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A Photosymbiotic Ascidian *Didemnum molle:* Trans-generational Transmission of Photosymbionts, and Colors Morphs.

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Algal symbiosis is known in various marine metazoans, such as sponges, cnidarians, flat worms, and mollusks. Interestingly, in the phylum Chordata, obligate algal symbiosis is only known in some colonial ascidians belonging to the family Didemnidae. About 30 photosymbiotic species have been described from tropical and subtropical waters, and they harbor prokaryotic algae: *Prochloron* sp. or *Synechocystis* spp. *Didemnum molle* (Herdman, 1886) is one of the most common photosymbiotic ascidians that are widely distributed in the Pacific ranging from about 30°N to 30°S. The dome-shaped colonies are conspicuous in shallow coral reefs and seagrass beds, and are sometimes found at 30 m in depth or deeper. In Ryukyus, *D. molle* has been recorded from Amami-ohshima Island (ca. 28°N) and the islands at lower latitudes. Here, I report our recent studies on this photosymbiotic species, with remarks on the mode of trans-generational transmission, so called vertical transmission, of photosymbionts (*Prochloron* sp.) and discuss color morphs of the colonies.

In obligate symbioses, the host organisms need to acquire their symbionts during their life cycle. In some species, the symbionts are incorporated from the environment (horizontal transmission), while in others the symbionts are maternally inherited (vertical transmission). In most photosymbiotic ascidians, vertical, or trans-generational transmission occurs. In the colonies of *D. molle, Prochloron* cells are exclusively distributed in the cloacal canal (cavity) and peribranchial cavity. The embryos, which begin life without algal symbionts, possess two layers of tunic covering the ectoderm. The massive inner layer of the tunic covers the larval trunk entirely, whereas the thin outer layer covers only the anterior half of the larval trunk. The inner layer appears to have a sticky surface, and, as the embryos age, algal cells grown in the cloaca tend to adhere to it, but they do not stick to the surface of the outer layer covering the anterior half of the trunk which bears the photolith and the larval adhesive organs. Consequently, when the larvae are released they bear green algae only on the posterior half of the body. The thin outer layer of the tunic is sloughed off during metamorphosis following larval settlement.

Various colony colors are known in *D. molle:* white, brown, dark-gray, white with colored (gray or brown) patches, and some intermediates. The colony color is determined by calcareous spicules (white) and pigment cells (brown or dark-gray) distributed beneath the colony surface. It apparently also depends on depth. Off Maeda Point (Okinawajima Is.), colonies in shallow sites are brilliant white, with densely distributed spicules, and often with brown or dark-gray pigmentation, while colonies in deeper sites are less pigmented, with sparsely distributed spicules. These bathymetrical color differences indicate that light protection may be one of the functions of the colony color. Since solar radiation contains harmful ultraviolet (UV) radiation, light protection is crucial to survival particularly for sessile animals. However, solar radiation is indispensable for photosynthesis in photosymbiotic animals, where dense spicules and pigments may decrease photosynthetically active radiation (PAR) as well as UV irradiation. One of the solutions for this dilemma is mycosporine-like amino acids (MAAs) that have absorption maxima in the UV-A (320-400 nm) and UV-B (290-320 nm) ranges but are transparent to PAR (400-700 nm). Both photosymbiotic ascidians and *Prochloron* sp. are known to contain several MAAs. Among the colonies from 5, 10, 15, and 20 m deep, the concentration of total MAAs decreases bathymetrically, indicating that MAA contents are regulated by the light condition. Although the colonies at 5 m depth contain less MAAs than those at 10 m depth, colonies inhabiting shallow and exposed sites seem to reduce solar radiation mainly with spicules and pigment cells, evidently as an economic device when the radiation is so strong. In *D. molle* colonies, UV radiation is screened by an appropriate combination of spicules, pigment cells, and MAAs, depending on the ambient light condition.

Recently we found two populations of *D. molle* in reef lagoons off Okinawajima Island, Japan, where the colonies at one site are all dark gray and those at the other site are mostly brown. The two populations are only about 20 km apart. The difference in colony colors could not be caused simply by depth and/or light shading, because both colonies occur at exposed sites at about 1 m depth or less. Spectroscopic and chromatographic analyses showed that chlorophyll *a* and total MAA concentration in the dark-grey colonies were estimated to be 1.4 and 2.4 times higher, respectively, than in the brown colonies. The contents of total MAAs per chlorophyll *a* (weight/weight) were significantly greater in the dark gray than in the brown colonies. Thus, there may be some physiological differences between the color morphs. Based on monthly samplings during a year from the two sites, we found that the dark-gray colonies posses gonads only in summer, whereas the brown colonies have gonads all year round. Moreover, the commensal crustacean fauna in the cloacal cavity is different: only one type of copepod occurs in the dark-gray colonies, but three types of copepods and one palaemonid shrimp are found in the brown colonies. These differences between the two color morphs may indicate a high adaptability of this species. Alternatively, they may be cryptic species, although to date taxonomists usually consider different color morphs of *Didemnum molle* as a single species.