論文の内容の要旨

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論文題目 Nutrient ecology of the xylophagous insect *Dorcus rectus* (Coleoptera: Lucanidae) in association with microbes
（微生物と結びついた材食性昆虫コクワガタ（鞘翅目：クワガタムシ科）の栄養生態学）

Insects have great difficulty in utilizing wood as food resource. Wood is composed of mostly of polymers that insects are difficult to digest, and almost lacking in essential nutrients. Therefore, most insects that reside in wood are known to have an association with microbes that help digestion of wood. Some wood-boring insects have a mutualistic association with fungi. For example, ambrosia beetles carry a specific ambrosia fungus into the wood where they deposit eggs, cultivate it in the wood, and feed on it. Wood wasps also have symbiotic fungi that may enhance the nutrient value of wood or help digestion of wood. For these insects the association with microbes is clear. However, there are other insect groups that do not have a definite symbiotic association unlike ambrosia beetles or wood wasps, but do utilize the fungi that have already existed in wood. The typical insects are stag beetles.

Adult females of the stag beetle *Dorcus rectus* (Motschulsky) (Coleoptera: Lucanidae) deposit eggs in dead trunks and boughs of broad-leaved trees that have been infested with white-rotting fungi. The mothers place granulated wood besides the eggs at oviposition. Hatched larvae initially feed on the granulated wood and then move to feed on the decaying wood. The granulated wood have been thought to harbor some microbes beneficial to the larval nutrition. In Chapter 1, I examined the effect of wood decay by different wood-rotting fungi on larval growth, and the effect of initial ingestion of the granulated wood on larval growth. To prepare decaying wood, autoclaved Japanese beech (*Fagus crenata*) wood sawdust were inoculated with each two white-rotting fungus *Bjerkandera adusta* and *Trametes versicolor*, and incubated at

25°C for 3 or 10 months. Newly hatched larvae of *D. rectus* were initially supplied with small amount of the granulated wood, and then reared on the decaying wood or non-decaying wood for 28 days. For a control, newly hatched larvae were soon placed on the decaying wood or non-decaying wood and reared in the same manner. Consequently, I demonstrated that the larvae cannot grow on non-decaying wood, and that initial ingestion of the granulated wood positively affected the larval growth although the larvae which were not supplied with the granulated wood also exhibits a substantial growth on decaying wood.

Insects that feed on decaying wood are usually classified as xylophagy although decaying wood contains substantial amount of fungal mycelia. In Chapter 2, I examined whether *D. rectus* larvae can utilize only fungal mycelia as food. For this purpose, newly hatched larvae of *D. rectus* were reared for 14 days on artificial diets containing a fixed amount of freeze-dried mycelia of the following fungi: *Bjerkandera adusta*, *Trametes versicolor*, *Pleurotus ostreatus* and *Fomitopsis pinicola*. The mean incremental gain in larval body mass was greatest on diets containing *B. adusta*, followed by *T. versicolor*, *P. ostreatus*, and *F. pinicola*. The growth rate of body mass correlated positively with mycelial nitrogen content of the different fungi. It also correlated positively with the mycelial content of *B. adusta* in the diet. Addition of antibiotics to diets with mycelia nearly halved larval growth, indicating that larvae were able to use fungal mycelia as food without the assistance of associated microbes although the microbes positively affected larval growth. Four newly hatched larvae reared on artificial diets containing *B. adusta* mycelia developed to the second instar in 21 to 34 d; and one developed to the third (= final) instar. These results provide evidence that fungi may constitute the bulk of the diet of *D. rectus* larvae.

Dead wood is decomposed by wood rot fungi, resulting in decaying wood. Because wood has a solid structure that remains for a long time, the chemical components of wood are mostly kept within wood. Some water-soluble components are released from decaying wood by reaching, however, most of the water-insoluble components remain in decaying wood. These water-insoluble components may be released by insects and incorporated into the nutrient circulation in forest. In Chapter 3, three independent experiments were conducted to clarify whether *D. rectus* larvae can utilize the water-insoluble fraction of decaying wood and fungal mycelia. In the first experiment, third instar larvae of *D. rectus* were reared on decaying willow (*Salix* sp.) wood and the decaying wood and larval feces were analyzed for the carbon and nitrogen contents in each fraction of hot-water- and hot-alkaline-extraction. Nitrogen of the water-soluble and alkaline-soluble-water-insoluble fraction exhibited 39% and 38%

decrease, respectively, from decaying wood to larval feces, although nitrogen of the alkaline-insoluble fraction shoed almost no change. Carbon contents showed the same tendency, however, the decrease was only 21% and 26% for water-soluble and alkaline-soluble-water-insoluble fraction, respectively. In the second experiment, larvae were reared on the same decaying wood and the absorption ratio was determined as the decrease in dry mass through the larval ingestion and excretion. The absorption ratio was calculated as 26%, and using this value, the net utilization rate of carbon and nitrogen of each extractive fraction were recalculated. The third experiments was conducted to determine whether the larvae can grow merely on either water-soluble or water-insoluble fraction of fungal mycelia. To make artificial diets, freeze-dried mycelia of *B. adusta* was extracted by distilled water at 100°C for 3 hours and the extract and residue that were equivalent to 100 mg dry mycelia were suspended in agar. Newly hatched larvae did not grow either on the diet containing mycelial extract or on the diet containing residue, although the larval growth on the mixed diet containing both extract and residue was as much as that on the diets containing the equivalent amount of the non-extracted mycelia.

Fungal decomposing activity can enhance the nutrient value of wood, however, the nitrogen and other nutrient levels in decaying wood are still much lower than those of insect bodies. Therefore, cannibalism usually plays an important role in nutrient ecology of wood-feeding insects. In Chapter 4, I examined the nutritional significance of cannibalism in *D. rectus* larvae, with reference to their interference competition in laboratory and field. A laboratory experiment, in which two larvae were placed on milled decaying wood in test tubes for two weeks, showed that cannibalism occurred in the first and second instar larvae. Cannibals tended to have larger head capsules than their victims. Cannibalizing larvae gained more body mass than non-cannibals. The carbon/nitrogen (C/N) ratio of decaying wood was much higher than that of larvae, explaining an increased body mass following cannibalism. Sixty-two percent of surviving second-instar larvae were wounded by their opponents. When cannibalism did not occur at the second larval instar, large-headed larvae grew but the growth of small-headed larvae was restricted, suggesting strong interference. However, a field study suggested low rates of interference competition between larvae.

Wood-boring insects that associate with mutualistic microbes usually possess special organs to store and convey the microbes. In Chapter 5, I newly discovered the microbe-storage organ (mycangium) in the stag beetles. The mycangium being located under the tergum was present in all adult females of 22 lucanid species examined, but absent in adult males. On the contrary, adult insects of both sexes of Passalidae and

Scarabaeidae, which are closely related to Lucanidae, had no mycangium unlike lucanids. Yeast-like microbes were isolated from the mycangium of five lucanid species. Base sequence of ITS/5.8S and D1/D2 rDNA indicated that the microbes are closely related to the xylose-fermenting yeasts *Pichia stipitis*, *P. segobiensis*, or *Pichia* sp.

This study showed two microbial associations of stag beetles in reference to their nutrition: one is the one-sided utilization of wood-rotting fungi by insects, and another is the symbiotic association with xylose-fermenting yeasts. Stag beetles are utilizing wood-rotting fungi within decaying wood as well as having a symbiotic association with xylose-fermenting yeasts to help digestion and/or detoxification of wood xylose. There are many ecologically and evolutionally interesting phenomena left in the association of stag beetles and microbes, therefore, further studies are needed for fully understanding of nutrient ecology of stag beetles.