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Uric acid recycling in the shield bug, *Parastrachia japonensis* (Hemiptera: Parastrachiidae), during diapause

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Abstract

Nymphs of the univoltine shield bug, *Parastrachia japonensis* grow by feeding on the drupes of their sole food plant, which are available for only 2 weeks a year. The new adults soon enter a reproductive diapause and survive without feeding for at least 10 months up to 2 years. Uric acid was found to be the predominant component among four waste nitrogenous compounds, i.e., uric acid, allantoin, allantoic acid and urea in the body of both nymphs and adults in all stages, and to be predominantly excreted by the nymphs and reproductive adults. However, adults in diapause excreted negligible amounts of these compounds. *Erwinia*-like bacteria were found exclusively in the cecum of midgut, in which three uricolytic enzymes, i.e., uricase, allantoinase and allantoicase were detected. Ninety % of adults in diapause could survive on water for 9 months, but those given 0.02% rifampicin aqueous solution all died within this period, with significant reduction of the bacteria and uricase activity in the cecum. Rifampicin treatment resulted in a considerable reduction of free amino acids, especially proline in the hemolymph. These results suggest that uric acid is recycled as an amino acid source with the aid of *Erwinia*-like bacteria, and uricase functions as a key enzyme for this process.

Keywords: Diapause; Uric acid recycling; Uricase; Uricolytic enzyme; Symbiont

1. Introduction

Most insects are evolutionally uricotelic and excrete uric acid as the predominant end-product of nitrogen catabolism, while some species convert it to allantoin and further to allantoic acid, and a few insects living in water-rich circumstances further catabolize them to urea or ammonia (Bursell, 1967; Cochran, 1985a). Some species are known or have been suggested to recycle nitrogen from waste products with the aid of symbionts. In the brown planthopper, *Nilaparvata lugens* which stores uric acid in the body, intercellular yeast-like symbionts with the activity of uricase (urate oxidase; EC 1.7.3.3) residing in the fat body have a key role in recycling of uric acid (Sasaki et al., 1996; Hongoh and Ishikawa, 1997; Hongoh et al., 2000), while in a termite *Reticulitermes flavipes* intracellular bacteria in the hindgut have the ability to metabolize uric acid to amino acids (Potrikus and Breznak, 1980a,b, 1981). In the cockroach, *Periplaneta americana*, a similar role of fat body endosymbionts was suggested (Mullins and Cochran, 1975a,b; Cochran, 1985b), though the presence of the key enzyme uricase has not been well demonstrated in the latter two species. In none of these insects, has the presence of other uricolytic enzymes, i.e., allatoinase (E.3.5.2.5) and attantoicase (E.3.5.3.4) been demonstrated. In an aphid *Acyrthosiphon pisum,* which does not excrete any uricolytic substances but instead excretes extra amino acids, recycling of aspartate with the aid of endosymbiotic bacteria has been suggested (Sasaki et al., 1990).

The provisioning shield bug, *Parastrachia japonensis* is monophagous. Its sole food source is the drupes of the deciduous tree, *Shoepfia jasminodora* (Santalales: Olacaceae) (Gyotoku and Tachikawa, 1980), and the mothers provide the nymphs with this food. Young nymphs develop by feeding on the mature drupes of *S. jasminodora*, which are suitable for the growth of the young but are available

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only for a short period (nearly 2 weeks) during early summer (Nomakuchi et al., 1998; Filippi et al., 2000a, 2001). The new adults soon enter reproductive diapause, mostly forming aggregations that are suspended from the leaves and branches of non-host broad-leaf evergreen trees or ferns until the following spring, although they aggregate close to the ground on hot summer days and underground in winter, from December to February (Tsukamoto and Tojo, 1992; Filippi et al., 2000b). We recently demonstrated that aggregate formation functions to reduce significantly the respiration, which seemingly contribute to their long-term survival during diapause (Tojo et al., 2005). Inseminated females in the following spring move to *Schoephia jasminodora* where they eat the endosperm of the non-mature drupes, as the first food-uptake 10 months after adult emergence, and then develop ovaries and oviposit (Filippi-Tsukamoto et al., 1995). Thus, the adults are required to survive for at least 10 months without feeding.

Hemipteran insects are generally known to have symbiotic bacteria (Boush and Coppel, 1974). In the brown-winged green bug, *Plautia slali* (= *crossota*), long bacilliform microorganisms were detected in the cytoplasm of epithelial cells (mycetocytes) of gastric cecum at the end of the midgut (Abe et al., 1995). Further, we found the presence of *Erwinia*-like bacteria in the cecum in various species of stink bugs (unpublished). These facts suggested a possible involvement of *Erwinia*-like symbionts in the efficient utilization of nutrients stored in newly emerged *Parastrachia japonensis* adults for long period survival. Here, we show data supporting uric acid recycling in this species in diapause with the aid of *Erwinia*-like bacteria residing in the cecum.

2. Materials and methods

2.1. Insects

The life cycle of *Parastrachia japonensis* at Hinokuma Park, Saga, Kyushu (33°N, 130°E), the northern boundary of this bug's range is as follows. Females and males enter the reproductive stage from late April to middle May, when *S. jasminodora* blooms and produces drupes. For laboratory experiment, non-inseminated females were collected in middle April, and inseminated females in early May after long-term copulation was ascertained by observation (Tsukamoto et al., 1994). The inseminated females move to *S. jasminodora* in late May where they extract the digested endosperm of the drupes and their ovaries develop over a period of about 2 weeks. They oviposit on the ground under the leaf litter from mid-June. The females guarding the egg masses and those gardening the hatched nymphs in the nests were also collected as those before and after oviposition. Young nymphs develop by feeding on the mature drupes of *S. jasminodora* provisioned by their mothers from mid-June to early July. Nymphs in early (1st–2nd) and late (4th and 5th) instar nymphs were collected. The fifth instar nymphs moult to adults in mid-July. Adults shortly after emergence and 3 month later were further collected.

In the laboratory, these insects collected were reared at 22 °C under 16L–8D photo-regime in plastic cups which contained square cotton plugs with absorbed distilled water, or mature drupes of *S. jasminodora*, as mentioned for respective experiments. The tops of the cups were covered with sheets of newspaper to avoid direct light exposure, since they live in dark forests.

For chemical analyses of nitrogenous waste products, nymphs and adults were frozen within 1 day after collection and kept at −20 °C until analyses. For analyses of feces, nymphs and adults in growing or reproductive stage were reared on mature drupes of *S. jasminodora*, in plastic cups (i.d. 12 cm × 4.5 cm height), each cup containing five nymphs of mixed sexes, or five adults of a specific sex. The feces were rinsed out with distilled water after 3 days of rearing. The aliquot part of the rinsing was kept at −20 °C until analyses. For adults in diapause, 100 females or 100 males collected in mid-October were reared in groups on distilled water in large-sized plastic container (20 cm × 15 cm, 15 cm height), and the feces were similarly rinsed out from the containers after 6 months of rearing. Two replicates were done with the insects collected at each designated stage.

For physiological and biochemical studies with adults in diapause, 3-months old adults (wet weight: females, 180–230 mg; males: 130–160 mg) were reared on water or 0.02% (w/v) rifampicin (an antibiotic) aqueous solution in the small-sized plastic cups, each cup containing five individuals of a specific sex, and their survival ratios were monitored for 10 months. Other groups of adults in diapause were similarly reared on water or rifampicin solution, and five individuals of the same sex were used at different intervals for the analyses of nitrogenous waste products and total nitrogen. Six replicates were done for the insects of each respective group. Some individuals were used for the detection of *Erwinia*-like bacteria, uricolytic enzymes and amino acid assays.

2.2. Determination of uric acid, allantoic acid, urea and total nitrogen contents

Five adults or nymphs were homogenized in a glass homogenizer with 5 ml of distilled water. The homogenizer was treated in water bath at 90 °C and then centrifuged at 800 *g* for 5 min. After transferring the supernatant to a 15 ml glass cylinder, same extraction procedure was repeated twice further with 5 ml of distilled water. Three supernatants were mixed in the cylinder and filled up to 15 ml with distilled water, and frozen at −20 °C until further analysis.

Five ml of the supernatant prepared as mentioned above was added to a column (1 × 4 cm) with Dowex 1 × 2 resin. The column was washed first with 40 ml of 0.004 M formate, 100 ml of 0.01 M formate, and finally with 40 ml