Behavioral changes of powderpost beetle, *Lyctus africanus* Lesne (Coleoptera: Bostrichidae): responses on female extract

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**Abstract.** Powderpost beetle, *Lyctus africanus*, is a common dry wood pest in the tropical region. The insect pest damages wood by converting the wood into powdery frass. The damaged wood is created by larvae of *L. africanus* which digests the starchy wood part into powdery form. The powdery form developed in exit holes from which *Lyctus* emerges is an indicator of an active infestation. However, the presence of frass could be detected in the delayed period. Developing prevention of *Lyctus* entrance into the wood is crucial to minimize the losses. Monitoring the *Lyctus* infestation regularly, as chemical communication practice, leads to green technology in the pest control system. The chemical communication in *Lyctus* beetle was identified as the pheromone detected in male *Lyctus* beetle. In contrast, the female crude extract was observed to show none of the attractant behavior. In this study, the activity of female crude extract of *Lyctus* on the behaviour of the beetle was observed. The extract was from hexane washed of newly emerged female beetle for 24 h. Chemical analysis by GC-MS indicated dominancy of hydrocarbon compounds in the female crude extract. Purification of the hydrocarbon fraction was then conducted in SiO\(_2\) column with hexane solvent. Then, a preference test of the compounds against both male and female beetle was supervised in the Petri dish method. Result of the preference test indicated a repulsion behavior of both female and male beetles. Furthermore, the purified compound increased the repulsion behavior of *Lyctus* beetle. The hydrocarbon is suggested as a specific compound of female beetle which alters the behaviour of *Lyctus* beetle.

1. **Introduction**

The *Lyctus* powderpost beetle is a Lyctinae member of Bostrichidae family [1]. The beetles are a cosmopolitan species which is commonly found in a tropical region, such as Africa, Southeast Asia, North America, Europe [2], Australia and Japan [3], either as a native or an introduced species. The beetles convert the wood components of sapwood into small particles in the form of powdery frass. The beetles are considered to be one of the major pests threatening timber and timber products, including plywood [2], furniture, doors and ceiling [4]. The damage is inconspicuous inside the wood; thus, infestations are hardly located and monitored.

Some monitoring techniques have been developed to inspect insect populations regularly. The use of insect traps for monitoring has been widely used in pest management [5]. Study on pheromone of *Lyctus africanus* male beetles has been initiated [6]. The male and female beetles were attracted and aggregated in the crude male extract of *L. africanus*. Furthermore, synthetic compounds of those
compounds detected in the male extract also invited the conspecific species to exhibit aggregation behavior [6]. Then, it is suggested that male beetles alter behavioral change of *Lyctus* beetles by specific compounds found in the body part of male beetles. On the other hands, lack information on the female role in a group of *Lyctus* beetles is existed.

In this study, the activity of female crude extract of *Lyctus* beetles on beetles’ behavior was observed. The study is crucial to understand ecological aspects of *Lyctus* to develop strategies for monitoring and controlling *Lyctus* beetles.

2. Materials and method

2.1. *Insect colony*

The adult stage of *L. africanus* beetles was used in this study. The beetles were reared on artificial diet consisting of dried yeast (24%, Asahi Food and Health Care, Japan), starch (50%, Nacalai Tesque, Japan) and Meranti wood (*Shorea* spp.) sawdust (26%) in a glass jar (450 mL) in controlled chamber (26 ± 2 °C and 65 ± 10% relative humidity). For determination of male and female beetles, observation on visible apical hairs along the hind margin of the abdominal segment of adult female was conducted [7].

2.2. Collection of chemical compound

The crude extract of female beetles was collected by a whole body extraction on newly emerged beetles using hexane in a self-lock tube (Eppendorf tube, 2 mL, Germany). The extraction was conducted for 50 female beetles with hexane (1000µL) for 24 hours at 26 °C and 65 % relative humidity. Then, the extract was transferred into a new tube.

2.3. Behavioral activity

Dual-choice bioassays [8, 9] were performed in a dark climatic chamber. The assays were conducted in a closed Petri dish (Ø 90 mm) with filter paper (Ø 90 mm, Whatman No. 1, GE Healthcare, UK) at the bottom. The crude extract (50 µL) of female was applied to a paper disc (Ø 10 mm, 60 mg, Advantec type 27, Toyo Roshi Kaisha, Japan). The same volume of hexane was applied to the control paper disc, and the discs were then air-dried for approximately 1 min before the bioassay. One pair of paper discs were placed in a Petri dish, with the discs were placed opposite one another (60 mm in the distance). A group of *L. africanus* beetles (20 individuals), both females and males, were placed in one side part of Petri dish, so they had the same starting point; thus the distance of each disc from the starting point was the same. The number of beetles located on either of the paper discs was observed for 5 min. Beetles settling in the free area (neither treated nor untreated paper discs) were considered as nonresponders. Ten replicates were made for each combination. To determine the attraction or repulsion responses with regard to the extract, an aggregation index (AI) value was calculated as \((T – C)/N\), where \(T\) is the number of beetles located on the treated paper disc, \(C\) is the number of beetles located on the control disc, and \(N\) is the total number of beetles used in the bioassay. A positive value of AI corresponds to an attraction response, while a negative value corresponds to a repulsion response.

2.4. Chemical analysis

One beetle was immersed in hexane (10 µL) for 5 min. To run the analysis, the extract (1 µL) was injected into gas chromatography-mass spectrometry (GC-MS) instrument. The GC-MS analyses were conducted with a Network GC System (6890N; Agilent Technologies, USA) coupled with a mass selective detector (5975 Inert XL; Agilent Technologies) operated at 70 eV. The column used was an HP-5MS capillary column (Agilent Technologies, 0.25-mm I.D. x 30 m, 0.25-µm film thickness). The carrier gas was helium with a constant flow rate of 1.00 ml/min. Samples were analyzed in the splitless mode with the temperature programmed to change from 60 °C (initially for 2 min) to 290 °C at a rate of 10 °C/min. The final temperature (290 °C) was then maintained for 5 min. The GC-MS data were recorded using Chemstation (Agilent Technologies) with reference to an MS database (Agilent NIST05 mass spectral library, Agilent Technologies). Column chromatography was performed on a Wakosil silica gel C-200 with the specified solvents. 1H- and 13C-NMR spectra were recorded on a Bruker...
Biospin AC400M spectrometer (400 MHz for $^1$H and 100 MHz for $^{13}$C) using TMS (tetramethylsilane) as the internal standard.

2.5. Solvent extraction and isolation

The adults (100 individuals) were transferred into a tube (self-lock Eppendorf tube, 2 mL, Germany), and dipped into hexane (2 ml) for 24 hours. The extracts, after being separated from the beetle bodies, were concentrated in vacuo and applied to an SiO$_2$ column (0.5 g, Wako-gel C-200). The column was eluted with hexane solvent. The fraction was subjected to GC-MS analyses and concentrated to 2-ml aliquots for bioassay.

3. Results and discussions

3.1. Bioassay for crude extract and purified fraction

The adult stage of $L. africanus$ beetles, both females and males, were exposed to various combination of female crude extract (FE) vs. control and its purified fraction (PF) vs. control. The beetles moved immediately after being released in Petri dish. The beetles, both male and female, preferred to stay away from treated paper discs either with female crude extract or those with a purified fraction. The AI value of crude extract and purified fraction combination expressed minor value, which was lesser than 0.1 for FE, and negative for a purified fraction (table 1). The AI values indicated a repulsion response for all treatments.

The AI values indicated that purification of FE in hexane solvent induced more repulsion behavior both male and female beetles. The chemical compounds in both FE and PF were considered as significant factors in this behavior.

Table 1. Response of adult $Lyctus africanus$ to treated discs with female crude extract (FE) or purified fraction (PF) and control, presented by aggregation index value ($N=20; n=10$).

| Tested beetles | Treatment  | FE vs Control | PF vs Control |
|----------------|------------|---------------|---------------|
| ♀              | 0.05 ± 0.04 | -0.2 ± 0.15   |               |
| ♂              | 0.03 ± 0.02 | -0.06 ± 0.11  |               |

3.2. Chemical analysis

Crude extract from female beetles (FE) and its purified fraction (PF) was compared using GC-MS analysis (figure 1). Four major peaks (detected as hydrocarbons of C$_{25}$, C$_{27}$, C$_{29}$, and C$_{31}$) were specifically detected in large amounts in both chromatograms. C$_{27}$ and C$_{29}$ were detected in higher quantity than other compounds. Those hydrocarbon compounds were also detected either smaller amounts or absent in male beetles than those in female beetles [6]. Kartika et al. 2015 reported that the hydrocarbons detected in hexane fraction were not involved in aggregation behavior of $L. africanus$.

In general, the hydrocarbons comprise a significant portion of the cuticular lipids of insects. The cuticular hydrocarbons compounds could prevent desiccation and are also important in chemical communication, which allow them in recognizing signals between individuals [10, 11]. It was reported in crickets, the cuticular hydrocarbon is known as chemosensory of female-specific scent imbued by female to the male cricket during mating [12]. The hydrocarbon labeled in male crickets used by the female to recognize the prior mates. As the female prefers novel mates in mating, the labeled male crickets are marked and avoided for re-mating by females [12]. In this study, the presence of hydrocarbon in both FE and PF extract, from which was identified as mated partners, was supposed to be avoided by both male and female $L. africanus$ beetles.

In general, females frequently mate more often than their necessary to ensure complete fertilization and it allows females to maximize the number of material resources obtained from males [13] and to derive indirect genetic benefits by mating with genetically superior sires [14]. Multiple mating with different partners is necessary to provide significant fitness benefits to females [15, 16]. Some studies also recorded that females of particular insects preferentially mate with novel males rather than with
males with whom they have recently mated [17, 18]. Hence, females should discriminate against previous mates using specific compound to find genetically superior sires.

As stated previously, in favor of seeking mating partners, female crickets (Gryllodes sigillatus) relied on self-referent cues to identify previous mates by marking the males with their unique chemical signs during mating [12]. In another case of male beetle, the rustic borer beetle, (Xylotrechus colonus F. (Coleoptera: Cerambycidae), recognized females by distinguishing the female’s cuticular hydrocarbon, which was identified as female-specific compounds.

In this study, the repulsion behavior of L. africanus in response to FE and PF might be the result of several factors, including the following: 1) females might prevent other females or unwanted mated males by profiling the hydrocarbon compounds, and 2) males discriminate the mated females by outlining the hydrocarbon patterns. Further examination is required in favor of profiling the actual function of hydrocarbons in the chemical ecology of L. africanus beetles.

4. Conclusions

The adult L. africanus beetles, both male and female, showed repulsion behavior against treated paper discs either with female crude extract or those with a purified fraction. The hydrocarbon compounds, which were identified as female-specific compounds, were considered as significant factors in this behavior. The female and male beetles were supposed to discriminate the unwanted female or male by relying on cuticular hydrocarbon.

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5. References

[1] Liu LY and Schönitzer K. 2011. Phylogenetic analysis of the family Bostrichidae auct. at suprageneric levels. *Mitteilungen des Münchener Entomologische Gesellschaft* 101: 99-132

[2] Halperin J and Geis KU. 1999. Lyctidae (Coleoptera) of Israel, their damage and its prevention. *Phytoparasitica* 27: 257-262

[3] Mito T and Uesugi T. 2004. Invasive alien species in Japan: the status quo and the new regulation for prevention of their adverse effects. *Global Environ Res* 8: 171-193

[4] Iwata R. 1982. Occurrence record of *Minthea rugicollis* Walker and an unrecorded species, *Lyctus africanus* Lesne (Col., Lyctidae) from Japan (in Japanese). House Househ Insect Pest: 60-63

[5] Burkholder WE and Ma M. 1985. Pheromones for Monitoring and Control of Stored-Product Insects. *Annu Rev Entomol* 30: 257-272

[6] Kartika T, N. Shimizu, T. Yoshimura (2015) Identification of Esters as Novel Aggregation Pheromone Components Produced by the Male Powder-Post Beetle, *Lyctus africanus* Lesne (Coleoptera: Lycitinae). *PlosOne*

[7] Gerberg EJ. 1957. *A revision of the new world species of powder-post beetles belonging to the family Lycitidae*, vol Technical Bulletin No. 1157., vol 1157, April edn. US Dept. of Agriculture, Washington, D.C

[8] Yinon U and Shulov A. 1967. New findings concerning pheromones produced by *Trogoderma granarium* Everts (Coleoptera, Dermestidae). *J Stored Prod Res* 3: 251-254

[9] Schulz S, Fuhlendorff J, Steidle JL, Collatz J and Franz JT. 2004. Identification and biosynthesis of an aggregation pheromone of the storage mite *Chortoglyphus arcuatus*. *ChemBioChem* 5: 1500-1507

[10] Howard RW and Blomquist GJ. 1982. Chemical ecology and biochemistry of insect hydrocarbons. *Annu Rev Entomol* 27: 149-172

[11] D'Ettorre P and Heinze J. 2005. Individual recognition in ant queens. *Curr Biol* 15: 2170-2174

[12] Ivy TM, Weddle CB and Sakaluk SK. 2005. Females use self-referent cues to avoid mating with previous mates. *Proc R Soc B* 272: 2475-2478

[13] Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60, 145–164

[14] Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? *Biol. Rev.* 75, 21–64

[15] Sakaluk, S. K., Schaus, J. M., Eggert, A.-K., Snedden, W. A. & Brady, P. L. 2002 Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. *Evolution* 56, 1999–2007

[16] Ivy, T. M. & Sakaluk, S. K. 2005 Polyandry promotes enhanced offspring survival in decorated crickets. *Evolution* 59, 152–159

[17] Archer, M. S. & Elgar, M. A. 1999. Female preference for multiple partners: sperm competition in the hide beetle, *Dermestes maculatus* (DeGeer). *Anim. Behav.* 58, 669–675

[18] Eakley, A. L. & Houde, A. E. 2004. Possible role of female discrimination against ‘redundant’ males in the evolution of colour pattern polymorphism in guppies. *Proc. R. Soc. B. 271*(suppl.), S299–S301