

# 環動昆

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## Identification of Benzoquinones and Hydroquinones as the Secretory Compounds from Three Species of Okinawan Millipedes

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沖縄産ヤスデ3種の分泌物成分、ベンゾキノン類およびヒドロキノン類の同定  
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沖縄本島で採集したマルヤスデ目ミナミヤスデ科のミナミヤスデ *Trigoniulus lumbricinus* Gerstaecker, マルヤスデ目カグヤスデ科のタカクワカグヤスデ *Spirobolellus takakuwai* Wong, ヒキツリヤスデ目ヒモヤスデ科リュウキュウヤハズヤスデ *Glyphiulus septentrionalis* Murakamiの防御分泌物の構成成分をガスクロマトグラフ (GC) 法, ガスクロ質量分析法及び核磁気共鳴吸収スペクトル分析法を用いて調べた。3種の分泌物は2-メトキシ-3-メチル-1,4-ベンゾキノンを共通の主成分とするが, その他副・微量成分のプロフィールは種それぞれに特異的と判断した。近年の侵入種ミナミヤスデには2-メトキシ-3-メチル-1,4-ベンゾキノン (GCピーク面積比72%) 以外に副成分トルキノン (25%) の存在が文献で知られているが, その他に微量成分として5成分を検出し, うち4成分を2,3-ジメトキシ-1,4-ベンゾキノン, 2,3-ジメトキシ-5-メチル-1,4-ベンゾキノン (3%) 及びトルヒドロキノン, 2-メトキシ-3-メチル-ヒドロキノンと同定した。タカクワカグヤスデからは2-メトキシ-3-メチル-1,4-ベンゾキノン (95%) と2,3-ジメトキシ-1,4-ベンゾキノン (5%) を主成分, その他4微量成分のうち3成分を2,3-ジメトキシヒドロキノン, 2,3-ジメトキシ-5-メチル-1,4-ベンゾキノン, 2-メトキシ-3-メチル-ヒドロキノンと同定した。リュウキュウヤハズヤスデからは主成分2-メトキシ-3-メチル-1,4-ベンゾキノン (100%) 以外に2微量成分を検出し, 2,3-ジメトキシ-1,4-ベンゾキノン及び2-メトキシ-3-メチル-ヒドロキノンと同定した。

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The defense secretions from three Okinawan millipedes, *Trigoniulus lumbricus* Gerstaecker, 1873 (Spirobolida: Pachybolidae), *Spirobolellus takakuwai* Wang (Spirobolida: Spirobolellidae), and *Glyphiulus septentrionalis* Murakami 1975 (Spirostreptida: Cambalo-psidae) were a characteristic mixture of quinones and hydroquinones. In addition to two quinones already known, 2-methoxy-3-methyl-1,4-benzoquinone (**2**) and toluquinone (**1**), four more compounds were found from *T. lumbricus* in decreasing order of abundance; 2,3-dimethoxy-5-methyl-1,4-benzoquinone (**6**), 2,3-dimethoxy-1,4-benzoquinone (**3**), toluhydroquinone (**4**), 1,4-dihydroxy-3-methylbenzene, and 2-methoxy-3-methylhydroquinone (**7**). From *S. takakuwai*, five compounds were identified: **2**, **3**, 2,3-dimethoxyhydroquinone (**5**), **6**, and **7**. *G. septentrionalis* contained **2**, **3**, and **7**. Quinone **2** was the major component in all three species together with traces of **3** and **7**.

**Key words:** Millipedes, Benzoquinones, Hydroquinones, *Spirobolellus takakuwai*, *Glyphiulus septentrionalis*, *Trigoniulus lumbricus*

## Introduction

Defensive secretions have been studied in the six of the ten orders of millipedes and been largely classified into three groups by their chemical structures. In the first group, of Polydesmida, are cyanogenic species that produce mandelonitrile or its decomposition products as a mixture of benzaldehyde and hydrogen cyanide. In the second group, of Spirobolida, Julida, and Spirostreptida, are benzoquinone emitters. In the third group, of Glomerida and Polyzoniida, are emitters of other alkaloids and nitro compounds. There are no publications reporting defensive compounds of species in the remaining four orders, Polyxenida, Chordeumatida, Platydesmida, and Siphonophorida (Eisner *et al.*, 1978).

The secretions were studied extensively in the 1980s when mass spectrometry coupled with gas-liquid chromatography (GC-MS) was developed, except for Japan. Most Japanese millipedes (277 species belonging to 27 families in 10 orders) were not studied, and because more than 97% of the

species in Japan are endemic (Murakami, 1993), details of their defensive secretions remained unknown. The chemical compositions of secretions of the following four cyanogenic species collected in Japan have been studied: *Epanerchodus japonicus* Carl (Polydesmida: Polydesmidae), with benzaldehyde, phenol, and mandelonitrile (Mori *et al.*, 1994), *Parafontaria laminata armigera* Verhoeff (Xystodesmidae: Polydesmida), with benzaldehyde, mandelonitrile, benzoyl cyanide, mandelonitrile benzoate, and benzoic acid (Mori *et al.*, 1995), *Nedyopus patrioticus patrioticus* (Polydesmida: Paradoxosomatidae), with *p*-cresol, benzaldehyde, phenol, and mandelonitrile (Noguchi *et al.*, 1997a), and the Okinawan millipede *Chamberlinius hualienensis* Wang (Polydesmida: Paradoxosomatidae) of seven compounds, benzoic acid, mandelonitrile, benzaldehyde, benzonitrile, methyl benzoate,  $\alpha$ -methoxybenzyl alcohol, and mandelonitrile benzoate (Noguchi *et al.*, 1997b). Except for benzoic acid in the last species, the other compounds have been listed in an earlier monograph (Eisner *et al.*, 1978).

Another compound, 2-nitroethenylbenzene, has been recently identified as a component of secretions of the white millipede *Eucondylodesmus elegans* Miyosi (Polydesmida: Doratodesmidae) (Kuwahara *et al.*, 2002). It is of interest to identify the defense compounds of Japanese millipedes as well, especially as new components may be found.

As part of our chemical analysis of Japanese millipedes, we collected three Okinawan species belonging to Spirobolida, and Spirostreptida, as benzoquinone emitters, and examined their defence secretions.

## Materials and Methods

### Millipedes examined

*Trigoniulus lumbricinus* Gerstaecker 1873 [Spirobolida: Pachybolidae, (*minami-yasude* in Japanese), body length, ca. 3-6 cm, body diameter, ca. 5 mm, and body weight, 719 mg ( $n=1$ )] was collected at a sugar-cane field in Ishikawa City, Okinawa, in November 1996. The species was first found in the area near a US Air Force base and has been considered to be one of the naturalized animals on the island (Anonymous, 1996). The species, originally from Southeast Asia, crawls periodically into houses, where it is considered a nuisance (Higa *et al.*, 1993). When disturbed, the millipede being to curl up with its head in the center to become a disk of about 3 cm in diameter. It has a mixture of toluquinone (**1**, methyl-1,4-benzoquinone) and 2-methoxy-3-methyl-1,4-benzoquinone (**2**) as its defence secretion (Monro *et al.*, 1962), and was used here as the standard in the elucidation of the chemical components of the other species.

*Spirobolellus takakuwai* Wang [Spirobolida: Spirobolellidae; *takakuwakaguya-yasude* in Japanese; body length, ca. 2 cm in length; body diameter, ca. 2 mm, weighing 72.6  $\pm$  12.9 mg (mean  $\pm$  SD,  $n=5$ )] was collected at a sugar-cane field in Kochinda,

Okinawa, in November 1996. The species is creamy white, with a pair of brown secretory glands on the sides of each body segment, and emits luminescence when disturbed in the dark (Shinohara and Higa, 1997).

*Glyphiulus septentrionalis* Murakami 1975 [Spirostreptida: Cambalopsidae; *ryuukyuu-yahazu-yasude* in Japanese; body length, ca. 2.5 cm; body diameter, ca. 1.5 mm, weighing 10.2  $\pm$  2.2 mg (mean  $\pm$  SD,  $n=5$ )] was collected together with *S. takakuwai* from the same soil and on the same date as mentioned above. The species is slender and characteristically seems to freeze for a moment, to look like a straight or bent rod, when disturbed. The species, which is a pale charcoal color, does not coil up like other species.

All three species were kept alive at 25 °C in a polyethylene bag with raw sliced potatoes as food.

### Analytical procedures and conditions

Individuals of *S. takakuwai* and *G. septentrionalis* were placed in MeOH (0.5 or 0.2 ml, respectively) for 3 min, and the yellow liquid obtained was decanted. Portions of extracts thus prepared were examined by gas-liquid chromatography (GLC) or GC-MS at the condition indicated below. Extraction and analysis were repeated three times with three individuals of each species. A 2-ml MeOH extract from one individual of *T. lumbricinus* was used as the standard for analyses.

GLC was done on an HP-5890 series II Plus apparatus (Hewlett Packard) equipped with a flame ionization detector and an HP-5 capillary column (0.32 mm  $\times$  30 m; film thickness of 0.33  $\mu$ m) at a programmed temperature increase condition from 60 °C to 290 °C at 10 °C/min with an initial pause for 2 min. Each sample was analyzed in a split-less mode with He as the carrier gas, and the chromatogram was processed by an HP3396 series II Integrator. GC-MS was done by an HP-5989B

mass spectrometer, operated at 70 eV, with the same column and operating conditions as above.

An NMR spectrum was obtained by a Bruker AC 300 MHz apparatus to elucidate the structure of benzoquinone, with crude extract of millipedes soaked in  $\text{CDCl}_3$  (800  $\mu\text{l}$ ) for 3 min. The yield of each component in MeOH extracts from the three species was determined by GLC with 2-tridecanone as the internal standard.

## Results and Discussion

All compounds found in the three species are listed in Fig. 1. The aromatic proton region ( $\delta$  6.40–6.80) of the NMR spectra (Fig. 2), indicates the proportion of the component; *T. lumbricinus* indicated spectrum of a mixture composed of more than two compounds, whereas that of *G. septentrionalis* was one almost pure compound. Two ortho-coupled protons (10.05 Hz) as two sets of doublets at  $\delta$  6.60

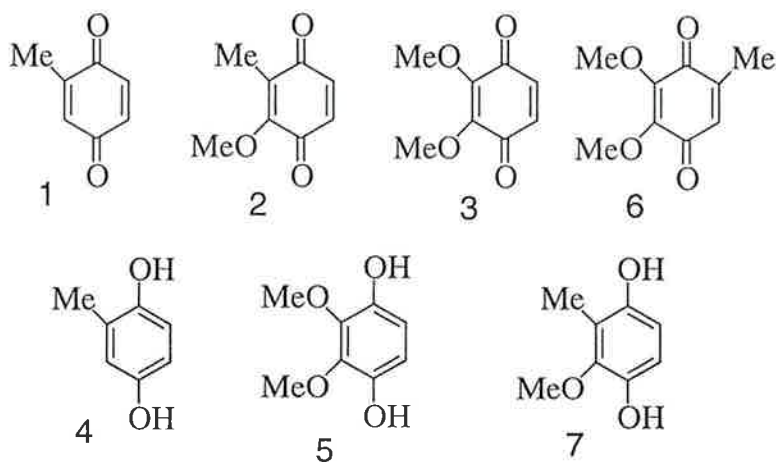


Fig.1 Structures of quinones and hydroquinones identified in three species of Okinawan millipedes.

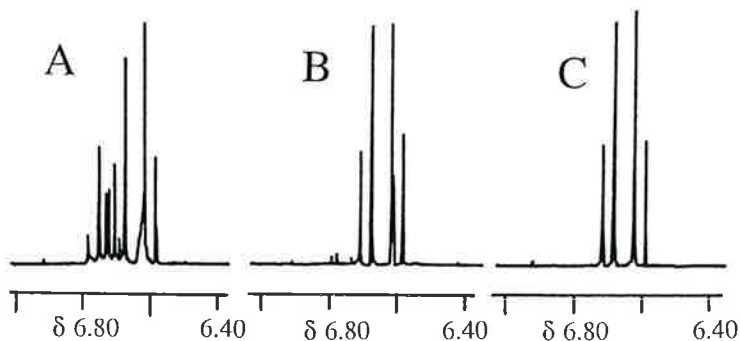


Fig. 2 NMR spectra ( $\delta$  6.4–6.8) of three species of Okinawan millipedes, measured in  $\text{CDCl}_3$  with a 300 MHz spectrometer.

A : *Trigoniulus lumbricinus*, B : *Spirobolellus takakuwai*,  
C : *Glyphiulus septentrionalis*

and 6.70, together with methyl and methoxy protons at  $\delta$  1.96 and  $\delta$  4.03 (not shown) suggested the shared major component to be 2-methoxy-3-methyl-1,4-benzoquinone (**2**). Results of GLC (Table 1) showed seven peaks (1-5, 7 and 8) for the extract of *T. lumbricinus*, among which two peaks (1, relative abundance, 25%; ca. 0.4 mg/millipede, and 2, 72%; ca. 1.1 mg/millipede) were of major compounds. The extract of *S. takakuwai* gave six peaks (2, 3, and 5-8), among which peaks (2 and 3) were of the major components, with relative abundance of 95% ( $241.6 \pm 92.7 \mu\text{g}/\text{millipede}$ , mean  $\pm$  SD,  $n = 5$ ) and 5%, respectively. The extract of *G. septentrionalis* gave three peaks (2, 3, and 8); peak 2 was major and the other two peaks were minor.

The major component (peak 2) in all three defense secretions gave an  $M^+$  and base ion peak at  $m/z$  152 (100%), with the following fragment ions: 137 (5%), 134 (3%), 122 (35%), 109 (15%), 94 (5%), 82 (12%), 66 (14%), and 53 (12%), and the structure was elucidated to be 2-methoxy-3-methyl-1,4-benzoquinone (**2**). The structure was consistent with the NMR spectrum mentioned above. This compound was reported earlier as being found in *T. lumbricinus*, together with toluquinone (**1**, peak 1) (Monro *et al.*, 1962). The presence of **1**

in *T. lumbricinus* was confirmed not only by GC-MS of the compound in peak 1, which gave an  $M^+$  and base ion at  $m/z$  122 (100%) together with the following diagnostic ions:  $m/z$  94 (57%), 82 (38%), 68 (16%), 66 (26%), and 54 (25%), but also by the following NMR data; methyl protons at  $\delta$  2.06 together with aromatic protons' multiplet around  $\delta$  6.78-6.58.

Besides these quinones **1** and **2**, two more quinones (peaks 3 and 7) were detected in *T. lumbricinus* and *G. takakuwai*, and peak 3 was detected in *G. septentrionalis*. Results of GC-MS of peak 3 indicated an  $M^+$  ion at  $m/z$  168 (98%) and base ion at  $m/z$  123 (100%), together with the following diagnostic ions:  $m/z$  153 (44%), 139 (14%), 125 (20%), 95 (18%), 82 (27%), 69 (31%), and 54 (19%). On the basis of this mass spectrum and NMR spectrum of the extract from *S. takakuwai*, in which the compound was found at 5% relative intensity, the structure of the compound in peak 3 was unambiguously elucidated to be 2,3-dimethoxy-1,4-benzoquinone (**3**), the O-CH<sub>3</sub> protons of which appeared by NMR to overlap at  $\delta$  4.03 with those of **2**. Peak 7 (3% relative intensity in *T. lumbricinus*) indicated an  $M^+$  and base ion at  $m/z$  182 (100%), together with the following

Table 1 Relative abundance of quinones and hydroquinones in three species of Okinawan millipedes

GC peak	$t_R$ min	Compound identified	Percentage in:		
			<i>T. lumbricinus</i>	<i>S. takakuwai</i>	<i>G. septentrionalis</i>
1	6.02	Toluquinone ( <b>1</b> )	25	nd <sup>2)</sup>	nd
2	8.60	2-Methoxy-3-methyl-1,4-benzoquinone ( <b>2</b> )	72	95	100
3	10.54	2,3-Dimethoxy-1,4-benzoquinone ( <b>3</b> )	tr <sup>1)</sup>	5	tr
4	10.82	Toluhydroquinone ( <b>4</b> )	tr	nd	nd
5	11.00	Structure not identified	tr	tr	nd
6	11.24	2,3-Dimethoxyhydroquinone ( <b>5</b> )	nd	tr	nd
7	11.86	2,3-Dimethoxy-5-methyl-1,4-benzoquinone ( <b>6</b> )	3	tr	nd
8	11.89	2-Methoxy-3-methylhydroquinone ( <b>7</b> )	tr	tr	tr

1) tr: Trace (intensity) less than 1%.

2) nd: Not detected.

ions: 167 (47%), 153 (15%), 139 (24%), 137 (89%), 136 (25%), 111 (17%), 83 (32%) and 69 (22%). The structure of the compound in peak 7 was suggested to be 2,3-dimethoxy-5-methyl-1,4-benzoquinone (**6**), methyl protons of which were confirmed at  $\delta$  2.06 overlapping with those of **1**.

Mass spectra of peaks 4, 6, and 8 were characteristically composed of an  $M^+$  and  $M^+ - m/z 2$  ion set ( $M^+$  and  $M^+ - 2$  ion set, hereafter) as follows: peak 4 gave an  $M^+$  (and base ion) at  $m/z$  124 (100%) and  $m/z$  122 (99%), together with ions at  $m/z$  123 (51%), 94 (52%), 82 (38%), 68 (18%), 66 (29%) and 54 (28%). Peak 6 indicated an  $M^+$  and base ion at  $m/z$  170 (100%) and  $m/z$  168 (74%), together with ions at  $m/z$  155 (48%), 153 (36%), 137 (11%), 123 (62%), 112 (16%), 109 (22%), 95 (11%), 82 (14%), 69 (23%), and 54 (11%). Peak 8 showed an  $M^+$  ion at  $m/z$  154 (47%) and base ion at  $m/z$  152 (100%), with ions at  $m/z$  139 (32%), 122 (35%), 111 (21%), 109 (15%), 94 (8%), 82 (15%), 66 (15%), and 53 (12%). Such characteristics suggested that these were hydroquinones (Budzikiewicz *et al.*, 1967).

Trial measurement of hydroquinone (1,4-dihydroxybenzene) as the model compound showed the same phenomena. Hydroquinone at a dose around its detection limit indicated an  $M^+$  (and base ion) at  $m/z$  110 (100%) and  $M^+ - 2$  ion at  $m/z$  108 (72%). In this mass spectrum, an  $M^+$  and all other fragment ions from benzoquinone also were present. However, intensities of the ions seemed to differ depending upon the sample size. At higher doses than its detection limit, the intensity of the  $M^+ - 2$  ion decreased to 11% or less against its  $M^+$  (100%), dose-dependently. Intensities of other fragments at a higher dose were as follows: 82 (19%), 81 (22%), 55 (16%), 54 (18%) and 53 (18%). Whereas the corresponding benzoquinone gave an  $M^+$  and base ion at  $m/z$  108 (100%), with

ions at  $m/z$  82 (22%), 80 (23%), and 54 (37%). We, therefore, concluded that peak 4 was of toluhydroquinone (**4**), that peak 6 was of 2,3-dimethoxyhydroquinone (**5**), and that of peak 8 was of 2-methoxy-3-methylhydroquinone (**7**), respectively.

Peak 5 indicated an  $M^+$  at  $m/z$  182 (5%) and base ion at  $m/z$  45 (100%), together with the following fragment ions; 151 (9%), 137 (15%), 122 (36%) 109 (4%), 93 (3%), 82 (8%), 67 (5%), 63 (6%) and 53 (8%). The structure of the component in peak 5, however, remained obscure at present.

The three Okinawan species, belonging to the orders, Spirobolida, and Spirostreptida, were quinone emitters, as reported by Eisner *et al.* (1978). A total of four benzoquinones were identified, three of which were accompanied by traces of the corresponding hydroquinone. A total of six hydroquinones have been reported in the secretion from the spirostreptid *Telodeinopus aoutii* by Deml and Huth (2000), among which hydroquinones **5** found in *S. takakuwai* was the second example. Those hydroquinones probably were biosynthetic precursors, as suggested by Deml and Huth (2000).

Quinone **1** is widely distributed among those three orders, as is **2** (Eisner *et al.*, 1978), but **1** was not detectable in *S. takakuwai* or *G. septentrionalis*. Compound **2** was the only component present in all three species, where it was the major component found with its corresponding hydroquinone **7** as a trace. The proportions and absolute amounts of the seven components differed with the species, and these profiles seemed to be species-specific. *T. lumbricinus* had compounds **2**, **1**, and **3** in decreasing order. Likewise, *S. takakuwai* had compounds **2** and **3**, and *G. septentrionalis* had only compound **2**.

The chemical components of secretions of four Okinawan millipedes have been published (Kiku-

Wang *et al.*, 1993); two of them, *C. haulienensis* Wang (Polidesmida: Stronglyosomidae) and *Riukiarlia pugionifera* Verhoeff (Polidesmida: Xystodesmidae) are cyanogenic. From *C. haulienensis*, three compounds (mandelonitrile, benzoic acid, and mandelonitrile benzoate) have been isolated and identified among seven reported by Noguchi *et al.* (1997b) as mentioned in the introduction. Mandelonitrile, benzoic acid, and benzyl alcohol from *R. pugionifera* have been identified. The other two species *Anaulaciulus okinawaensis* Shinohara (Julida: Julidae) and *Prospirobolus joannise* Attens (Streptida: Rhinocricidae), are quinone emitters. Three compounds (**4**, **2**, and **6**, in the amount of 1100.4, 195 and 31 mg, respectively) are isolated and identified from 1.5 kg of *A. okinawaensis*, and two compounds, **4** (30 mg) and **2** (9 mg) were obtained from 800 g of *P. joannise* (Kikunaga *et al.*, 1993). That toluhydroquinone (**4**) was the main component in two species is unexpected, because hydroquinones are usually distributed as traces (Deml and Huth, 2000).

In this study, quinones **1**, **2**, and **3** were accompanied by a minute amount of corresponding hydroquinones **4**, **7**, and **5** (quinone **6** was the only exception). Quinones **2** and **3** and hydroquinone **7** were found in all three species, including the case as traces. Quinone **2** was the major component of the defense secretions.

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## Effects of Humidity Changes on the Feeding Activity of a Pest Termite, *Reticulitermes speratus* (Kolbe)

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湿度変化がヤマトシロアリの摂食活動に及ぼす影響 中山友栄<sup>1)</sup>・築瀬佳之<sup>2)</sup>・吉村 剛<sup>1)</sup>・藤井義久<sup>2)</sup>・今村祐嗣<sup>1)</sup> ( <sup>1)</sup>京都大学木質科学研究所, <sup>2)</sup>京都大学大学院農学研究科)

日本全国に分布するヤマトシロアリ (*Reticulitermes speratus* (Kolbe)) の摂食活動に及ぼす環境湿度の影響を、アコースティック・エミッション (AE) モニタリングを用いて明らかにすることを試みた。湿度を固定および変動させた環境下で、ヤマトシロアリ職蟻の摂食活動をAE事象率 (5分間当たりのAE事象数) によって観察した。固定湿度実験では、各湿度間で明らかな差は観察されなかったが、70%、80%RHにおいて検出されたAE事象数が他の湿度条件下と比べて高い値であった。また、50-90%RH間を段階的に変動する変動湿度実験では、職蟻の摂食活動によって発生するAE事象率は周囲の湿度が上昇するに伴って増加し、逆に低下すると急激に減少した。この変動湿度実験では、80%、90%RH下において摂食活動が最も活発であった。これらの結果から、ヤマトシロアリ職蟻の摂食活動は、環境湿度を低下させることによって抑制されると考えられる。

The effect of the environmental relative humidity (RH) on the feeding activity of *Reticulitermes speratus* (Kolbe), which is one of the major pest species and is distributed throughout almost the whole country, was investigated by acoustic emission (AE) monitoring. Two trials of fixed and variable RH tests were conducted. In the fixed RH test, although no significant difference was observed, higher AE events were observed at 70% and 80% RH than at other RH levels. The AE event rates generated by the feeding activities of workers under stepwisely-changed RH gradient (50-90-50% RH) increased with environmental RH rising, and decreased rapidly when RH lowered.

The highest feeding activities were detected at 80% and 90%RHs in the variable test. From these results, it seems that the feeding activity of *R. speratus* workers is restrained by lowering environmental RHs.

**Key words:** *Reticulitermes speratus* (Kolbe), RH change, Feeding activity, AE monitoring

## Introduction

Termite is the most serious pest affecting wood and wood-based materials in Japan. Environmental concerns have been promoting the development of novel termite control strategies, i.e. Integrated Termite Control (ITC) based on the ecological knowledge of the target species. Relative humidity (RH) is one of the most important environmental factors affecting termite activity (Wigglesworth, 1965). The effect of environmental RH on the feeding activity of *Coptotermes formosanus* Shiraki, a major pest species in southern Japan, has been studied by acoustic emission (AE) monitoring (Suda *et al.*, 2000; Yusuf *et al.*, 2000), which has been applied to investigate the feeding activity of termites not only in laboratory trials (Imamura and Fujii, 1995; Matsuoka *et al.*, 1996) but also in field situations (Fujii *et al.*, 1998; Yanase *et al.*, 1999, 2001) as a non-destructive and useful tool. For *C. formosanus* it was reported that the highest feeding activity occurred around 75%RH (Suda *et al.*, 2000; Yoshimura *et al.*, 2000; Yusuf *et al.*, 2000).

Another major pest species, *Reticulitermes speratus* (Kolbe), which is distributed throughout almost the whole country (Mori *et al.*, 2002), is thought to need to ingest wet woods, while *C. formosanus* ingests relatively dry woods because the latter has a water reserving capacity (The Society of Materials Science, Japan, 1982). So it is likely that the RH-dependence of these two major

pest species strongly differs even though both insects are subterranean species classified in the same family, Rhinotermitidae.

In this study, AE monitoring was used to examine the effects of environmental RH on the feeding activity of *R. speratus*.

## Materials and Methods

### Termite and wood specimen

Workers of *Reticulitermes speratus* (Kolbe) collected from a field colony maintained at the Uji Campus of Kyoto University were used in this study. Test insects were starved for 1 day prior to the experiments to activate their feeding activities.

Wood blocks of Japanese red pine (*Pinus densiflora* Sieb. et Zucc.), measuring 35mm (R) × 35mm (T) × 50mm (L), with 5 holes (5mm in diameter and 20-30mm in depth) were air-dried and served for the experiments. Mean water content of the blocks was 12.8%.

### AE sensor

A piezoelectric AE sensor with a resonant frequency of 150Hz (NF, AE-901s) was attached on the surface of the wood block. The signals from the sensor were filtered through a high-pass filter with a cut-off frequency of 100kHz, amplified 66 dB, and discriminated at a threshold voltage of 0.1 V with an AE apparatus (NF, AE-9501).

### Experimental unit

An acrylic cylinder (80mm in diameter and 50mm in height) with the plaster bottom was used as a test container. A test wood block with an AE

sensor was put in the bottom center of a test container with 100 workers of *R. speratus*. The assembled test container was set on moist cotton pads (Fig. 1), so that the workers could take up water from the plaster bottom. The control unit without workers was set up to detect background (electric) noises.

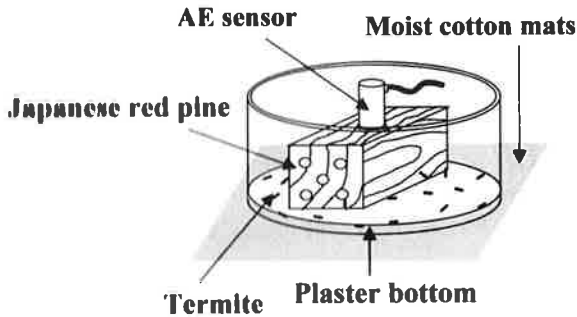


Fig. 1 An experimental unit.

#### Periodicity of the feeding activity under constant environmental conditions

To recognize the periodicity of the feeding activity of *R. speratus* workers under constant environmental conditions, a test unit was installed in a temperature- and RH-regulating chamber (RYEIA, KCL-1000) with 70% RH and 25 °C,

and AE measurement was carried out for 2 days.

#### Relative Humidity (RH) conditions

Two trials were conducted in the present investigation. As the first trial, the fixed RH test, a test unit was installed in the chamber at 50, 60, 70, 80 and 90%RH respectively at 25 °C for 8 hours. Six replicates (3 times x 2 channels) were employed at the fixed RH test. For the second trial, the variable RH test, the RH was increased and then decreased in a stepwise manner from 50-60-70-80-90-80-70-60-50%RH at 2-hour intervals at 25 °C, and the cycle was repeated 2 times (1 cycle required 1 day). Three replicates (total 6 cycles) were employed in the variable RH test.

#### Statistical analyses

A moving average was calculated for smoothing the fluctuation under constant environmental conditions. In this study, every continuous 12 data of cumulative AE events for 5 min were averaged to discuss the periodicity of feeding activities. In fixed and variable RH tests, relationships between RH and AE events were statistically analyzed using *F*-test and *t*-test, respectively.

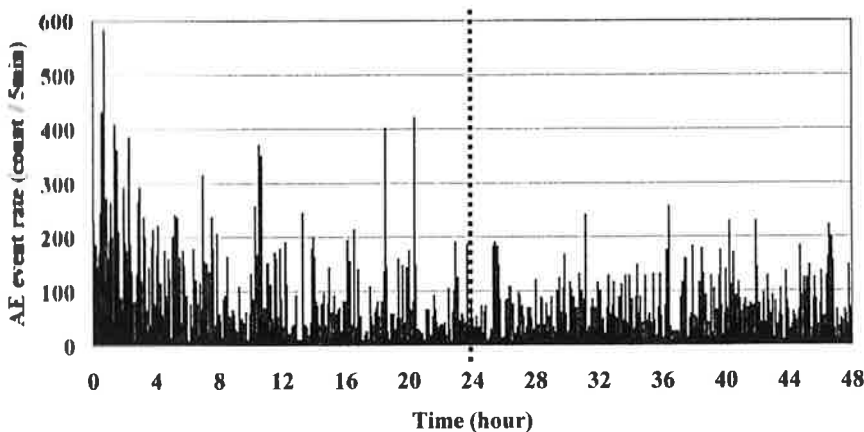


Fig. 2 AE event rates produced by workers of *Reticulitermes speratus* for 48 hours maintained at 25 °C and 70%RH.

## Result and Discussion

### Periodicity of the feeding activity

Figure 2 shows typical patterns of AE events per 5 min (AE event rate) for 2 days at 25 °C and 70%RH. AE event rates fluctuated for 2-4 hour intervals throughout 2 days with the highest and lowest AE event rates of 100-200 events/5min and less than 50 events/5 min, respectively.

Transforming the data into the moving averages clarified the trend during the 2 days, showing 2-4 hour periodic fluctuations (Fig. 3). These results suggest the 2-4 hour periodicity of the feeding activity of workers of *R. speratus* under constant environmental conditions. Therefore, AE events were monitored for 8 hours and 24 hours in the fixed RH test and the variable RH test, respectively, to separate the effects of environmental factors and the endemic factors.

### Fixed RH test

Figure 4 shows the relationship between RH and cumulative AE events over 8 hours at various fixed RH levels. The cumulative AE events were averages for 6 replicates. Although there were no significant

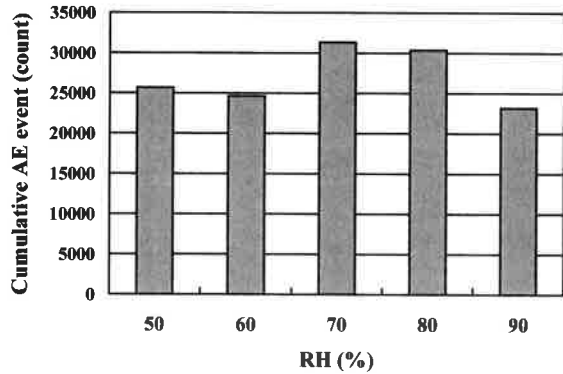


Fig. 4 Cumulative AE event for 8 hours by workers of *Reticulitermes speratus* at various RH levels.

differences (*F*-test,  $p < 0.05$ ), more frequent AE events were obtained at 70% (ca. 31,000 events/8 hours) and 80%RH (ca. 30,000 events) than those at 50% (ca. 26,000 events), 60% (ca. 25,000 events) and 90%RH (ca. 23,000 events). It was reported that the feeding activity was highest at 75%RH (ca. 21,000 events/8 hours) when 100 workers of a field colony of *C. formosanus* collected from Kagoshima Prefecture, were AE-monitored under a fixed RH condition (Yusuf *et al.*, 2000).

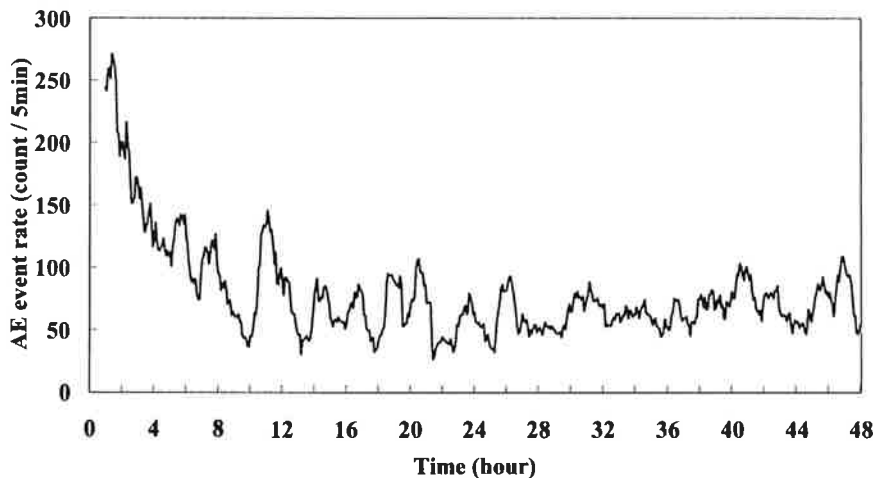


Fig. 3 Sixty-minute moving averages (12 data points) transformed from the data in Fig. 2.

The yearly average RH of Kyoto Prefecture and Kagoshima Prefecture, collecting sites of *R. speratus* and *C. formosanus*, were 68% and 74%RHs (National Astronomical Observatory, 1992), respectively. It seems natural that the feeding activity of termites is optimized at the yearly average environmental conditions such as RH.

However, it also should be noted that the feeding activity of the termite was not significantly lowered even in the lower RH conditions (Fig. 4). This suggests the environmental flexibility of *R. speratus*, which is distributed widely in Japan. The lack of a water-holding capacity in *R. speratus* (The Society of Materials Science, Japan, 1982) may have facilitated the flexibility.

**Variable RH test**

Figure 5 shows a typical change in AE event rates generated by the feeding activities of workers under stepwise-changes in the RH gradient (50-90-50%RH). The AE event rates increased with environmental RH rising, and decreased rapidly when RH lowered. In 2 other replicates (4 cycles), AE event rates fluctuated with RH gradient in the similar manner.

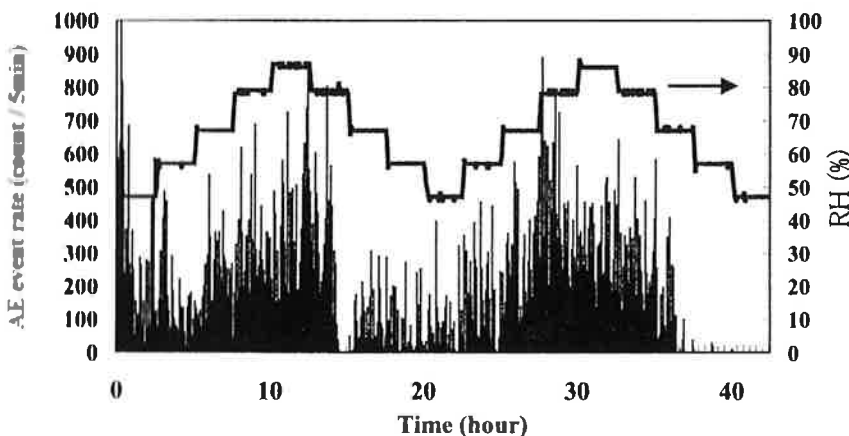


Fig. 5 A typical change in AE event rates produced by workers of *Reticulitermes speratus* under stepwise-changes in the RH gradient (50%-90%-50%RHs).

Averaging the AE event rates per 5 min for each RH condition in 2 cycles (Fig. 5), RH-dependence of the AE event rates could be assessed quantitatively (Fig. 6). Figure 6 shows the data in the 60-90-60%RH gradient, because the complete data set was not obtained at 50%RH condition. The highest AE event rates were obtained at 80% and 90%RHs in the rising stage (350-400 events/5 min). On the other hand, the lowest AE event rate was observed

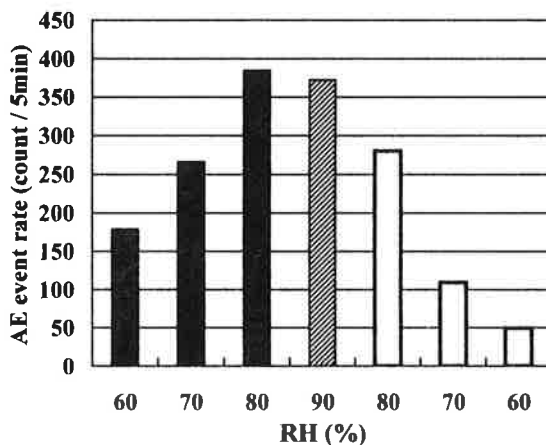


Fig. 6 AE event rate per 5 min at rising (■) and lowering (□) RH stages averaged from the data in Fig. 5. ▨ : not served for statistical analyses.

at 60%RH in the lowering stage (ca. 50 events / 5 min) (Fig. 6). A significant difference was observed by the *t*-test ( $p < 0.01$ ) between AE event rates at the rising stages (black bar : 170-450 events / 5 min) and the lowering stages (white bar : 5-300 events / 5 min), with each 6 samples (3 RH stages  $\times$  2 cycles). When comparing AE event rates at the same RH in both the rising and lowering stages, a significant difference was admitted only at 70%RH (*t*-test,  $p < 0.05$ ). Based on these results, it is probable that the feeding activity of workers of *R. speratus* is restrained by lowering environmental RHs.

The optimum RH condition for the feeding activity of *C. formosanus*, which was categorized as the subterranean species like *R. speratus*, was in 70-75%RH at the fixed RH (Yoshimura *et al.*, 2000; Yusuf *et al.*, 2000). In this study, the highest feeding activities of workers of *R. speratus* were obtained at 70-80% and 80-90% RH in the fixed (Fig. 4) and variable RH tests (Fig. 6), respectively. As described above, *R. speratus* does not have a water-holding capacity (The Society of Materials Science, Japan, 1982). This fact might result in the development of a sensitivity against lowering environmental RH levels in the species. From the present results, it is suggested that the positive control of humidity in houses would contribute to the development of the Integrated Termite Control (ITC) in the future. As a next step, the water-dependence of workers of *C. formosanus* and *R. speratus* is now under investigation.

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## 京都府南部におけるオオゴキブリの 採取と飼育

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Collecting a *Panesthia* Cockroach Species, *Panesthia angustipennis spadica* (Shiraki) in Kyoto and Its Rearing Experiments. Kinuko Fukumoto<sup>1)</sup>, Teruo Fukumoto<sup>2)</sup>, Hitoko Yamaguchi<sup>1)</sup>, Hiroko Yamaguchi<sup>3)</sup> and Hideakira Tsuji<sup>4)</sup> (1) Osaka Seikei Womens College, 10-62, Aikawa Ichome, Higashi-Yodogawa-ku, Osaka 533-0007, Japan, (2) 19-10 Yuden, Tanabe, Kyo-Tanabe, Kyoto 610-0331, Japan, (3) Imperial College Science, Technology and Medicine, London SW7, 2AZ UK, (4) KSK Institute for Environmental Biology, F-409, 2-1 Nishino-Rikyu-cho, Yamashina-ku, Kyoto 607-8345, Japan). *Jpn. J. Environ. Entomol. Zool.* 13: 133-137 (2002)

Adults and nymphs of an outdoor wood-eating cockroach species, *Panesthia angustipennis spadica* (Shiraki), were collected from rotting wood in a southern area of Kyoto and were reared under unheated room conditions. A couple of adults survived for more than two and a half years, and the female produced a batch of less than thirty nymphs only once in summer every year. Newly born nymphs grew very slowly, and they could not become adults within three years, suggesting that the nymphal stage should extend for four years or more.

**Key words:** Wood-eating cockroach, *Panesthia angustipennis spadica*, Nymphal period, Adult longevity, Reproduction

### はじめに

オオゴキブリ, *Panesthia angustipennis spadica* (Shiraki), はゴキブリ目オオゴキブリ科に属し, 日本の青森県から鹿児島県屋久島にかけて分布する大型の野外性ゴキブリである(朝比奈, 1991). 森林の朽木中で木質を食して集団で生息し, その生息環境は自然の保全状態が良好であることを示している.

著者らは, 京都府下においてすでに標本の採取が知られている亀岡市の千歳区や京都市東山一帯その他を数年間探索したが, いずれも土地開発が進んだためか発見できず, ようやく1992年5月に京都府綴喜郡田辺町(現在の京田辺市)の山林中で本種の成虫および幼虫を発見し採集することができた.

本邦の野外性ゴキブリの周年経過としてモリチャバネゴキブリ, *Blattella nipponica* Asahinaのサンプリングと飼育による周年経過観察(Tsuiji, 1985)のほか, 屋内にも見られるヤマトゴキブリ, *Periplaneta japonica* Karnyの越冬時期のサンプリング(田原・小林, 1971; Tsuiji and Tabaru, 1974)や休眠と発育の確認(Tsuiji and Mizuno, 1972, 1973; 小宮山・緒方, 1981; 岩崎, 2000), クロゴキブリ, *Periplaneta fuliginosa* (Serville)の休眠と発育の確認(辻, 1988, 2000; Tsuiji and Mizuno, 1973; 高木, 1974, Takagi, 1978; 辻・種池, 1993等), 屋外でのトラップ記録(中野, 1996, 2000), キョウトゴキブリ, *Asiablatta kyotensis* (Asahina)の飼育周年経過観察(辻ら, 1993)などがある. しかし, 森林の朽木に住むオオゴキブリについては発育が極端に遅いこともあり, 少なくとも京都付近の個体の長期にわたる観察や飼育記録はない.