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Symbiosis between Euglena and Damselfly Nymphs Is Seasonal

Abstract. *An endosymbiotic association has been demonstrated between* Euglena *and nymphs of three species of damselfly. The hindgut of the nymphs is inhabited by the euglenoid only during the winter. Symbiotic associations involving green euglenoids and insects are virtually unknown.*

Damselfly nymphs (Zygoptera: Odonata) collected during the last three winters from freshwater, interdunal ponds (*1*) have their rectums so densely packed with green unicells that the terminal four segments of the abdomen are dark green (Fig. 1). These algae (tentatively identified as *Euglena*) infect almost the entire population of each of three damselfly species, *Anomalagrion hastatum* (Say), *Lestes vigilax* Hagen, and *Ischnura verticalis* (Say). We have not seen this condition in other localities, nor do we find the algal masses on or in other members of the macrofauna, such as dragonfly nymphs (Anisoptera: Odonata), other aquatic insects, isopods, amphipods, and snails.

The rectum of damselfly nymphs is functionally modified as an auxiliary respiratory organ. Although this is not the primary site of oxygen exchange, as it is in the dragonfly nymphs, water can be pumped in and out through the anus bringing oxygen to additional absorptive surfaces (*2*). The abdomen of damselfly nymphs is sufficiently transparent that the cells of its green partner can be observed through the body wall. The gentle water current created by respiratory movements of the insect causes the green cells to swing back and forth, but it does not flush them out of the chamber. The cells are embedded in the folds of the rectum wall (Fig. 2), or they are attached by their posterior end to the cuticular lining and to each other by an adhesive substance. Cells removed from the rectum are spherical or cuboidal, 13 to 14 μm in diameter, having 8 to 12 large chromatophores and small red or brown granules, which probably represent the dispersed eyespot. As the cells are warmed by the microscope lamp for 10 to 30 minutes, they exhibit an apparent euglenoid movement or metaboly. During this period, many cells elongate to dimensions of 7 to 11 by 30 to 35 μm. In each a single flagellum emerges, and the cell swims away with the characteristic euglenoid gyrating swimming motion (Fig. 3). Studies with light and electron microscopes reveal the presence of pyrenoids, paramylon, two flagella within a reservoir, an eyespot made up of eight or more granules, a large endosome in the nucleus, and a striated pellicle underlain by microtubules and muciferous bodies. These are characteristic of the genus *Euglena*. The shape and size of the cells and the arrangement of the chromatophores render the species unidentifiable at this time (*3*).

The damselflies complete one life cycle each year. Eggs are laid during the summer; they then hatch, and the young nymphs grow rapidly until the ice closes the ponds in winter. Then growth ceases, and the nymphs feed only occasionally. In the spring, they resume growth, and the adults emerge in June. The life history of the damselfly and *Euglena* are synchronized by the seasonal changes in the pond. Both forms live separately during the summer, and nothing is known of the ecology of this *Euglena* during this time. Late in the fall, the *Euglena* is somehow attracted to the insect.

We have collected damselfly nymphs, in November, with the external surface of the abdomen around the anus and the bases of the caudal lamellae coated with elongate, green cells. The rectum of each of these nymphs had many spherical cells within it. Both inside and outside the insect, the *Euglena* were nonflagellated, and we assume that they enter the rectum through the anus by a combination of metaboly and gliding motion. The damselfly nymphs present a protected, motile, translucent microhabitat and probably a source of dissolved organic nutrients for the *Euglena*. Since the ponds are shallow and eutrophic, the insects move, as the periphery freezes to the bottom, into deeper water or congregate in spring-fed areas where the ice remains thin. When the ice thaws in March, the damselflies move rapidly into the relatively warm shallows (8° to 10°C) carrying the *Euglena* into regions of relatively higher light intensity where the cells may transform back to the motile, flagellate form and leave their winter host or may be discarded with the cast skin, which includes the rectal lining, when the insect molts. If, during the winter, we bring an insect collected from under the ice into the warm laboratory, the cells will

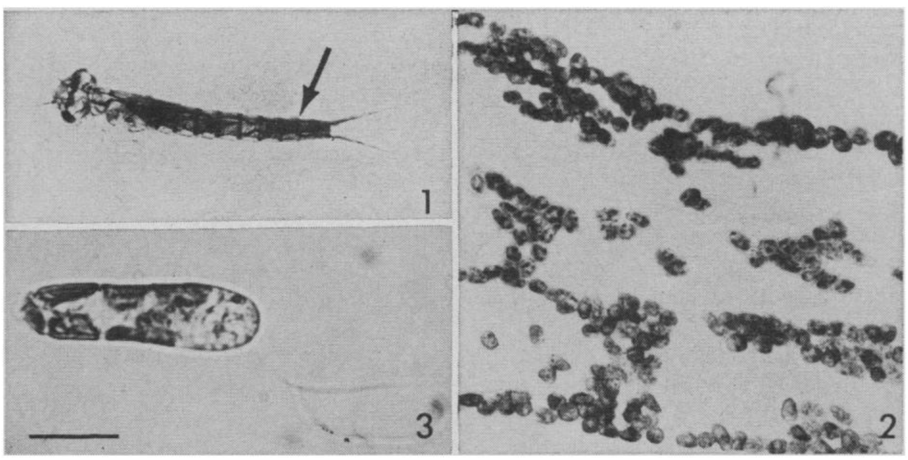

Fig. 1. Nymphal damselfly (*Anomalagrion hastatum*) carrying euglenoids in rectum (arrow). Fig. 2. Rectum removed from damselfly to show arrangements of euglenoids. Fig. 3. Flagellated *Euglena* (scale represents 10 μm).

leave the insect rectum within a week. Whether the damselfly benefits from its symbiotic partner as a potential source of oxygen is unknown.

Symbiotic associations involving either damselflies or green euglenoids are virtually unknown. Lackey (*4*) mentions only the epizoic *Colacium* and the endozoic *Euglenamorpha hegneri* Wenrich. Michajlow (*5*) discusses euglenoids as possible parasites of the guts of copepods. Although not recorded as such, damselflies may, like the closely related dragonfly nymphs, act as an alternate host for trematode parasites (*2*) and might even carry the epizoic *Colacium cyclopicola* Gicklhorn which has been observed on copepods, cladocerans, ostracods, and larval water mites (*6*). We know of no other instance in which green species of euglenoid flagellates form a phycobiotic association with damselfly nymphs except where the latter's cuticle acts as a casual substrate for temporary attachment. We define symbiosis here as "a close, long-lasting association of dissimilar organisms" (*7*). The repeated occurrence of this relationship over three successive years and the seasonal synchrony constitute, we believe, valid evidence that the interaction of *Euglena* and the damselfly nymph represents a case of seasonal symbiosis.

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Aspartylphenylalanine Methyl Ester: A Low-Calorie Sweetener

Abstract. *Sensory evaluation indicated that aspartylphenylalanine methyl ester is about 160 times sweeter than sucrose in aqueous solution. Blends of this dipeptide with (i) sodium saccharin, (ii) sodium saccharin and sucrose, and (iii) sodium saccharin, sucrose, and calcium cyclamate did not differ significantly from 4 or 12 percent sucrose in bitterness, off-flavors, or aftertaste.*

Aspartylphenylalanine methyl ester (*1*) is about 160 times sweeter than sucrose in aqueous solution. This dipeptide does not differ significantly from sucrose in bitterness, aftertaste, off-flavors, or general acceptability in concentrations comparable in sweetness to 4 percent sucrose. Also, blends of the dipeptide with (i) sodium saccharin, (ii) sodium saccharin and sucrose, and (iii) sodium saccharin, sucrose, and calcium cyclamate do not differ significantly from 4 percent sucrose in bitterness, aftertaste, or off-flavors (Table 1).

Two and four percent sucrose solutions are approximately equal to 1 and 2 teaspoons of sugar per 8 ounces of liquid (2.1 and 4.2 g per 100 ml), respectively. Thus, this would be comparable to amounts of sugar commonly used in tea and coffee. Tea (*2*) had little effect on the sweetness of blends of the sweeteners at the concentrations used. However, slightly more dipeptide was required in tea than in water for sweetness equal to 2 percent sugar (Table 2).

Initially, the dipeptide in a concentration [(6.7 mg per 100 ml) $\times 10^{-2}$] slightly less sweet than 10 percent of sucrose was characterized by a marked off-flavor. However, at concentrations equal to 12 percent sucrose, blends were acceptable in flavor and did not differ significantly from sucrose in bitterness, off-flavors, or aftertaste. Twelve percent sucrose, and the blend containing calcium cyclamate, were significantly preferred to the other two blends in flavor and general acceptability (Table 1). Because carbonated soft drinks range from 8 to 12 percent in sugar content (*3*), these findings are applicable to the beverage industry.

For the above evaluations, all solutions were prepared (weight to volume) at 20°C with odor-free distilled water. Blends of sweeteners were formulated so that approximately equal sweetness was contributed by each. Synergism was evident when solutions were formulated as though sweetness contributed by each compound was additive. Therefore, it was necessary to adjust sweetness by reducing the concentration of each compound in the blends.

Equivalents for sweetness were established by means of ranking tests with at least 20 judges. The following formula for chi square of ranks (*4*) was used for analysis of data from these tests:

$$\chi_r^2 = \frac{12}{np\,(p+1)} \times \text{sum(rank totals)}^2 - 3n(p+1)$$

where p equals the number of treatments, n the number of replications, and d.f. equals $p - 1$; 12 and 3 are constants. Sweetener solutions were reformulated and retested if they were significantly different from sucrose

Table 1. Mean ($n = 20$) panel scores of flavor attributes of sweeteners and blends of sweeteners. Means followed by the same letter do not differ significantly within columns within concentrations of sweetness (*6*). Abbreviations are: S, sucrose; D, dipeptide; SS, sodium saccharin; and CC, calcium cyclamate.

Sweetener	Scale: 1 (low) to 9 (high)		Scale: 0 (none) to 3 (strong)		
	Flavor	General acceptability	Bitter	Off-flavor	Aftertaste
Sweetness equivalent to 4 percent sucrose					
S	6.4[a]	6.0[a]	0.5[a]	0.1[a]	0.4[a]
D	5.3[b]	5.3[a]	0.8[a]	0.2[a]	0.8[a]
D + SS	6.0[ab]	5.7[a]	0.6[a]	0.3[a]	0.7[a]
D + SS + S	5.9[ab]	5.8[a]	0.4[a]	0.1[a]	0.7[a]
D + SS + S + CC	5.6[ab]	5.4[a]	0.7[a]	0.1[a]	0.8[a]
Sweetness equivalent to 12 percent sucrose					
S	7.1[a]	7.1[a]	0.6[ab]	0.1[a]	1.0[ab]
D + SS	6.3[b]	6.0[b]	1.0[a]	0.2[a]	1.5[a]
D + SS + S	5.8[b]	6.0[b]	0.8[ab]	0.1[a]	1.1[ab]
D + SS + S + CC	7.3[a]	7.0[a]	0.4[b]	0.2[a]	0.8[b]