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Nitrogen fixation in the stag beetle, *Dorcus* (*Macrodorcus*) *rectus* (Motschulsky) (Col., Lucanidae)

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Abstract: Stag beetles are xylophagous insects that feed mainly on dead wood. They play an important role in the decomposition of dead wood in forest ecosystems. Most dead wood contains 1% nitrogen at most. It is suspected that stag beetles can utilize atmospheric nitrogen. We show that the larvae of *Dorcus* (*Macrodorcus*) *rectus* exposed to nitrogen reduce acetylene to ethylene in a time-dependent fashion. No reaction was detected with the dead wood or autoclaved larvae, suggesting that living larvae use the reaction for fixing nitrogen. Acetylene reduction to ethylene by larvae increased with incubation time. This effect was not seen using decayed wood only, autoclaved wood only or autoclaved larvae. Acetylene reduction by the larva proceeded at 1.25 ± 0.37 nmol acetylene/h/g (fresh wt), corresponding to the fixation of 0.25 μg nitrogen per day per larva.

Keywords: acetylene reduction, dead wood, xylophagous insect

1 Introduction

Nitrogen gas (N_2) accounts for 79% of the Earth's atmosphere, but very few organisms are able to use it as a nitrogen source (Dixon and Kahn, 2004). Most biologically available nitrogen originates from nitrogen fixation mediated by diazotrophic bacteria that are widely distributed in both marine and terrestrial ecosystems (Nardi et al., 2002). These bacteria are either free-living or associated with higher organisms, such as legumes (e.g. Sprent and Sprent, 1990). Evidence for nitrogen fixation has been found in the form of acetylene reduction activity in insects with nitrogen-poor diets: several termite species (e.g. Benemann, 1973; Breznak et al., 1973), a bark beetle, *Dendroctonus terebrans* (Bridges, 1981); a wood-eating cockroach, *Cryptocercus punctulatus* (Breznak et al., 1974); a scarabaeid beetle, *Cetonia* spp. (Citernesi et al., 1977); and a fruit fly, *Ceratitis capitata* (Behar et al., 2005). Stag beetles are xylophagous insects that mainly feed on dead wood, and are thus termed saproxylic. Saproxylic insects play an important role in the decomposition of dead wood in forest ecosystems (Harmon et al., 1986). Wood mould contains 0.8–1.0% nitrogen (Kelner-Pillault, 1974; Jönsson et al., 2004), while most animals comprise about 10% nitrogen. It is suspected that they have efficient ways of acquiring nitrogen. Little is known about the origin of the nitrogen supply for saproxylic insects other than termites. This paper is the first to report the N_2 fixing ability of the stag beetle larvae, *Dorcus* (*Macrodorcas*) *rectus* using acetylene reduction method.

2 Materials and Methods

2.1 Insects

Larvae of *D. rectus* were collected from naturally decayed Japanese oak wood in mid-April at Tsukuba, Japan. All larvae had terminated their diapause. Each larva was individually placed in a polyethylene cup containing decayed wood pieces and maintained for 5 days at 25 ± 1°C. Only third-instar larvae of similar size were used for the assay.

2.2 Acetylene reduction assay

Acetylene reduction was used as a functional assay for nitrogen fixation (Stewart et al., 1967; Hardy et al., 1968). Acetylene was purchased from Higashi-Nihon Koatsu Co. Ltd. The gas was purified by passing it through a 25 M NaOH solution, conc. H_2SO_4 and then water before use. It was injected into closed vessels (20 ml) to a final concentration of 20% (v/v) by replacement of 4 ml of air. The vessels contained an individual larva or decayed wood pieces from which the larvae had been collected. Autoclaved larvae and decayed wood pieces were used as negative controls. Reduction of acetylene to ethylene was measured by flame ionization gas chromatography (Shimadzu, GC-8AIF) using 0.5 ml gas samples taken at 0, 2 and 4 h after incubation at 25 ± 1°C. The experiments were replicated eight times for larvae and six times for wood pieces.

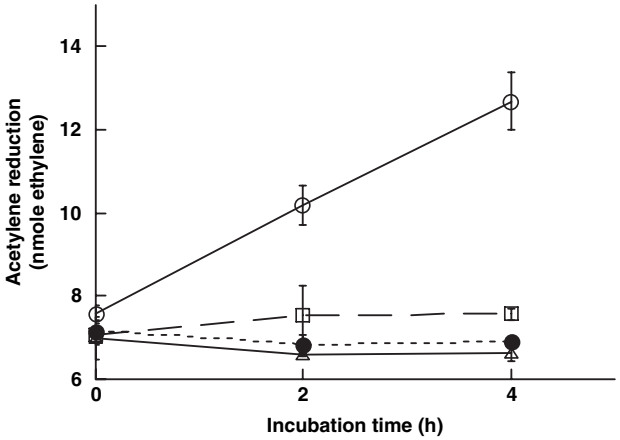

Fig. 1. *Acetylene reduction activity as a function of incubation time. Acetylene reduction is shown as the production of ethylene (nmole) in each sample. Bars represent standard errors.* ○—○; *living larvae,* □-□; *wood pieces,* ●···●; *autoclaved larvae,* △—△; *autoclaved wood pieces*

3 Results and Discussion

Acetylene reduction to ethylene by the larvae linearly increased with incubation time, but was not seen for decayed wood pieces, autoclaved wood pieces or autoclaved larvae (fig. 1). The low level of ethylene seen at 0 h was originally present in the purchased acetylene. Larval fresh weight was 1.41 ± 0.16 g. The true reduction activity by the larvae was calculated as 1.25 ± 0.37 nmol acetylene/h/g (fresh wt), corresponding to 0.25 μg fixed nitrogen per day per larva, calculated based on an ethylene:nitrogen molar ratio of 4:1 (Postgate, 1978). The estimated rate of nitrogen fixation for the stag beetle, *D. rectus*, is higher than seen in other Coleoptera: 0.03–0.05 nmol acetylene/day/g (corresponding to 0.00125–0.0021 nmol/h/g) by a scarabaeid beetle, *Cetonia* spp., (Citernesi et al., 1977), and no *in situ* acetylene reduction by a bark beetle, *D. terebrans* (Bridges, 1981). The acetylene reduction activity by the stag beetle may be caused by the association of nitrogen-fixing bacteria in the gut, as is the case with termites, the bark beetle and the fruit fly (Bridges, 1981; Breznak, 1982; Behar et al., 2005).

Termites, especially saproxylic species, are well-known for their role in nitrogen cycling in forest ecosystems in the tropics, in association with their symbiotic gut bacteria (Breznak, 1982). However, termites are not distributed in the cold temperate regions to which stag beetles are adapted: for example, *D. rectus* inhabits the northern part of Hokkaido in Japan, where there are no termites. In these regions, nitrogen fixation by stag beetles contributes to the nutrient supply to forest ecosystem. Further studies will be necessary to elucidate the nitrogen-fixing ability of stag beetles.

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