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Are stag beetles fungivorous?

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ABSTRACT

Stag beetle larvae generally feed on decaying wood; however, it was unknown whether they can use wood-rotting fungi alone as food. Here, to clarify this, newly hatched larvae of *Dorcus rectus* (Motschulsky) (Coleoptera: Lucanidae) 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 21–34 days; and one developed to the third (=final) instar. This study provides evidence that fungi may constitute the bulk of the diet of *D. rectus* larvae.

1. Introduction

Fungivory is widely observed in insects (Kukor and Martin, 1987). Insects that inhabit the fruiting bodies of macrofungi such as mycetophilid flies (Diptera) and erotylid beetles (Coleoptera) are obligate fungivores (Kukor and Martin, 1987). There are also many insect species that feed exclusively or predominantly on fungal tissue by grazing on the substrate on which the fungus is growing. Leaf-cutting ants (Hymenoptera) and macrotermitine termites (Isoptera) culture specific fungi in their nests and feed directly on the fungal tissues (Chapela et al., 1994; Wood and Thomas, 1989). Ambrosia beetles culture and consume the ambrosia fungi that grow within their galleries in wood (Beaver, 1989). For these insects, evidence of fungivory is clear. However, for insects that feed in substrates containing fungi, it can be difficult to determine if the fungi are used for nutrients or merely consumed along with the substrate. For example, insects such as *Drosophila melanogaster*, whose larvae eat yeast on rotten fruits (Simmons and Bradley, 1997), are difficult to confirm as fungivorous.

Stag beetles (Lucanidae), some cerambycid beetles, and higher termites inhabit and feed on wood decayed by wood-rotting fungi (Araya, 1993; Saint-Germain et al., 2007; Abe et al., 2000). Wood is composed mostly of cellulose as well as lignin and hemicelluloses which together comprise about 90% of the total volume (Parkin, 1940). These compounds are difficult to digest. In contrast, wood contains extremely low contents of nitrogen, sugars, and starch (Haack and Slansky, 1987). Such nutrient conditions make wood a poor food resource. However, colonization by wood-rotting fungi reduces polymers to simpler compounds (chemically altered), resulting in a physically altered structure as well as chemically altered structure which may increase the value of wood as a nutritional resource. Hanula (1993) pointed out five possible advantages of fungal-infested wood over fresh wood as food for insects: (1) increased concentrations of nitrogen and other elements in fungal mycelia, (2) increased ingestion and digestion of wood made fragile by wood-rotting fungi, (3) increased moisture content of wood, (4) increased digestion of woody tissue by enzymes originating from fungi, and (5) detoxification of toxic or repellent allelochemicals in wood. However, another potential advantage of feeding on wood colonized with wood-rotting fungi is direct nutrient acquisition from the fungal mycelia.

The stag beetle, *Dorcus rectus* (Motschulsky), is distributed widely in Japan (Kurosawa, 1985). Adult females locate decaying wood of broad-leaved trees affected by white-rot fungi, make a small pit with their mandibles on the surface of the wood, pack granulated wood into the pits, and then insert their ovipositor into the granulated wood and deposit an egg there (personal observation by Tanahashi). Larvae hatching from these eggs then feed on the granulated wood before moving inwards to feed on

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decaying wood (Kojima, 1996). The granulated wood is believed to harbor microbes beneficial to larval development. The total duration of larval development (three instars) varies from about 6 months to 2 years depending on environmental and nutritional conditions (Kojima, 1996). In contrast to larvae, adults feed by sucking on sap exuded from tree trunks and rotten fruits. Adults live for 1 year or more in the field because new adults, that have sharp mandibles and intact elytra, and old adults, that have blunt mandibles and wounded elytra, hibernate together in decaying trunks (Tanahashi unpubl.).

Decaying wood that *D. rectus* larvae consume includes a substantial amount of fungal mycelia as shown by Jones and Worrall (1995); however, it is unknown whether they can use fungal mycelia alone as food. The first aim of this study was to determine if *D. rectus* larvae are fungivorous; for this purpose, we performed rearing experiments with artificial diets containing freeze-dried mycelia of four wood-rotting fungi. Second, to determine the larval growth response to low nutrient conditions, we reared the larvae on artificial diets containing different amounts of fungal mycelia. Finally, to determine whether associated microbes influence the growth of *D. rectus* larvae feeding on fungal mycelia, we reared the larvae on diets with fungal mycelia present both with and without antibiotics.

2. Materials and methods

2.1. Insects

More than 30 males and 60 females of *D. rectus* adults were collected on Mt. Takao, 599 m above sea level (Hachioji City, Tokyo, Japan) in late August 2005 and 2006. Since *D. rectus* females captured in 2005 rarely oviposited, they were fed with commercial food for beetles (Power Jelly; LIS, Tokyo, Japan) for 2 months and then placed at 5 °C in the dark for 5 months to simulate overwintering conditions. The adult females readily oviposited after the low-temperature treatment. The adults were placed individually in transparent, polystyrene containers (179 mm $\times$ 89 mm $\times$ 47 mm deep) with *Quercus acutissima* wood chips of 1 cm deep and fed commercial food under constant conditions of 25 °C and L:D 16:8 or 8:16 h. Males and females were randomly paired every 2 weeks. A piece of moistened *Q. acutissima* wood decayed by the white-rot fungus *Lentinula edodes*, 30 mm $\times$ 30 mm $\times$ 50 mm, was supplied individually to female adults every 1–3 days for oviposition. The wood was then removed and the eggs collected. Eggs were sterilized by soaking them in deionized water, 99.5% ethanol, and 70% ethanol twice in that order, and finally rinsed in sterile deionized water for 20 s. Sterilized eggs were placed on moistened filter paper in Petri dishes and then incubated at 25 °C in the dark. Newly hatched larvae were weighed individually within 1 day of hatching. Intermittent deposition of a small number of eggs by individual females and high mortality of the surface-sterilized eggs required about 1 year for the collection of all larvae needed in our studies. Newly hatched larvae were allocated to three experiments at the time of hatching to randomize the effect of time of collection.

2.2. Fungi

Three white-rot fungi, *Bjerkandera adusta* (Willd.) P. Karst., *Trametes versicolor* (L.) Lloyd, *Pleurotus ostreatus* (Jacq.) P. Kumm., and the brown-rot fungus, *Fomitopsis pinicola* (Sw.) P. Karst., subcultured separately on potato dextrose agar (PDA; Sigma–Aldrich, St. Louis, MO, USA) plates at 25 °C, were used in this study. *B. adusta*, *T. versicolor*, and *F. pinicola* are the dominant wood-rotting fungi in temperate deciduous forests (Coates and Raynor, 1985; Chapela et al., 1988; Nordh, 1997; Takahashi and Kagaya, 2005), while sawdust on which *P. ostreatus*, an edible fungus, is cultured, is used for larval rearing of stag beetles in Japan.

A 5-mm diameter disk of each fungal culture was removed from the plates, floated on 15 ml of potato dextrose broth (Sigma–Aldrich) (24 g/l) in 50-ml flasks, and incubated at 25 °C for 10 days. Mycelia were removed from the broth using suction filtration, rinsed with deionized water several times, and then freeze-dried for 24 h. Dried mycelia were ground with mortar and pestle to pass through 0.5 mm mesh.

To determine whether fungal mycelia grown on a natural substrate and those grown on artificial media have different effects on *D. rectus* larval growth, we selected *B. adusta*, the most effective fungus on the larval growth, and *Fagus crenata*, a broad-leaved, deciduous tree. Seven to 8-year-old *F. crenata* trees were cut into 40-cm-long logs (4–6 cm in diameter) at the Tokyo University Forest nursery in Chichibu in December 2006. The logs were soaked in water at room temperature for 1 day, placed by in groups of six in autoclavable plastic bags, and then autoclaved at 121 °C for 2 h. A mycelial culture of *B. adusta* on PDA plates (9 cm diameter) was put on the logs in each bag and then incubated at 23–25 °C for 6 months. After that, mycelial mats growing on the surface of the logs were collected, rinsed with deionized water several times, and then freeze-dried as mentioned above.

To determine carbon and nitrogen contents of dried mycelia of the four fungi, a small sample (30–50 mg) of mycelia was analysed by an elemental analyser (CN Corder MT-700, Yanaco, Kyoto, Japan). This determination for *B. adusta* was repeated five times to confirm homogeneity of sample and then for three other species of fungi it was repeated three times.

2.3. Artificial diets

A basic artificial diet was prepared as follows. Agar powder (450 mg) (Nacalai Tesque, Kyoto, Japan), mycelial powder (100 mg), and deionized water containing the preservatives described below (15 ml) were placed in a 50-ml glass test tube (27.3 mm inner diameter) with a flat bottom. Test tubes were capped with a polypropylene cap and autoclaved at 121 °C for 15 min. When cooled to 70 °C, test tubes were taken out of the autoclave and the molten diets were stirred several times, and then allowed to cool on a clean bench. Deionized water containing the preservatives was prepared by dissolving sorbic acid (0.828 g), L(+)-ascorbic acid (1.0 g), and sodium hydrocarbonate (1.1 g) in deionized water (1000 ml). We used sorbic acid as an anti-microbial agent, which is commonly used in artificial diets of longhorned beetles (Kosaka and Ogura, 1990; Dubois et al., 2002), and ascorbic acid was applied to prevent oxygenation of the artificial diets during autoclaving. Sodium hydrocarbonate was added to pre-autoclaved diets to raise the pH to ca 5.5. Preliminary experiments indicated that the preservatives did not affect larval growth. A control diet free from mycelial powder was prepared with 450 mg of agar and 15 ml of deionized water with preservatives.

2.4. Rearing procedure

Immediately after weighing, newly hatched larvae were placed individually on artificial diets in test tubes aseptically and reared at 25 °C in the dark. After 14 days, larvae were removed from test tubes and their body mass was determined in Experiments 1, 2, and 3.

2.5. Effect of different fungal species on larval growth (Experiment 1)

To determine the effect of mycelia from the four different fungal species on initial larval growth, newly hatched larvae were placed on artificial diets containing powdered mycelia of PD broth-