Notes on the Associated Fungi and the Mycetangia of the Ambrosia Beetle, *Crossotarsus niponicus* BLANDFORD (Coleoptera : Platypodidae)

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The associated fungi and the spore storage organs of the ambrosia beetle *Crossotarsus niponicus* were investigated. Stained serial sections of the adult beetles showed that at the back of the preoral cavity of the adult female, a single spherical mycetangium existed, and surrounding this sphere a sponge-like tissue which also contains fungus material was present. From the fluid contained in the preoral cavity of all of the female specimens, *Absidia glauca* HAGEM was isolated. From 2 or 3 individuals, *Cephalosporium mycophilum* (CORDA) TUBAKI, *C. acremonium* CORDA, *Chaetomium indicum* CORDA were isolated together with *A. glauca*. In the beetle *C. niponicus*, the male bored the galleries, while the female stored and propagated the fungi; in other words, one pair of the beetles formed one gallery system.

INTRODUCTION

Ambrosia beetles bore deeply into the wood of felled trees, logs or stumps, etc., cultivate ambrosia fungi in the galleries and feed on them. The relationship among the following three items, the ambrosia beetles, the fungi, and the host trees, have recently become of special importance from a viewpoint of ecology of the beetles and also from a nutritional physiological point of view together with the desirability of protecting the wood material of logs and felled trees. The ambrosia beetle has within its body gland-like organs called mycetangia in which it stores and protects the spores of ambrosia fungi, and to date the presence of this organ has been verified in approximately 50 species of the beetles. However the majority of the cases belong to the family Scolytidae, and a few have been revealed to be of the family Platypodidae.

Crossotarsus niponicus BLANDFORD belongs to the subfamily Crossotarsarinae of the family Platypodidae. In 1894 this species was originally described by BLANDFORD from Japan. This species has been found in Hokkaido, Honshu, Shikoku and Kyushu including Formosa. The trees which it is reported to attack are the beech, the Japanese oak, the zelkova tree, the Japanese walnut, etc., coming to a total of 20 or more species. Thus, it may be said that *C. niponicus* is an important enemy in forestry. *C. niponicus* bores so-called pin holes deeply into the wood of the trunk while the surroundings of the galleries are contaminated by ambrosia fungi and show a characteristic double layer of black ring-like spots (Figs. 8, 9, 10). In the present report the ambrosia fungi and mycetangia of this beetle and some associated habits were examined.

ISOLATION OF THE AMBROSIA FUNGI

Materials and methods

Pinholed beech samples (*Fagus crenata* BLUME, logs and stumps of about 20–35cm in

diameter) were selected from a beech stand at Kaminokuni in the Hiyama district, Hokkaido, in the autumn of 1968 and 1969. These beech samples were transported to Sapporo and allowed to lie over winter under snow. The samples were transferred to the experimental laboratory at the end of April, and the adult beetles of *C. niponicus* appearing during the beginning and middle of May were captured within 24 hr of their appearance and used for the experiments.

Generally, in the ambrosia beetles having mycetangia, the spores of ambrosia fungi are found stored within the mycetangia and are protected from desiccation by secretions from the beetles (FRANCKE-GROSMANN, 1956; 1967). Therefore, in the present experiment, the fractional sterilization method of FRANCKE-GROSMANN (1956) was utilized to remove general fungi attached to the bodies of the beetles. The adult beetles were first placed in a petri dish in which a sterilized filter paper was placed; after 48 hr these beetles were transferred to a dry sterilized petri dish and kept there a further 48 hr. This procedure was repeated 3 times. After the third treatment, the beetles were left in a petri dish containing a moistened sterilized filter paper. The remaining fungi were thus allowed to germinate and were cultured and isolated on a potato-dextrose agar culture medium. The temperature was maintained at 23°C and the number of beetles of both sexes were 20.

Results

No growth of mycelia was seen on the male adults, and outward gloss of the body surface remained normal, showing a clean state.

Each individual female expectorated a mildly viscous, light yellowish-white fluid from its mouth, and from this fluid a light greyish-brown mycelia grew and covered the surface of the female beetle. From both the fluid adhering to its mouth and from the mycelia covering the body, *Absidia glauca* HAGEM was isolated. Further, from 2 or 3 of the individuals, minute white fungi clumps and viscous fluid pale yellow in color were noted on the mesocoxae and abdominal segments. From these were separated: *Absidia glauca* HAGEM, *Cephalosporium mycophilum* (CORDA) TUBAKI, *C. acremonium* CORDA, *Chaetomium indicum* CORDA together with several species of *Bacillus*.

MYCETANGIA

Materials and methods

Adult beetles of *C. niponicus* were collected from the beechwood samples in the same manner as the above experiment. Forty-six females and 40 males were examined. Individuals of both sexes were fixed in A. F. T. fixing fluid (CARAYON method). The entire body was double embedded with Celloidin-Paraffin following the BRIGGS method (HUMASON, 1962), and 20 μ thin sections were stained using a modification of the GRAM-WEIGERT procedure (LEACH, 1940), which differentiates yeasts, fungi, Gram-positive bacteria in blue and insect tissues in red.

Results

In the head of the female beetle, two tissues were stained blue (Figs. 1-5). One was a single sphere shaped tissue with a diameter of about 0.2 mm, situated at the back of the preoral cavity, forward from, but under, the suboesophageal ganglion. The other was a spongelike tissue which surrounded the sphere from the left, right, above and rear, and extended forward along the cibarium. Further, the fluid

- Fig. 1. Sagittal section of the head of female *C. niponicus*. (My. 1, My. 2: mycetangia; FL: fluid contained in preoral cavity; Br: Brain; SG: suboesophageal ganglion).
 Fig. 2. Longitudinal section (slightly to the right of Fig. 1).
 Fig. 3. Same as above (slightly more to the right of Fig. 2).
 Fig. 4. Horizontal cross section through the head of adult female.
 Fig. 5. Same as above (slightly forward of Fig. 4).
 Fig. 6. Front leg of adult male.
 Fig. 7. Front leg of adult female.
 Fig. 8. Beech sample suffering ambrosia beetle damage (G: gallery; C: contamination extending from gallery).
 Fig. 9, 10. Gallery formation of *C. niponicus* in beech wood samples, and the larvae.
 Fig. 11. Gallery formation in an artificial rearing medium (AF: adult female; Arrow: expectorated fluid daubed on the gallery walls).

present in the preoral cavity was stained slightly reddish violet as compared with the above 2 tissues. As a result of dissecting non-stained individual specimens, the observed results showed milky-white for the 2 tissues, and the sphere tissue could be readily detached from the head. The fluid contained in the preoral cavity was pale yellow.

In the head of the male beetle no tissue as described above for the female head was seen, while better developed muscles than those of the female were observed.

In the proctoderm of both sexes, using the above staining method, some minute portions were stained pale violet.

DISCUSSION

Using adult beetles as a result of a certain moist and dry alternating sterilization procedure, it was shown that absolutely no growth of mycelia was seen on the body of the male beetles. This indicates that the various fungi on the body of the male beetle were not under any state of protection by the body of the male. FRANCKE-GROSMANN (1956) sterilized the bodies of several species of Xyloterini and Xyleborini, and demonstrated that a complete sterilization occurred. In addition BATRA (1963) shortened the alternating period of moist and dry to 12 hr and showed that the desired effect could be obtained.

As for the female beetles, in spite of the same method of sterilization, in all specimens mycelia grew from the mouth, and in all specimens *Absidia glauca* HAGEM were isolated. These facts suggest the presence of some storage organ in the mouth which protects the fungi. Examination of serial sections of the female beetles stained with the modified GRAM-WEIGERT stain showed two tissues containing blue colored fungus material. One was a single sphere shaped tissue situated at the back of the preoral cavity and the other was a sponge-like tissue surrounded the sphere. The slightly viscous fluid filling the preoral cavity was stained slightly reddish violet as compared with the blue of the above tissues. This may be because the fungi excreted from the above tissues may have been diluted by some secreted fluid in the preoral cavity.

In the proctoderm of both the male and female adult limited areas are stained; however, these may not be ambrosia fungi but are rather microorganisms related to digestion. FARRIS and FUNK (1965) reported such stainable portions in the digestive tract of *Platypus wilsoni*.

When a pair of adult male and female beetles are reared together in order to observe their boring activities, it was noted that invariably the male took the lead in boring. When the gallery is nearly finished, the male and female change the lead, and the female moves forward and smears the gallery walls with a pale yellow fluid from its mouth (Fig. 11). Mycelia growth is seen 3 or 4 days after the smearing. When a female alone is reared, not infrequently the boring fails, or in most cases the female dies after penetrating a distance of several centimeters.

From the difference in activities of the male and female in the boring of the galleries, the fact that in the culture tests mycelia growth is seen only in the female, together with the results in staining of the body tissue, it is apparent that in this species the female beetle has the symbiotic fungi and that the mycetangia are the spherical body and sponge-like tissue located at the back of the preoral cavity of the female.

The mycetangia of this species, according to the classifications of BATRA (1963)

and FRANCKE-GROSMANN (1963), belong to the category of oral mycetangia. The species in which oral mycetangia were found until now belong to the genera *Xyleborus*, *Dryocoetes* and *Pterocyclon* all of them limited to the family Scolytidae. In both the genera *Xyleborus* and *Dryocoetes*, a pair of mycetangia are found at the base of the mandible while in the genus *Pterocyclon* one is found between the mentum and the pregula, so the mycetangia in these cases are different tissues from that of the present species. As far as the family Platypodidae is concerned, so far as the author is aware, those in which mycetangia were confirmed are limited to 3 species of the genus *Platypus*. In these 3 species, hollows or small punctures in the pronotum play the role of mycetangia, and are also different from the case of the present species. It seems that the mycetangia of *C. niponicus* rather resemble the infrabuccal pouch of the queen of the leaf-cutting ants.

Mycetangia usually are found in one sex only, and in the case of the family Scolytidae, the mycetangia are developed in the sex which first attacks the host plant. In most cases they are reported to be the female (FARRIS and FUNK, 1965; FRANCKE-GROSMANN, 1967). However, in *Platypus wilsoni* of the family Platypodidae, in spite of the fact that the male first attacks the host plant, the sex that has the mycetangia is the female (FARRIS and FUNK, 1965). FRANCKE-GROSMANN (1967), in her outstanding review, reported that in the family Platypodidae, it is the male which attacks first, but in the few cases where true mycetangia have been found, they are present in the female. Similarly in the present case, the sex which first attacks the host plant and bores the galleries is the male as shown in the rearing tests in the present work, however the sex that possesses the mycetangia is the female. In the head of the male well-developed muscles connected with the mouth parts are present, and on the outer surface of the front tibia several deep grooves can be noticed (Fig. 6). In all cases these seem to be related to the boring habit of the male. The sex ratio as shown in Table 1 is 1 : 1, and within the galleries a pair of male and female parents share abode invariably as shown in Table 2 during the period of rearing the progenies. This monogamous

Table 1. THE FLUCTUATIONS OF THE ADULT APPEARANCE FROM BEECH SAMPLES COLLECTED AT THE END OF APRIL, 1968 (COUNTED IN THE LABORATORY)

			No. of		Percentage females
			♀	♂	
1968	May	16—20	0	0	—
		21—25	39	110	26.2
		26—30	188	139	57.5
		31—4	44	22	66.7
	June	5—9	4	2	66.7
		10—14	0	0	—
		15—19	1	2	33.3
		20—24	55	55	50.0
		25—29	21	24	46.7
		30—4	13	8	61.9
	July	5—9	0	3	0
	Total		365	365	50.0

Table 2. FAMILY STRUCTURE WITHIN EACH GALLERY SYSTEM
(BEECH SAMPLES, AUGUST 14-27, 1968)

		No. of			
Parent		Eggs	Larvae	Pupae	New adults
♀	♂				♀ ♂
1	1		7		
1	1		5		
1	1		4		
1	1		9	3	
1	1		10	4	
1	1		4	2	
1	1		11	5	
1	1		10	5	
1	1	18	64	18	
1	0		13	5	1 0

habit may be based on the role of the male as the gallery borer and the function of the female which stores and propagates the fungi.

As regards the symbiosis of the fungi and the ambrosia beetles, numerous reports are available on the fungi. However, by reason of the fact that these fungi have adapted themselves to a special symbiotic environment and have attained a high degree of specialization, as far as classification and ecology is concerned, various problems remain unsolved. Hitherto the fungi which have been reported to be symbionts of ambrosia beetles are numerous. They are : *Phycomycetes-Mortierella*; *Ascomycetes-Ascoides, Ceratocystis*; *Fungi imperfecti-Botryodiplodia, Monilia, Cephalosporium, Penicillium, Sporothrix, Sporotrichum, Leptographium, Monacrosporium, Cladosporium, Tuberculariella, Ambrosiomyces, Ambrosiella* (BAKER, 1963; FRANCKE-GROSMANN, 1967). As regards the 4 species of the fungi isolated in the present work, since no rearing tests of the present beetles on these fungi were made, nor any comparative studies among the fungi growing in the galleries, verifications were not obtained. But since *Absidia glauca* HAGEM was isolated from the fluid contained in the preoral cavity of the female, it is suggested that this is the symbiotic fungus of the present beetles. Further since it seems that *Chaetomium indicum* CORDA, *Cephalosporium mycophilum* (CORDA) TUBAKI, *C. acremonium* CORDA receive some protection by the beetle, it is surmised that they, together with *A. glauca*, from the ambrosia fungi flora.

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REFERENCES

- BAKER, J. M. (1963) Ambrosia beetles and their fungi, with particular reference to *Platypus cylindrus* FAB. 13th Symp. Soc. Gen. Microbiology, 232-265.

- BATRA, L. R. (1963) Ecology of ambrosia fungi and their dissemination by beetles. *Transac. Kansas Acad. Sci.* **66** : 213—236.
- FARRIS, S. H. and A. FUNK (1965) Repositories of symbiotic fungus in the ambrosia beetle *Platypus wilsoni* SWAIN (Coleoptera : Platypodidae). *Can. Ent.* **97** : 527—532.
- FRANCKE-GROSMANN, H. (1956) Hautdrüsen als Träger der Pilzsymbiose bei Ambrosiakäfern. *Z. Morph. u. Ökol. Tiere* **45** : 275—308.
- FRANCKE-GROSMANN, H. (1963) Some new aspects in forest entomology. *Ann. Rev. Ent.* **8** : 415—438.
- FRANCKE-GROSMANN, H. (1967) Ectosymbiosis in wood-inhabiting insects. *Symbiosis* (S. M. HENRY ed.), **2** : 141—205. Academic Press, New York and London.
- HUMASON, G. L. (1962) *Animal tissue techniques*. W. H. FREEMAN and Co., San Francisco and London, 468 pp.
- LEACH, J. G. (1940) *Insect transmission of plant diseases*. McGraw-Hill Book Co. Inc., New York and London, 615 pp.