Observations on the Alga Cladophora conchopheria on Shells of the Intertidal Gastropod Turbo coronatus coreensis

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Abstract: The occurrence of the green alga Cladophora conchopheria on shells of the intertidal gastropod Turbo coronatus coreensis was examined at a bay in central Japan. The shells were consistently fouled predominantly by C. conchopheria. Observation of cross-sections of the shells by SEM revealed that false roots of C. conchopheria penetrated through the periostracum and into the outer ostracum of the shells. Field observations showed that C. conchopheria was uncommon or absent in the innermost parts of the bay, where the substrata were composed partially of mud and T. coronatus coreensis was common. A transplant experiment revealed that the alga could not newly colonize shells or maintain their colonization at one of the innermost stations. After the death of the snails, the abundance of C. conchopheria on shells did not change for 3 months and then decreased gradually, probably as a result of tearing of the periostracum layer. We saw no new algal colonization on dead snail shells even after 8 months. Most colonization of living snails occurred on the shell part nearest to the aperture, indicating that algal colonization occurred primarily in the area of the new shell growth.

Key words: Cladophora, intertidal gastropod, symbiosis, epibiosis, algal colonization, transplant experiment

INTRODUCTION

The external shells of marine molluscs provide substrata for sessile organisms such as algae, bryozoans and sponges, and the epibionts and the molluscs have been known to exhibit either positive or negative relationships with each other (Vance 1978; Barkai & McQuaid 1988; Wahl 1997; Laudien & Wahl 1999).

The turbinid gastropod Turbo coronatus coreensis (Recluz, 1853) occurs along Japan’s intertidal rocky shore, and its shell is commonly covered by the filamentous green alga Cladophora conchopheria (Sakai, 1964). This epizoic alga occurs only on the shells of this species.

The basic reproductive biology and natural history of T. coronatus coreensis have been reported by Yukihira et al. (1995a, 1995b, 1995c). For C. conchopheria, the life cycle under cultured conditions (Wang & Sakai 1986), the perforating form through the snail shell (Matsuyama & Aruga 1993; Matsuyama et al. 1999) and the seasonality of reproduction (Matsuyama et al. 1999) have been studied. Xing & Wada (2002) described its natural distribution in terms of tidal levels, snail size and season, and examined temporal changes in algal coverage of living and dead snails in the laboratory. However, the relationship between the alga and the snail has not been examined.

To explore this relationship, the present study aimed to (1) understand how the false roots of C. conchopheria perforate the shell of T. coronatus coreensis, (2) elucidate the effect of snail death on the persistence of and colonization by C. conchopheria in the field, and (3) examine whether the prevalence of the alga on the snails is influenced by the conditions of the snail habitat.
MATERIALS AND METHODS

Survey area

Collection of samples, periodical surveys and experiments were carried out at an intertidal rocky shore in Tanabe Bay, central Japan (33° 41′ N, 135° 22′ E) from April to December 2000. Tides were semidiurnal with a range of 0.2 to 2.0 m.

SEM observations of shell sections

To understand how the false roots of *C. conchopheria* perforate the shells of *T. coronatus coreensis*, sections of the shells were examined using a scanning electron microscope (SEM). Both snails fouled by *C. conchopheria* and those unfouled were examined. The shells were fixed in 10% formalin, the soft parts were extracted, and then the shells were embedded in P-resin. The samples were cut and ground with Carborundum and polished with diamond paste. For SEM observation, polished samples were etched with 0.5 mol/l HCl and then coated with gold.

Spatial difference in abundance of *C. conchopheria*

The abundance of *C. conchopheria* on living snails was examined at seven stations (St. A – G) in Tanabe Bay (Fig. 1) during 16 – 17 August 2000. Sampling at each station was carried out at two points: a mid tidal level and a low tidal level. More than 50 snails were collected from each point by setting 1 – 11 quadrats (50 × 50 cm). Shell lengths and shell heights were measured, and the coverage (%) of *C. conchopheria* on snails larger than 10 mm in shell length was evaluated visually using five ranks: 0 (0%), 1 (0 – 25%), 2 (25 – 50%), 3 (50 – 75%), 4 (75 – 100%).

At each station during 4 h around the slack low tide of 17 August, we measured water temperature, salinity, and wave intensity and noted the substrate type (rock, boulder, cobble, sand, muddy sand or mud) where the snails occurred. Wave intensity was recorded as the maximum amplitude of surface water level during 30 s on a vertical pole established in an area of ca. 30 cm depth. Salinity was measured using a portable salinometer (CM-21P: Toa Electronics Ltd.).

A field transplant experiment was carried out at two stations, one where the alga was abundant on the snails (St. G) and one where the alga did not occur on the snails (St. F). We collected 40 snails with no attached algae at St. F and 40 snails with 100% alga coverage at St. G. Half (20) of the snails without the alga and half of those with the alga were tethered at each station. Each snail was bound on one end of a cotton thread (about 100 cm in length), and the other end of the thread was attached to a thin stake inserted into a rock. Shell length did not differ significantly between snails tethered at St. F (with the alga: $\bar{x} \pm SD = 19.6 \pm 1.2$ mm; without the alga: $20.3 \pm 1.3$ mm) and those at St. G (with the alga: $19.5 \pm 0.9$ mm; without the alga: $20.1 \pm 1.6$ mm) (Mann-Whitney U-test, with the alga: $P > 0.8$; without the alga: $P > 0.4$). The tethering started on 30 September, and on 10 November, the tethered snails that were still alive were checked for the occurrence of *C. conchopheria*. For snails that were initially fouled by the alga, we recorded if the amount of the alga increased or decreased. This amount was determined to have decreased if the algal mat had a concave appearance (there was no concavity initially). For snails that were initially unfouled, new colonization by the alga was checked visually.

Fig. 1. Locations of Sts. A – G in Tanabe Bay and coverage of *C. conchopheria* on live *Turbo coronatus coreensis* at mid tide and low tide levels of each station.
Coverage of *C. conchopheria* following the death of *T. coronatus coreensis*

To examine whether *C. conchopheria* can colonize or persist on empty shells, both shells without *C. conchopheria* and those densely covered with *C. conchopheria* were placed on an intertidal rock flat where many fouled snails lived. The coverage on these shells was monitored monthly from April to November.

Shells were obtained from 20 snails without the alga at St. D and 20 snails densely covered with the alga at St. G. Shell lengths were not significantly different between the two groups (without the alga: $\bar{x} \pm SD = 21.0 \pm 0.9$ mm, with the alga: $20.5 \pm 1.2$ mm, Mann-Whitney U-test: $P > 0.2$). The collected snails were killed by extruding the soft parts, and their shells were then attached to two concrete plates (30 cm × 30 cm × 5 cm H) with an adhesive agent. The groups were arranged in alternating 5 rows at 5 cm intervals. The plates with the shells were set at St. G on the day following the killing. Coverage (%) of *C. conchopheria* on these shells was evaluated visually using the five ranks described above. During the experimental period, we observed no discernable change in the coverage of *C. conchopheria* on 10 live snails in the same area.

Colonization of living snails by *C. conchopheria*

To examine which part of the shell is colonized, 40 snails without algae (17.9–26.8 mm shell length) were collected from St. F, marked with color pens and released on 3 June at St. G where *C. conchopheria* was abundant, and recaptured on 15 August. The recaptured snails ($n = 9$) were scanned for the occurrence of the alga and, if present, the shell part colonized was noted using the classification in Figure 2.

**RESULTS**

Perforating form of *C. conchopheria*

Electron micrographs of the snail shell revealed that false roots of *C. conchopheria* penetrated through the periostracum and into the outer ostracum (Fig. 3).

Spatial difference in abundance of *C. conchopheria*

Algal abundance on living snails differed among stations (Fig. 1). Coverage was markedly low at Sts. D and F in the innermost parts of the bay. In particular, St. F had no *C. conchopheria* at all. In contrast, the alga was remarkably abundant at Sts. C and G. Algal coverage was higher at mid tide levels than low tide levels at most stations.

Water temperature and salinity were similar at all stations, and wave amplitude was smaller at Sts. D and F, where the alga was less abundant; these two stations had a muddy substrate (Table 1).

In the transplant experiment, snails that survived showed some difference in the amount of the alga between groups (Table 2). Snails initially covered with dense alga had no change in the amount of coverage at St. G, but 5 of 6 snails at St. F showed a reduction. One of the 14 snails initially without algae at St. G exhibited new colonization, whereas none of those at St. F were colonized after 1.5 months.
Table 1. Water temperature, salinity, wave amplitude and substratum recorded at low tide of 17 August 2000 at each station.

<table>
<thead>
<tr>
<th>St.</th>
<th>Water temp. (°C)</th>
<th>Salinity (psu)</th>
<th>Wave amplitude (cm)</th>
<th>Substratum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28.3</td>
<td>31.3</td>
<td>9.0</td>
<td>rock</td>
</tr>
<tr>
<td>B</td>
<td>29.1</td>
<td>31.4</td>
<td>6.0</td>
<td>rock</td>
</tr>
<tr>
<td>C</td>
<td>29.2</td>
<td>31.4</td>
<td>13.0</td>
<td>rock, boulder, mud</td>
</tr>
<tr>
<td>D</td>
<td>29.5</td>
<td>31.7</td>
<td>1.5</td>
<td>boulder, pebble, cobble, sand</td>
</tr>
<tr>
<td>E</td>
<td>29.3</td>
<td>31.5</td>
<td>0.5</td>
<td>boulder, pebble, mud</td>
</tr>
<tr>
<td>F</td>
<td>29.9</td>
<td>31.2</td>
<td>0.0</td>
<td>rock, pebble, muddy sand</td>
</tr>
<tr>
<td>G</td>
<td>30.3</td>
<td>31.7</td>
<td>0.5</td>
<td>rock, boulder, muddy sand</td>
</tr>
</tbody>
</table>

Table 2. Results of the transplant experiment between St. F and St. G. For snails originally fouled by *C. conchopheria*, the proportion of the snails showing a decrease in the alga at the end of experiment was compared between the two stations by Fisher's exact probability test. For snails originally unfouled by the alga, the proportion of the snails showing colonization of the alga was compared between the two stations by Fisher's exact probability test.

<table>
<thead>
<tr>
<th>Fouled snails</th>
<th>Unfouled snails</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in Cladophora</td>
<td>Fisher's exact probability test</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>St. F</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 4. Temporal change in the coverage of *C. conchopheria* on snail shells after the death of the snails. Circles indicate snails that were fully fouled by the alga at the start. Triangles indicate snails that had no alga at the start. Coverage is represented by 5 ranks (0: 0%; 1: 0–25%; 2: 25–50%; 3: 50–75%; 4: 75–100%). Bar: standard deviation.

Fig. 5. Frequency of snail shell parts colonized by *C. conchopheria* 2 months after release at site where *C. conchopheria* was abundant.

Colonization of living snails by *C. conchopheria*

On the recaptured snails (9 among the 40 released), colonization by the alga was most frequent on part 1 of the growing margin of the lip; no colonization occurred on part 6 (Fig. 5).

DISCUSSION

Field observation showed that *C. conchopheria* rarely occurred on living *T. coronatus coreensis* at the innermost sites of the bay, where the substrata contained mud and wave energy was low. This finding suggests that extremely sheltered habitats are unsuitable for this alga.
This was confirmed by the transplant experiment between St. F where no alga occurred on the snails and St. G where the alga was abundant. Snails fully fouled by Cladophora conchopheria exhibited some reduction in overgrowth when they were maintained at St. F, whereas the alga appeared healthy on those snails that were maintained at St. G. Snails initially without algae that were maintained at St. G were colonized by the alga, whereas those that were maintained at St. F were not. These results suggest that at the sheltered environment at St. F, it may have been difficult for the alga to colonize the shells or to survive once attached.

In another field experiment, Cladophora conchopheria persisted on the shells of dead snails without a decrease in coverage for 3 months, but after 4 months the coverage began to decrease and fell to below 25% after 7 months. This persistence of the alga indicates that it does not require live snails to survive. Xing & Wada (2002) also reported that the coverage of Cladophora conchopheria in the laboratory decreased similarly between live snails and dead snails. However, after 7 months, most of the periostracum layers of dead shells were found to be torn off. Since the alga attaches to shell by perforating the periostracum, as recognized by SEM observation, a tearing away of the periostracum could detach the alga. We suggest that the principal cause for the decreased algal abundance that began 4 months following snail death is periostracum disintegration. Although the periostracum might be important for the sustained attachment, the alga did not appear to gain additional benefit from being attached to live snails.

Colonization of Cladophora conchopheria did not occur on dead shells for 8 months in our experiment. Wang & Sakai (1986) reported that zygotes could not be planted on cultured Turbo coronatus coreensis. But when live snails without algae were released at a site where the alga was abundant, colonization occurred after a few months and was concentrated at the part nearest to the shell aperture. This indicates that colonization occurs primarily in the area of shell growth.

Our study has demonstrated that the alga Cladophora conchopheria requires shells of live Turbo coronatus coreensis for colonization as well as to maintain the colonization. In contrast, the snail does not appear to require the alga, because snails survive without the alga (Fig. 1). This is also supported by the observation of Xing & Wada (2002) that the survival pattern of Turbo coronatus coreensis in the laboratory does not differ between snails with and without Cladophora conchopheria. Even if the alga is not essential for the snail, the two may somehow interact. Shell perforation by the alga could weaken the shell, increasing the snail’s susceptibility to predation. Conversely, algal fouling may reduce predation pressure on the snail, as has been demonstrated for epibionts on mussels (Laudien & Wahl 1999). Algae might also affect shell growth, as has been shown for epibionts on littorinid snails (Wahl 1996). These and other possible interactions should be tested in future studies to better understand the symbiotic relationships between the alga and the snails.

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