agilent 6890N equipped with a flame ionization detector (FID). DB-1MS and DB-FFAP columns (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA) were used for resolving the analytes. The oven temperature was programmed as follows: isothermal at 40 °C for 1 min, then rising at 6 °C/min to 250 °C, and held at this temperature for 4 min. After analysis, the oven temperature was raised to 300 °C and held at this temperature for 10 min as postrun. Helium was used as the carrier gas at a rate of 1.5 mL/min. GC-MS analyses of the extracts were performed by using an Agilent mass selective detector (MSD, Agilent 5973N MSD) interfaced with a 6890N GC equipped with a DB-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific). The analytic condition was the same as for GC analysis.

Extraction of Insect Body. Whole bodies of male and female beetles (50 or 100 individuals) were homogenized with hexane (100 μL/beetle) for detecting sex-specific compound. The solution was filtered through the glass wool. After the addition of *n*-decanol as internal standard (IS), the solution was concentrated under a gentle nitrogen stream. To find pheromone reservoir, the body of each sex (20 individuals, $N = 8$ male, $N = 5$ female) was dissected into head, thorax, and abdomen and then extracted with hexane for 1 h. The filtered extracts were treated as done in whole body extraction. The mean amount of male-specific components between male and female whole body extract was transformed into $\log_{10}(x + 1)$ and compared by *t* test ($P = 0.05$).

Collection of Volatiles in Boring Dust. Logs of *Q. mongolica* uninfected by *P. koryoensis* (15 cm long, 15−20 mm i.d.) were soaked in water for 14−20 days (*18*). Artificial entrance holes (2 mm i.d. and 1.5−2 cm in depth) were drilled with an electric drill into the logs at 3−5 cm intervals. A log has 45−80 of these entrance holes ($N = 8$). An unmated male adult was introduced into each hole through a plastic micropipet chip (1 mL volume). Within 1 day, they piled up thread-like boring dust like a bird's nest. This male-produced boring dust was less strongly attached around the entrance holes. Five days after males were introduced, the male-produced boring dust on the log was collected carefully with forceps, weighed, and extracted with hexane (200 μL of hexane per 100 mg of boring dust) for 1 day. After the boring dust had been removed, a female beetle was introduced into the hole that the male was in for mating. When females succeeded in mating, the shape of the boring dust changed into a ball-like form. Five days after the females had been introduced, the boring dust on the log was collected, weighed, and extracted with hexane for 1 day. The extracted solution of boring dust was filtered through the glass wool filter, and IS was added. As a control, artificial sawdust (1 g) prepared by using an electric drill was collected from the beetle-infected log, extracted, and analyzed.

Collection of Male-Produced Airborne Volatiles. A daily change of male-produced airborne volatiles was investigated. A *Q. mongolica* log containing 45 *P. koryoensis* males was placed into a three-neck separable flask. Volatiles were collected in a 10 cm long glass (6 mm o.d., 4 mm i.d.) packed with ca. 95 mg (2 cm in length) of Super Q column (Alltech, Deerfield, IL) held in place between two glass wool plugs. The same type of Super Q column was installed to supply purified air into the flask. The Super Q column was connected to an air pump, and the flask was aerated at a rate of 300 mL/min for 10 days. The Super Q column was exchanged everyday. The captured volatiles were eluted with 2 mL of hexane, and after the addition of IS, the elute was concentrated and analyzed on GC and GC-MS. The Super Q column was conditioned at 200 °C for 3 h under a nitrogen stream before using.

Electroantennography (EAG) Activity. Each pheromone blend component (10 μL) was applied onto a strip of filter paper (50 × 5 mm). The filter paper was placed into a Pasteur pipet. A glass electrode was filled with 0.1 N KCl solution, in contact with a silver wire. The head of the beetle was mounted on the reference electrode, and the distal tip of an antenna was on the recording electrode. A charcoal filtered and humidified air stream (1.5 L/min) was directed continually over the preparation, and a pulse stimulus of 0.5 s duration was added to the air stream every 30 s (stimulus controller CS-55, Synthec). Electroantennographic signals were amplified (Combi EAG probe, Synthec), transferred to IDAC-2 (Synthec), and recorded on a personal computer (EAG 2000, Synthec). A stimuli sequence was air, pheromone blend component (three times), and air. The recorded EAG activities were normalized as a percentage of the amplitude to the response of the air, which was defined as 100%. Mean responses between each pheromone component were compared by ANOVA followed by Bonferroni-corrected post hoc test at $P = 0.05$.

Field Assay. The experiment was carried out during May 7−28, 2008, in a natural *Quercus* spp. forest located in Paju, Gyeonggi province, Korea (37° 44′ N, 126° 54′ E). Two multifunnel traps (custom-made, overall height of ca. 1.5 m) composed of 20 clear plastic funnels (upper i.d. = 9 cm, lower i.d. = 2.5 cm, 8 cm in height) were stapled on a *Q. mongolica* tree. The lowest funnel was 20 cm above the ground. One was on the upside slope and the other on the downside slope. A plastic cup filled with approximately 100 mL of water was placed beneath the lowest funnel of the trap to collect captured beetles. Fifteen milligrams of pheromone blend diluted with hexane (200 μL) was applied on a dental cotton wick placed in a rubber septum. As a control, only hexane was applied. The rubber septum was hung on the trap at 50 cm above the ground. A pheromone blend treated rubber septum was place on each trap of the tree; thus, overall 30 mg of pheromone blend was loaded on a tree. The ratio of each compound in pheromone blend was 1:1:2:1:5 (citronellol/nerol/neral/geraniol/geranial). Thirty-two traps were deployed in 2 rows of 16 trees, in which traps in a row were separated by ca. 10 m. The distance between the rows was about 30 m. New baits were set between 10:00 and 12:00 a.m. The captured beetles were collected every 7 days, and the pheromone blend was renewed on the same day. The captured beetles were brought to the laboratory, sexed, and counted. The effect of pheromone blend on beetle capture was evaluated using the Wilcoxon test. After the test of pheromone blend effect was finished, all traps were set without pheromone blend, to evaluate positional effect among the traps. Two days later, the number of captured beetles was counted and compared.

Statistics. Statistical data were analyzed using S-Plus 2000 (MathSoft, Seattle, WA).

RESULTS

Extraction of Insect Body. GC-FID analysis of whole body extracts of male and female *P. koryoensis*showed the presence of male-specific compounds (peaks a−d in **Figure 1**). GC-MS analysis of the male body extract revealed the existence of nerol (a), neral (b), geraniol (c), and geranial (d). Comparison of retention indices of each compound with authentic compound on DB-1MS and DB-FFAP columns confirmed the structures of those compounds. The amount of each compound in both sexes is shown in **Table 1**. Among the male-specific compounds, geranial was the most abundant compound followed by neral, geraniol, and nerol. From the female whole body extract, the four compounds were detected in a small amount. The mean

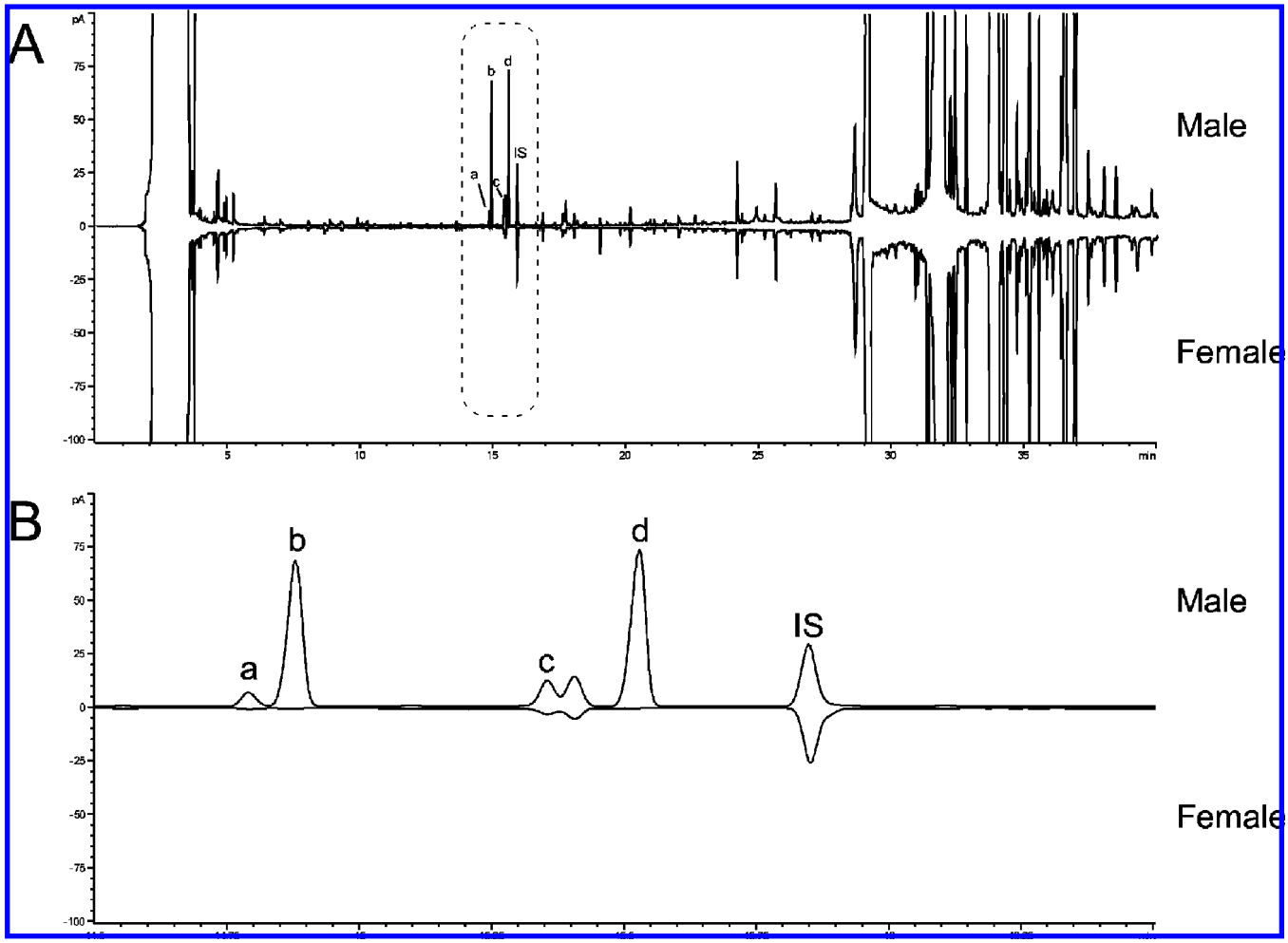

Figure 1. Representative gas chromatogram of whole body extract of *Platypus koryoensis*: (**A**) male body (upper), female body (lower); (**B**) magnification of dashed line in **A** [upper trace, male; lower trace, female; a, nerol; b, neral; c, geraniol; d, geranial; IS, internal standard (*n*-decanol, 10 ng)].

Table 1. Mean Amounts of Nerol, Neral, Geraniol, and Geranial Obtained from Whole Body Extract of Male and Female *Platypus koryoensis*

	amount of each component (mean ± SE, ng/beetle, $N = 8$) and occurrence frequency (OF)			
sex	nerol[a]	neral[b]	geraniol[c]	geranial[d]
male	1.85 ± 0.64 (OF = 7/8)	22.29 ± 5.86**[e](OF = 8/8)	3.07 ± 0.86 (OF = 8/8)	32.19 ± 9.03** (OF = 8/8)
female	0.54 ± 0.28 (OF = 3/8)	0.12 ± 0.10 (OF = 2/8)	1.40 ± 0.34 (OF = 7/8)	0.21 ± 0.14 (OF = 2/8)

[a] $t = -2.13$, df = 14, $P = 0.051$. [b] $t = -10.76$, df = 14, $P < 0.001$. [c] $t = -1.50$, df = 14, $P = 0.16$. [d] $t = -10.23$, df = 14, $p < 0.001$. [e] **, significantly different between male and female at $P < 0.01$ (*t* test).

amounts of geranial and neral in male whole body extract were significantly greater than those in female whole body extract. Although there was no significant difference, much more nerol was detected in male whole body extract than in female whole body extract. The four compounds (peak a−d in **Figure 1**) were detected from the abdomen extract of male beetles, but not detected from head or thorax, except one sample of thorax extract in which was detected 1.59 ng/beetle of neral (**Figure 2**; **Table 2**), whereas in female body part extract, no component described above was detected. The average proportions of the four compounds in male whole body extract and abdomen extract were 3:38:5:54 and 1:37:2:61 in the sequence of nerol, neral, geraniol, and geranial, respectively.

Analysis of Volatiles in Boring Dust. When males were introduced into an artificial hole, 72.4% of males succeeded in gallery formation and 6.4 mg/hole of boring dust was emitted. After females were introduced into the hole and mated, emitted boring dust was 14.8 mg per hole. GC-FID and GC-MS analyses revealed that male-produced boring dust extract contained citronellol in addition to the four male-specific compounds (peaks a−d in **Figure 1**; **Table 3**). Citronellol was identified by comparison of mass spectrum and co-injection with authentic compound on GC-MS. The peaks of citronellol and nerol were not resolved on our GC-FID system using a DB-1MS column but were resolved on the GC-MS system using DB-5MS. Thus, the integrated area of citronellol and nerol in the total ion chromatogram of GC-MS was used to determine the ratio of citronellol and nerol, and it was 1:1. The proportion of the five compounds was 22:4:18:55 in the sequence of nerol/citronellol, neral, geraniol, and geranial, respectively (**Table 3**.) In the case of artificial boring dust extract, no component above was detected. The amount of each compound in boring dust extract is summarized in **Table 3**.

Collection of Male-Produced Airborne Boring Dust Volatiles. The daily change of five components (nerol, citronellol, neral, geraniol, and geranial) in male-produced boring dust is shown in **Figure 3**. The production of five components was continued through the experiments (10 days). The amount of

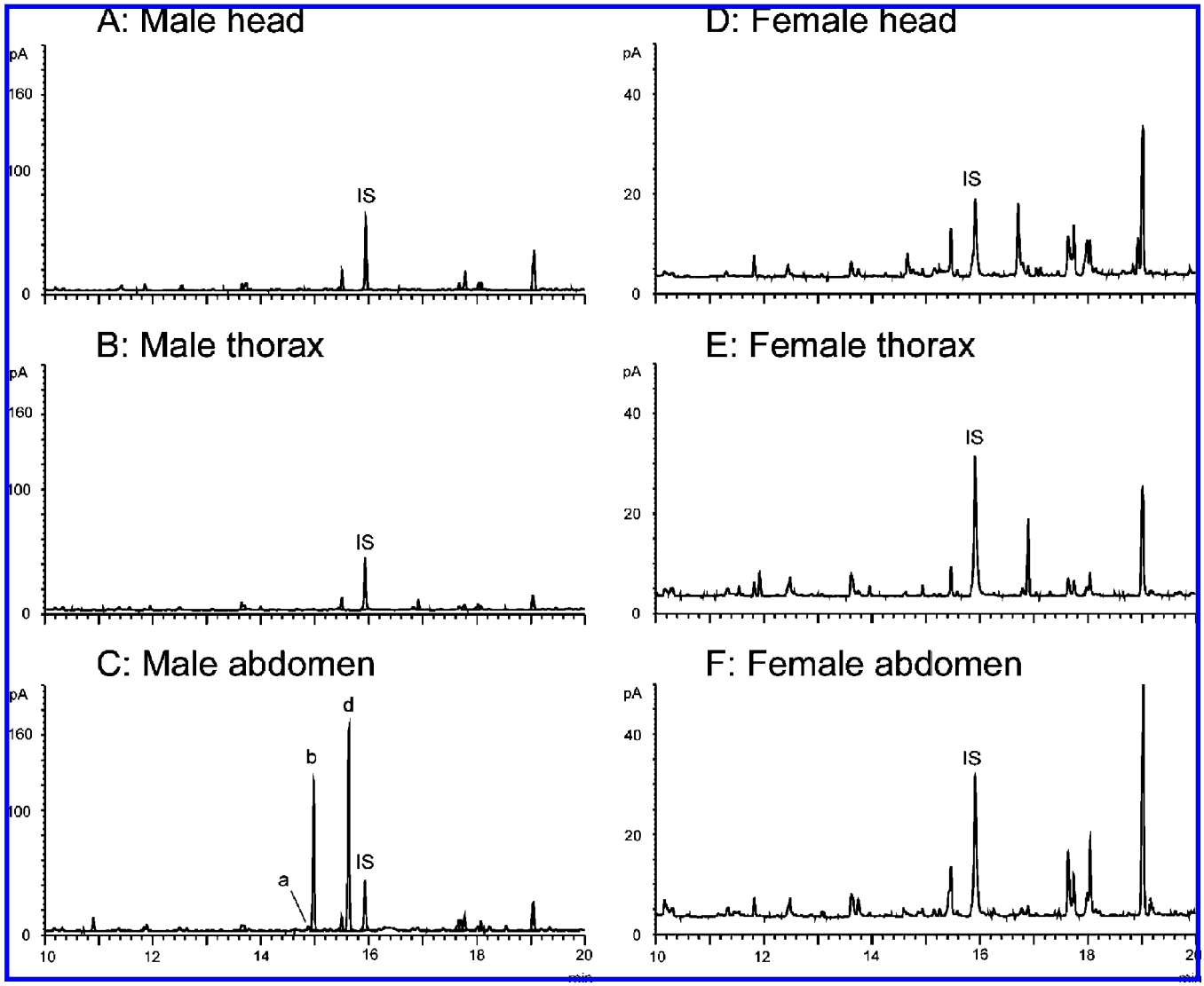

Figure 2. Representative gas chromatograms of body part extract of *Platypus koryoensis*: (**A**−**C**) male; (**D**−**F**) female body; a, nerol; b, neral; d, geranial (c, geraniol, is not shown); IS, internal standard (*n*-decanol, 10 ng).

Table 2. Mean Amounts of Pheromone Blend Components Obtained from Male *Platypus koryoensis* Body Part Extracts

	amount of compound (mean ± SE, ng/beetle, $N = 5$)			
body part	nerol	neral	geraniol	geranial
head	nd[a]	nd	nd	nd
thorax	nd	1.59 ± 1.59[b]	nd	nd
abdomen	0.55 ± 0.23	26.62 ± 3.52	1.12 ± 0.87	44.16 ± 5.58

[a] nd, not detected. [b] Detected in one of five samples.

geranial reached a peak at the second day after male introduction into the hole, whereas the other components reached peaks at the third day. After the fourth day, the emission amounts of all compounds decreased. Daily emitted amounts and proportions of each compound per beetle were 50:64:44:143 (ng/beetle) and 16:22:12:51 (%) in the sequence of nerol/citronellol, neral, geraniol, and geranial, respectively.

EAG. Male and female antennae responded to the five components and showed relatively high EAG activities (**Figure 4**). Neral was the most active compound for male antennae. Female antennae were more sensitive than male antennae to the compounds except for citronellol. Citronellol was shown to have the weakest antennal activity for both sexes.

Field Assay. The result of the field trap test is shown in **Table 4**. The number of captured male beetles in pheromone blend treated trap (treatment) was significantly greater than that in untreated trap (control) through the experiment period. In the case of female capture, except for the second week, the captured number of females in treatment was also significantly greater than that in control. The numbers of captured beetles were not significantly different at all positions (Wilcoxon test, $P = 0.13$, $Z = -1.52$).

DISCUSSION

Our results showed that the identified citronellol, nerol, neral, geraniol, and geranial detected from male body extract and male-produced boring dust volatiles comprised the male aggregation pheromone of *P. koryoensis*. These compounds were satisfied to pheromonal characteristics (sex-specific, consistently present, readily sensed by antennae, attractive), which had been suggested by Bartelt et al. (*20*). However, it remained to be investigated whether all five compounds are necessary to attract *P. koryoensis* or only one or parts of them are biologically active.

Nerol, neral, geraniol, and geranial were also detected from female whole body extract. However, considering occurrence frequency (**Table 1**) and the result of female body part extract,

Table 3. Mean Amounts of Each Pheromone Blend Component from Boring Dust Produced by *Platypus koryoensis*

boring dust type	*N*	amount of each component (mean ± SE, ng/beetle)			
		nerol and citronellol	neral	geraniol	geranial
unmated male produced	5	18.33 ± 2.69	3.48 ± 1.00	14.93 ± 7.34	44.93 ± 5.76
mated male and female produced	3	nd[a]	nd	0.78 ± 0.78[b]	1.01 ± 1.01[b]
control	3	nd	nd	nd	nd

[a] nd, not detected. [b] Detected in one of three samples.

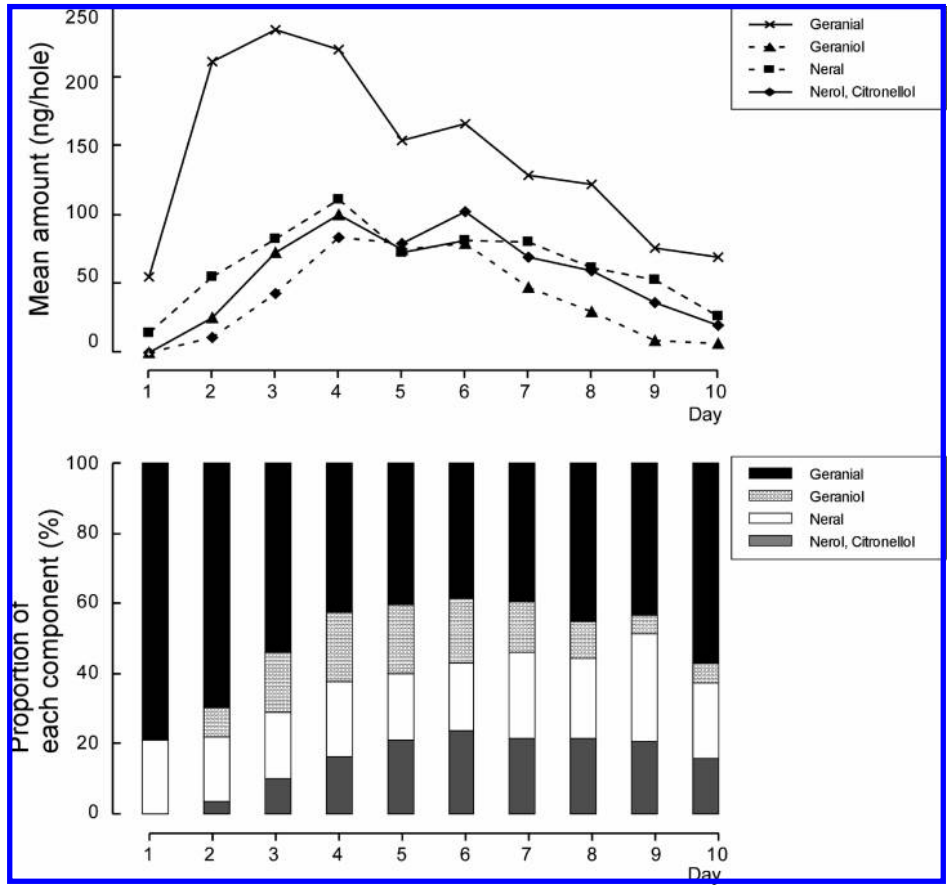

Figure 3. Daily changes of mean amount of each pheromone blend component (top) and its proportions (bottom). The pheromone blend component was obtained by collecting volatiles from *Quercus mongolica* log inoculated with the unmated male *Platypus koryoensis*, using Super Q.

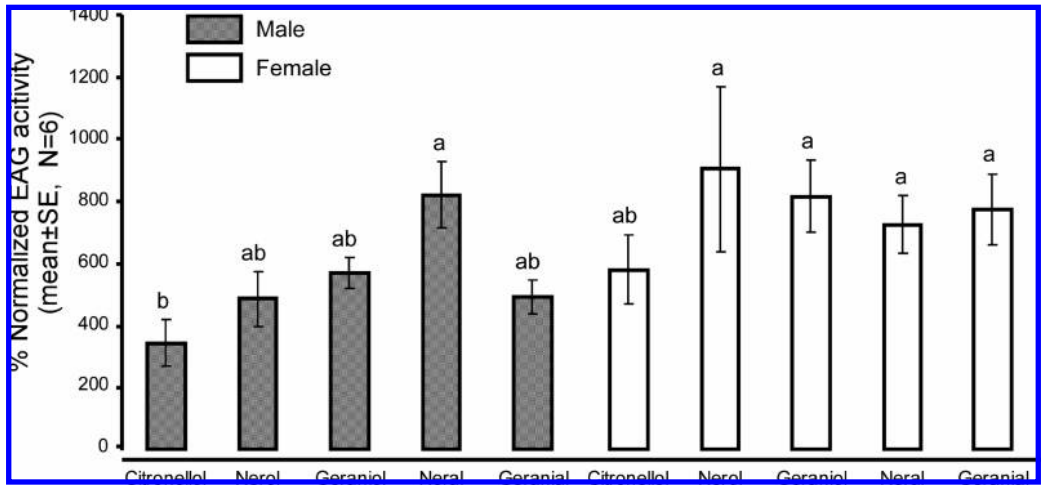

Figure 4. Electroantennographic activities of male and female *Platypus koryoensis* to each pheromone blend component. The same letters on error bars are not significantly different (Bonferroni-corrected post hoc test at $P < 0.05$).

those four compounds could be defined as male-specific compounds. The existence of the four compounds in female whole body extract may be due to small numbers of males mixed with female whole body extract, although sufficient attention was paid. Citronellol, which was not detected from any male body extract, was detected only from boring dust produced by

Table 4. Mean Numbers of *Platypus koryoensis* Caught in Multifunnel Traps[a] at Paju[b] in 2008

	number of beetles in a trap per week (mean ± SE)					
	male			female		
period	treatment $N = 16$	control $N = 16$	Z value	treatment $N = 16$	control $N = 16$	Z value
first week (May 7−14)	5.5 ± 2.1**[c]	1.0 ± 0.8	−3.012	1.4 ± 0.5*	0.3 ± 0.2	−2.2278
second week (May 14−21)	9.2 ± 4.9*	0.6 ± 0.3	−2.4034	1.1 ± 0.5	0.3 ± 0.3	−1.6614
third week (May 21−28)	68.4 ± 36.6**	5.9 ± 3.3	−2.6541	44.4 ± 24.6*	2.0 ± 1.2	−2.5296

[a] As a pheromone lure, the following mixture was used: citronellol (3 mg), nerol (3 mg), neral (6 mg), geraniol (3 mg), and geranial (15 mg). [b] Field assay was performed at a natural *Quercus* spp. forest located in Paju, Gyeonggi (37° 44′ N, 126° 54′ E). [c] The number of caught beetles in treatment and control traps was compared by Wilcoxon test; significant difference at *, $P < 0.05$, and **, $P < 0.01$, respectively.

unmated males. One possible exploration is that, unlike other components, citronellol would be biosynthesized after boring the entrance hole. To clarify this hypothesis, further investigation should be performed.

In this study, 75% of males introduced into the artificial holes bored the tunnel, and the amount of boring dust increased after mating. This result suggested that males initiated the gallery system and females enlarged and completed the gallery system. A similar gallery formation pattern has been reported for *P. quercivorus* (*21*). From the male-produced boring dust, four male-specific compounds and citronellol were detected, and those acted as an aggregation pheromone. A sex pheromone of *P. mutatus* (= *sulcatus*) and an aggregation pheromone of *P. quercivorus* have been identified from male-produced boring dust (*16*, *17*). Our result and early studies suggested that *Platypus* spp. cover their boring dust with pheromone compounds.

After mating, the pheromone blend of *P. koryoensis* was not detected from boring dust. In this study, geraniol and geranial were detected from boring dust produced by mated beetles. However, they were detected in only one replicate. It seemed to occur by contamination with male-produced boring dust. *P. koryoensis* is monogamous (personnel observation, J. Kim). Thus, cessation of pheromone production after mating would be of benefit for individual males with respect to energy conservation and completing gallery formation. After male *P. quercivorus* mated, the production of quercivorol, an aggregation pheromone, ceased and other sesquiterpenes were produced (personnel communication, T. Nakashima).

The amount of neral in the body extract and in Super Q collection was >37 ng/beetle and >100 ng/beetle at the fourth day, and its proportions in the pheromone blend were 37 and 22%, respectively. However, the amount of neral in hexane extract of male-produced boring dust was dramatically decreased to 3.48 ng/beetle. This difference may be attributed to sampling method difference or the different vapor pressures of each compound. Hexane extraction of boring dust extracted only the pheromone blend remaining on the boring dust; however, aeration using Super Q would collect not only pheromone blend that remained on the boring dust but also that emitted by male *P. koryoensis* simultaneously. This could be supported by the observation of the behavior of male *P. koryoensis* that thrust out his abdomen and moved it frequently up and down in the afternoon in the field, which was thought to emit pheromone.

Although the amounts of each component produced by male *P. koryoensis* were different in beetle body and boring dust extract, nerol, neral, geraniol, and geranial evoked the same levels of EAG activities to female antennae. However, the most abundant compound, geranial, evoked weaker EAG activities than neral to male antennae. This result suggested that each component could act differently on attraction to males and females irrespective of amount. This question could solved by an attraction test using as sole compound a pheromone source. Although the mean amount of citronellol in male-produced boring dust was almost the same as that of nerol, citronellol evoked the lowest EAG activities among the five components. In this study, the configuration of citronellol was not determined due to its tiny amount. Thus, an enantiomeric mixture of citronellol was used for testing its EAG activities to *P. koryoensis* antennae. The lowest EAG activity evoked by citronellol may be its low amount of proper enantiomer or interruption by enantiomer of each other. Determination of configuration of citronellol could solve this question.

Geranial and neral could be prepared by oxidation of the corresponding alcohols, geraniol and nerol, with MnO_2 or chromate. However, during the oxidation geometric isomerization occurred; thus, it should be chromatographed to obtain pure isomer (*22*, *23*). Matsuo et al. reported that *N-tert*-butylbenzenesulfenamide-catalyzed oxidation prepared pure isomers of geranial and neral (*24*). However, during the purification (distillation and/or chromatography), geranial and neral obtained by *N-tert*-butylbenzenesulfenamide-catalyzed oxidation produced ca. 30% of geometric isomers of each other, whereas the method of Piancatellia and Leonelli (*19*) used in this study gave satisfactorily pure isomers of geranial and neral.

All five components produced by male *P. koryoensis* have been identified as pheromones or attractants of a broad rage of insect species and arachnid orders (*25*). Lacey et al. (*26*) suggested that although terpenoid compounds are common in host plants, it seems more likely that male *Megacyllene carya* produced the compounds de novo. In our experiment, pheromone blend components were not detected from sawdust of the host tree but detected only from unmated males or boring dust produced by unmated males. This suggested that *P. koryoensis* biosynthesized those compounds de novo.

Most insets caught in the field bioassay were *P. koryoensis* adults, and this indicated the pheromone blend selectively attractive to *P. koryoensis*. The pheromone blend baited trap caught 5−15 times males and 4−20 times females compared to the control trap through the experiment. The number of male beetles captured in a trap was larger than the number of females. This may be attributed to the ecological characteristic of *P. koryoensis*. Similarly to *P. quercivorus* (*21*), male *P. koryoensis* aggregated on a host tree first and bored holes for the initiation of the gallery system. Females came later, mated, and completed construction of the gallery system. Because our field test period was the season that males emerged and aggregated on a host tree, the number of captured males was greater than that of females. Kamata et al. (*27*) pointed out that those ecological differences between sexes effected male-biased capture in *P. quercivorus*.

In this study, we identified an aggregation pheromone blend of *P. koryoensis*. To develop effective methods for semiochemical-based pest control, further analyses for the role of each

component and the combined effect of the pheromone and host kairomone component are needed.

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