Nestmate Recognition and Cuticular Hydrocarbons of Two Sympatric Species of *Reticulitermes* in Japan (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The nestmate recognition of two sympatric species, *R. kanmonensis* and *R. speratus,* was investigated in terms of agonistic behavior and trophallactic behavior. *R. speratus* showed strong agonistic behavior against different species, but no trophallactic contact with them. However, agonistic behavior against different colonies of the same species was weak, and trophallactic exchange of food was observed. On the other hand, *R. kanmonensis* showed strong agonistic behavior not only against different species but also against different colonies of the same species, and trophallactic contact was absent. These results indicate that *R. kanmonensis* does not exhibit colony fusion, unlike *R. speratus*, which is known to exhibit colony fusion. This marked difference in the occurrence of colony fusion can be related to the difference in the distribution pattern of the two species. Cuticular hydrocarbons of both species were also analyzed. Relatively high hydrocarbon homogeneity was observed among colonies in *R. kanmonensis* compared to *R. speratus*.

Key words: nestmate recognition; cuticular hydrocarbons; agonistic behavior; trophallaxis; colony fusion; sympatric distribution; invasive species.

INTRODUCTION

Genus *Reticulitermes* is one of the common genera that cause serious damage to woodwork in temperate regions. *R. speratus* is widely distributed throughout the Japanese mainland and had been known as the only species of this genus. However, it was found that another species, *R. kanmonensis*,

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is distributed sympatrically with *R. speratus* in southernmost Honshu and is thought to be an alien species (Kitade & Matsumoto 1993; Takematsu 1999; Morimoto 2000). Since the first report of this species as *R. flaviceps* by Nawa (1911), it has not been distinguished from *R. speratus* morphologically and has been controlled using the same method as for *R. speratus.* However, Takematsu (1999) presented the characters distinguishing this species from *R. speratus*, and since then the two species have been treated separately in the ecological study of *Reticulitermes*.

Kambara & Takematsu (2009) showed that *R. kanmonensis* tend to choose less favorable habitats (less damaged wood) when coexisting with *R. speratus*, and this fact predicted that even living woods and houses are vulnerable to termite damage by *R. kanmonensis*. In order to control this species more effectively, it is important to clarify the ecological features of *R. kanmonensis*.

Kitade & Hayashi (2002) reported the detailed distribution range of this species. It is remarkable that the distribution area of *R. kanmonensis* is restricted to the coastal areas on both sides of the Kanmon strait located between Honshu and Kyushu Islands, and that this species has not expanded its distribution range for a century since the first record in 1911 (Nawa 1911; Nawa 1912a,1912b; Nawa 1917; Kitade & Matsumoto 1993; Takematsu 1999; Morimoto 2000). Within the whole distribution area, *R. kanmonensis* and *R. speratus* were distributed sympatrically. However, the local distribution of the two species was not homogeneous; the distribution ratio of some local areas was strongly biased toward *R. kanmonensis* (Kitade & Hayashi 2002; Kambara & Takematsu 2009). Why did *R. kanmonensis* show such a biased distribution pattern without expanding their distribution range? Here we consider whether one of the reasons for the distribution pattern might be associated with the interspecific and intraspecific interactions between the two species. Kitade *et al.* (2004) reported the highly aggregated distribution of incipient colonies of *R. kanmonensis* in a small area and the lack of mitochondrial haplotype variation within a mature nest, and they assumed the absence of worker mingling and the strong intraspecific competition among colonies.

In the present study, we investigate the recognitions for nestmate and nonnestmate of *R. kanmonensis* and *R. speratus* in terms of the agonistic behavior and trophallactic behavior. We also analyze the cuticular hydrocarbons of both species, which are reported to play a key role as cues for nestmate recognition, and discuss the relationship between nestmate recognition and cuticular hydrocarbon composition.

MATERIALS AND METHODS

Termites

Four collecting sites were chosen according to the local distribution ratio of the two species: *R. kanmonensis*-area (Site1: distribution ratio of *R. kanmonensis* was 100%), *R. kanmonensis* dominant-area (Site2: distribution ratio of *R. kanmonensis* was 85%), *R. speratus* dominant-area (Site3: distribution ratio of *R. kanmonensis* was 35%), and *R. speratus*-area (Site4: distribution ratio of *R. kanmonensis* was 0%). Sites 1-3 were in a forest in Ejio Park, Onoda (131°12'E, 34°2'N), Yamaguchi Prefecture. Site 4 was in Yoshida, Yamaguchi (131°27'E, 34°9'N), Yamaguchi Prefecture. Three colonies for each species were collected from each site, respectively.

Bioassay

Bioassays were conducted using a plastic container (82mm diameter x 46mm height) with a sheet of filter paper at 25°C. To stain the termite gut for discriminating the test individuals and target ones, termites of each colony were divided into two groups and were fed a filter paper stained by red food coloring and blue food coloring respectively for 24 hrs. Thirty workers were introduced into the test arena as test individuals, and after 3 hrs a target individual was introduced into the arena. Survival and occurrence of body color change of the target individual were recorded after 24hrs. In these bioassays, we regarded the death of target individual as "agonistic behavior" (i.e. non-nestmate recognition) and body color change of a target individual as "trophallaxis behavior" (i.e. nestmate recognition).

The following three experiments were carried out in order to evaluate nestmate and non-nestmate recognitions for *R. kanmonensis* as well as for *R. speratus*.

Experiment 1: Recognition between individuals from the same colony was tested. Both test individuals and target ones were sampled from the same colony in each trial. For *R. kanmonensis*, one colony collected from each of sites 1 to 3 was used. One colony was used from site 4 for *R. speratus*. Every trial was replicated 15 times.

Experiment 2: Recognition between individuals from different colonies of the same species was tested. Test individuals and target ones were sampled from the same species but different colonies in each trial. Colonies collected from sites 1 to 3 were used for the tests of *R. kanmonensis* and for *R. speratus* samples from site 4 were used. In each site, 3 colonies were used for 6 possible combinations of test/target sets. Every set was replicated 3 times, and in total 18 replication were done for each site.

Experiment 3: Recognition between individuals from the different species was tested. Test individuals and target ones were sampled from different species in each trial. For the tests of *R. kanmonensis*, individuals of one colony of *R. kanmonensis* collected from each of sites 1 to 3 were used as test individuals and individuals of one colony of *R. speratus* collected from each of sites 2 to 4 were used as target individuals. In each site, every trial was replicated 5 times and in total 15 replications were done. For the tests of *R. speratus*, individuals of a colony of *R. speratus* collected from site 4 were used as test individuals and individuals of one colony of *R. kanmonensis* from each of sites 1 to 3 were used as target individuals. Every trial was replicated 10 times.

GC analysis

Cuticular hydrocarbons were extracted from 3 colonies of *R. kanmonensis* from sites 1 to 3 respectively and 3 colonies of *R. speratus* from sites 2 to 4 respectively. Three replications were done for each colony except the colony of *R. kanmonensis* from site 3 which admitted no replication due to a lack of individuals. Cuticular hydrocarbons were extracted by immersing 100 workers in 2 ml of *n*-hexane for 5 min. After evaporation of the hexane, the extracts were redissolved in 1 to 10 µl of *n*-hexane for gas chromatography (GC). The gas chromatography was conducted by a Shimadzu GC-14B (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and DB-1HT capillary column (Frontier Lab; length 30m, film thickness, 0.1 µm). Helium was used as the carrier gas. The injection temperature was programmed at 65°C for 0.01 min and then raised at 30°C/min to 355°C, with a final hold for 26.3 min. The oven temperature was kept at 60°C for 0.1min and then raised at 20°C/min to 200°C, 10°C/min to 275°C, 5°C/min to 355°C, with a final hold for 10 min. The detection temperature was

355°C. Hydrocarbons were identified according to the results of the GC-MS analysis reported in Takematsu & Yamaoka (1999) and quantified as the percent of the total hydrocarbon component.

The differences of cuticular hydrocarbons within a colony and within a site were analyzed using principal component analysis (PCA), employing the component ratio of cuticular hydrocarbons. The calculations were performed using the Excel-Toukei software program (Social Survey Research Information Co., Ltd., 2006).

RESULTS

Bioassay

Numbers of survival and trophallaxis of target individuals of *R. kanmonensis* and *R. speratus* are shown in Table 1. In the assay for the individuals from the same colony (Experiment 1), all target individuals of both *R. kanmonensis* and *R. speratus* survived, and occurrences of trophallaxis were more than 86%. These facts indicated that both *R. kanmonensis* and *R. speratus* have strong nestmate recognition for individuals from the same colony. Conversely, in the assay for the individuals from the different species (Experiment 3), most individuals of both *R. kanmonensis* and *R. speratus* were dead and no trophal-

Table 1. Number of survivors and trophallaxis of target individuals of *R. kanmonensis* and *R. speratus*.

Experiment	Species	Site	N	Number of Surviving Targets (%)	Number of color changed targets (%)
Experiment1	*R. kanmonensis*	Site 1	15	15 (100.0)	15 (100.0)
(Same colony)	*R. kanmonensis*	Site 2	15	15 (100.0)	15 (100.0)
	R. kanmonensis	Site 3	15	15 (100.0)	13 (86.7)
	R. speratus	Site 4	15	15 (100.0)	14 (93.3)
Experiment 2	*R. kanmonensis*	Site 1	18	7 (38.9)	3 (16.7)
(Different colony)	*R. kanmonensis*	Site 2	18	9 (50.0)	2 (11.1)
	R. kanmonensis	Site 3	18	3 (16.7)	0 (0.0)
	R. speratus	Site 4	18	18 (100.0)	8 (44.4)
Experiment 3	*R. kanmonensis*	Site 1	15	0 (0.0)	0 (0.0)
(Different species)	*R. kanmonensis*	Site 2	15	2 (13.3)	0 (0.0)
	R. kanmonensis	Site 3	15	1 (6.7)	0 (0.0)
	R. speratus	Site 4	10	0 (0.0)	0 (0.0)

lactic behavior was observed. These indicated that both species have strong agonistic behavior against the different species.

On the other hand, in the assay for the individuals from the different colonies of the same species (Experiment 2), the results showed a marked difference between the two species. In the case of *R. speratus*, all target individuals survived, and the ratio of trophallactic behavior was 44.4%. While, in the case of *R. kanmonensis*, more than 50% of target individuals were dead and only a few target individuals changed their body color: especially in site 3 (*R. speratus* dominant-area), the survival rate of target individuals of *R. kanmonensis* was remarkably low and no trophallactic behavior was observed.

GC analysis

From *R. kanmonensis*, 21 components of hydrocarbons were identified and quantified. From *R. speratus*, 22 components of hydrocarbons were identified and quantified. Cuticular hydrocarbon components of both species were different (Takematsu & Yamaoka 1999). Nine substances were in

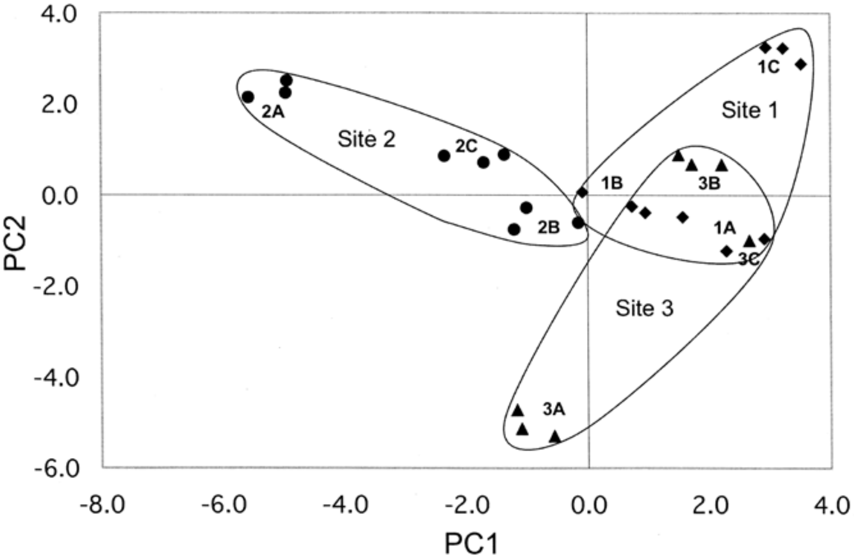

Fig. 1 PCA evaluating the differences of cuticular hydrocarbons within and among colonies of *R. kanmonensis* based on the proportion of 20 major CHC components. A-C show the colony of each site; solid diamond, solid circle and solid triangle show Site 1, 2 and 3 respectively.

common. The results of the PCA were plotted using the first and second principal component axes. Fig. 1 shows the result of the PCA evaluating the differences of cuticular hydrocarbons within and among colonies of *R. kanmonensis* based on the proportion of 21 components. The first principal component (PC1) explained 32.08% of the total variance and the second principal component (PC2) explained 25.92% of the total variance. Fig. 2 shows the result of the PCA evaluating the differences of cuticular hydrocarbons within and among colonies of *R. speratus* based on the proportion of 22 components. The first principal component (PC1) explained 43.28% of the total variance and the second principal component (PC2) explained 15.99% of the total variance.

In both species, within each colony there was a very similar cuticular hydrocarbon profile, and they could be separable with other colonies. On the other hand, variations among colonies within a site of *R. kanmonensis* (Fig.

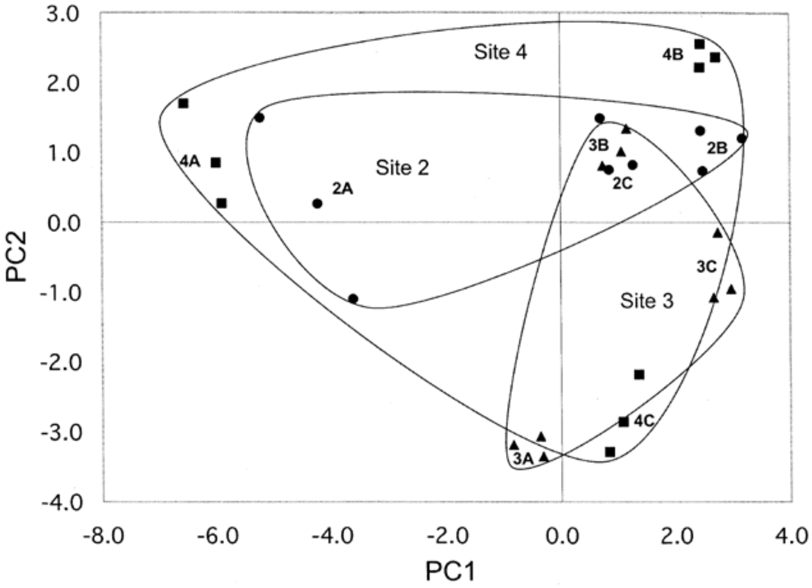

Fig. 2 PCA evaluating the differences of cuticular hydrocarbons within and among colonies of *R. speratus* based on the proportion of 20 major CHC components. A-C show the colony of each site; solid circle, solid triangle and solid square show Site 2, 3 and 4 respectively.

1) were much smaller than those of site 4 for *R. speratus* (Fig. 2 solid squares), which was used in the bioassays.

DISCUSSION

R. kanmonensis and *R speratus* are distributed sympatrically and *R. kanmonensis* is thought to be an alien species having been introduced into the native distribution range of *R. speratus*.

R. speratus showed strong agonistic behavior against different species and no trophallactic contact with them. However, agonistic behavior against different colonies of the same species was weak and trophallactic behaviors were often observed. On the other hand, *R. kanmonensis*, a sympatric species with *R. speratus*, showed strong agonistic behavior against different colonies of the same species as well as of different species and no trophallactic contact was observed. These results suggest that *R. speratus* has colony fusion as already described in Matsuura & Nishda (2001), while *R. kanmonensis* so strongly exclude non-nestmate individuals that colony fusion can not occur. Kitade *et al.* (2004) showed the lack of mitochondrial haplotype variation in *R. kanmonensis* within a mature nest and the aggregated distribution of founding colonies nested in small branches. This indicated that worker mingling did not occur in *R. kanmonensis* and founding colonies were under a strong intraspecific competition with mature colonies, resulting in a low survival rate of incipient colonies. In agreement with these results of Kitade *et al.* (2004), our data showed strong intraspecific competition and at the same time a lack of colony fusion in the case of *R. kanmonensis*.

For some *Reticulitermes* species, occurrences of colony fusion have been reported, and it is known that the frequency of the phenomenon is highly variable depending on populations (Bulmer & Traniello 2002; DeHeer & Vargo 2004,2008; Leniaud *et al.* 2009; Matsuura & Nishida 2001; Nobre *et al.* 2008; Perdereau *et al.* 2010a). Perdereau *et al.* (2010a), among others, investigated the social organization of *R. flavipes* with a special interest in invading species and reported that colony fusion occurred more frequently in introduced populations of France than in native populations of North America. The occurrence of colony fusion may be thought of as a key to lowering the cost for the intraspecific competition. This provides one possible mode of ecological advantage. In invaded habitats, colony fusion can be understood as a mechanism that is favorable for the invasive success to attaining high worker densities and interspecific dominance. In many social insects, shifts in the social organization have already been observed and thought to contribute to invasive success (as was summarized by Perdereau *et al.* 2010a). Leniaud *et al.* (2009) also reported unicoloniality and low intraspecific aggression of *R. urbis*, an invading species in France, resulting in invasive success in the introduced region.

Since this is a case of *R. kanmonensis* being introduced into ranges where another ecologically more potent species is dominant, then it might have been thought that *R. kanmonensis* without the ability of colony fusion is unlikely to survive in such ecologically recessive environments. However, *R. kanmonensis* persisted for more than a century in such an area. The present result suggests that this long-term persistence was attained without resort to the ability of colony fusion. In this respect, it is to be noted that *R. kanmonensis* has a specific flexibility in the choice of habitats; that is *R. kanmonensis* tends to choose less favorable habitats (houses or living woods) when coexisting with *R. speratus* (Kambara & Takematsu 2009). To date, the original distribution of *R. kanmonensis* is not clear. It may be of interest to clarify the original distribution of *R. kanmonensis* and the social organization in that area.

Cuticular hydrocarbons are generally considered as an important cue for interspecific and colony recognition (Clément & Bagnères 1998). It has been well accepted that the cuticular hydrocarbon compositions are different qualitatively at the specific level, whereas there are quantitative differences at the intraspecific level (Howard 1993; Haverty *et al.* 1997). Perdereau *et al.* (2010b) investigated the cuticular hydrocarbons of native and introduced populations of *R. flavipes* and reported the remarkable hydrocarbon homogeneity observed within the introduced populations compared to the native populations due to a reduction of genetic diversity through a genetic bottleneck or a selective process for the less common alleles of recognition. Perdereau *et al.* (2010b) also assumed that the homogeneity of cuticular hydrocarbons in introduced populations of *R. flavipes* could explain the lack of intraspecific aggression and, indirectly, the high rate of colony fusion within these introduced populations

The present study shows the homogeneity of cuticular hydrocarbons in *R. kanmonensis* compared to *R. speratus*. This phenomenon can be explained as a reduction of genetic diversity in the invading species as reported in Perdereau *et al.* (2010b). However, based on the results of agonistic behavior, *R. kanmonensis*, an invading species, showed strong intraspecific competition in spite of the homogeneity of cuticular hydrocarbons. Therefore in the present study, the intensity of agonistic behavior seems to depend not only on cuticular hydrocarbon-related factors but on other features of each species such as ecological or genetic traits.

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REFERENCES

Bulmer, M.S. & J.F.A. Traniello 2002. Lack of aggression and spatial association of colony members in *Reticulitermes flavipes*. J. Insect Behav. 15: 121–126.

Clément, J.L. & A.G. Bagnères 1998. Nestmate recognition in termites. *In*: R.K. Vander Meer, M.D. Breed, K.E. Espelie & M.L. Winston (eds.), Pheromone communication in social insects. Ants, wasps, bees, and termites. Westview Press, Boulder, CO, US: 126–155.

DeHeer, C.J. & E.L. Vargo 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. Mol. Ecol. 13: 431–441.

DeHeer, C.J. & E.L. Vargo 2008. Strong mitochondrial DNA similarity but low relatedness at microsatellite loci among families within fused colonies of the termite *Reticulitermes flavipes*. Insectes Sociaux 55: 190–199.

Kambara, K. & Y. Takematsu 2009. Field habitat selection of two coexistence species of Reticulitermes, *R. speratus* and *R. kanmonensis* (Isoptera, Rhinotermitidae). Sociobiology 54: 65–75.

Kitade, O. & Y. Hayashi 2002. Localized distribution of an alien termite *Reticulitermes kanmonensis* (Isoptera: Rhinotermitidae). Entomological Science 5: 197–201.

Kitade, O., Y. Hayashi, Y. Kikuchi & S. Kawarasaki 2004. Distribution and composition of colony founding associations of a subterranean termite, *Reticulitermes kanmonensis*. Entomological Science 7: 1–8.

Kitade, O. & T. Matsumoto 1993. Symbiotic protistan faunae of *Reticulitermes* (Isoptera: Rhinotermitidae) in the Japan Archipelago. Sociobiology 23: 135–153.

Howard, R.W. 1993. Cuticular hydrocarbons and chemical communication. *In*: D.W. Stanley-Samuelson & D.R. Nelson (eds.), Insect lipids: Chemistry, biochemistry and biology. University of Nebraska Press, Lincoln, NE, US: 179–226.

Haverty, M.I., M.S. Collins, L.J. Nelson & B.L. Thorne 1997. Cuticular hydrocarbons of termites of the British Virgin Islands. J. Chem. Ecol. 23: 927–964.

Leniaud, L., A. Pichon, P. Uva & A.G. Bagnres 2009. Unicoloniality in *Reticulitermes urbis*: A novel feature in a potentially invasive termite species. Bulletin of Entomological Research, 99: 1–10.

Matsuura, K. & T. Nishida 2001. Colony fusion in a termite: What makes the society "open"? Insectes sociaux 48: 378–383.

Morimoto, K. 2000. Termite. *In*: Japan Termite Control Association (ed.), Termite and Control. Japan Termite Control Association, Tokyo: 1–126.

Nawa, U. 1911. On "kiashi-shiroari" (*Reticulitermes flaviceps*). Konchu Sekai 16: 33.

Nawa, U. 1912a. On a termite that emerges earlier. Konchu Sekai 15: 194–195.

Nawa, U. 1912b. Nuptial flight of termite that emerges earlier. Konchu Sekai 17: 154.

Nawa, U. 1917. Distribution of "kanmon-shiroari". Konchu Sekai 21: 72–73.

Nobre, T., L. Nunes & D.E. Bignell 2008. Colony interactions in *Reticulitermes grassei* population assessed by molecular genetic methods. Insectes Sociaux 55: 66–73.

Perdereau, E., A.G. Bagnères, S. Dupont & F. Dedeine 2010a. High occurrence of colony fusion in a European population of the American termite *Reticulitermes flavipes*. Insectes Socaux 57:393–402.

Perdereau, E., F. Dedeine, J.P. Christidès & A.G. Bagnères 2010b. Variations in worker cuticular hydrocarbons and soldier isoprenoid defensive secretions within and among introduced and native populations of the subterranean termite, *Reticulitermes flavipes*. J. Chem. Ecol. 36: 1189–1198.

Takematsu, Y. 1999. The genus *Reticulitermes* (Isoptera: Rhinotermitidae) in Japan, with description of a new species. Entomological Science 2: 231–244.

Takematsu, Y. & R. Yamaoka 1999. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) in Japan and neighboring countries as chemotaxonomic characters. Applied Entomology and Zoology 34: 179–188.