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INTERTIDAL SANDY BEACHES AS A HABITAT WHERE PLASTID ACQUISITION PROCESSES ARE ONGOING

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Edge of the sea is a strange and beautiful place.
— R. Carson, in The Edge of the Sea (1955)

1. Introduction

An intertidal sandy beach is a constantly changing habitat, and, in that sense, it could be regarded as an extreme environment. It alternates between a seabed and a land with every tidal transition, and this alternation changes physical conditions such as beach morphology, water level, nutrients, oxygen level, salinity, temperature, light intensity, etc. Sand is an unstable substratum. Tides and waves constantly move sands on the submerged shore face. Even a single rainfall during the low tide changes the physical conditions, and a one-night storm could change even the landscape of the shore resulting in a catastrophe for its microbial communities.

The intertidal sandy shore often appears not to be vegetated. Yet, there is an unexpectedly rich microalgal community under the beach. Many ecological studies have revealed that shallow sand/mud seabeds, including intertidal beaches are “secret gardens” that are rich in microalgal communities (for review, MacIntyre et al., 1996). Well-known members of these gardens are cyanobacteria and diatoms. There are also a variety of flagellate algae, such as dinoflagellates, cryptophytes, euglenophytes, chlorophytes, and prasinophytes. Nevertheless, our understanding of the flora and fauna of such communities is still fragmentary compared to our understanding of those of coastal waters. Spatial and temporal heterogeneities of the environment make extensive investigations of microbial communities in these extreme environments difficult. The intertidal sandy shore remains an unexplored frontier for modern phycology and protistology.

Our focus here is to introduce a unicellular flagellate that we discovered from an intertidal sandy shore in Japan (Okamoto and Inouye, 2005; Okamoto and Inouye, 2006). The organism, Hatena arenicola, possesses a green algal symbiont, and most likely represents an early intermediate stage of the plastid acquisition process via secondary endosymbiosis. As the majority of the extant algae are thought to have acquired the plastid via a similar process (McFadden,
In this chapter, we focus on selected features of the intertidal sandy shore as an extreme habitat, and then, we take up the endosymbiosis of *H. arenicola* and comparative examples that are also known from similar habitats. The intertidal beach is an important habitat to which phycologists should pay special attention.

2. Variation of Intertidal Sandy Beaches

Geomorphological features and detailed classifications of the intertidal sandy beach have been reviewed (e.g., Short, 1999a). Brown and McLachlan (1990) covered broad topics about a sandy beach ecosystem that are helpful in understanding how physical features affect the biota, although their main subject is the meiofaunal community. Elliott et al., (1998) and Asmus et al., (2004) include useful links to a wide range of references for further reading. Here we point out some important features that affect the microalgal distribution.

2.1. VARIATION OF SANDY BEACHES

Light and water are essential for algal growth. Because the euphotic zone is at most several millimeters deep, algae grow on the shore face where the water is more or less continually present. Such a condition is found at the sheltered beaches (Short, 1999b), where the waves brake before they reach the shore face. Sheltered beaches have a shallow slope composed of finer sand grains. The smaller the particle size is, the larger total volume of the interstitial space is, and the more effective capillary action becomes. This allows larger volume of the water stay in the interstitial space and keeps the shore face wet even during the low tide. This wet area at low tide is called the seepage face (Brown and McLachlan, 1990). Fig. 1 illustrates a shore profile. The seepage face is developed where ground water level decouples from the sea level during the low tide (Masselink and Turner, 1999).

The extreme of the sheltered beach is a tidal flat, where the water rarely moves. Less water exchange result in an accumulation of organic matter and a less aeration, which enhances bacterial growth and makes the environment reductive. Under such a condition, a bacterial loop dominates the ecosystem and algal growth is limited to the surface layer.

The opposite extreme is the exposed beach. The exposed beach has a steeper slope with coarse sand grains. Waves directly brake at the shore face. Coarse particles create less capillary action, so that the water directly penetrates in and out, but does not stay. Consequently, the shore face is dry during the low tide, so the algal distribution is restricted to the littoral zone at the lowest of intertidal range, where waves constantly bring water (Brown and McLachlan, 1990).
On a continuum between those two extreme, we can find a moderately sheltered beach. Unlike the tidal flat, the physical mixture during high tide and the continuous flow of the ground water near or on the shore face at low tide keep the environment aerated. This surface water often has a dense algal population. Isonoura beach in Japan, where we found *H. arenicola* falls into this category.

### 2.2. HETEROGENEITY WITHIN A SANDY BEACH

A sandy shore is a place where physico-chemical conditions vary drastically within a small distance, which means the algae with very different requirements can occur right next to each other. One of the most influential conditions is the decline of light intensity along the depth gradient. The euphotic zone is restricted to the surface layer of less than 5 mm deep in most cases (Brown and McLachlan, 1990), which is strikingly different from the open water where the boundary is located more than 100 m deep in the water column.

The oxic-anoxic state also varies along the depth. The oxic-anoxic condition is much more dependent on the grain size. The boundary is located within a several millimeters depth (at the tidal flat) or at a more than 1 m depth (at the exposed beach) (Brown and McLachlan, 1990). The oxic-anoxic state affects the type of the nitrogen source available (NO\(_2\), NO\(_3\), or NH\(_4\)), and therefore restricts the distribution of some algae (e.g., Kingston, 1999 and references therein).

The vertical gradation of physio-chemical conditions together with the effect of the waves induces the vertical migration of microalgae (e.g., Kingston, 2002; Saburova and Polikarpov, 2003; see references therein). Vertical migration of intertidal benthic microalgae has been known for 100 years for a wide range of algae such as cyanobacteria, diatoms, dinoflagellates, and euglenophytes. They often form a visible bloom on the sand surface. The typical pattern is to migrate upwards and stay in the surface photic layer during the daytime at the low tide, then to

![Figure 1. Profile of a sandy shore.](image-url)
migrate downwards during the daytime at the high tide and throughout the night to avoid wave disturbance and to take up nutrients depleted in the surface layer.

3. Nature’s Ongoing Experiments at the Intertidal Sandy Beach

As outlined above, the intertidal sandy shore is a dynamic and complex environment. Although there seem to be rich microbenthos communities including a variety of algae inhabiting sandy beaches, our knowledge of these communities is insufficient and many undescribed species remain. One of them is *H. arenicola*, a unicellular flagellate we discovered from a sandy shore in Japan, was one of these. This organism is most likely currently undergoing plastid acquisition via endosymbiosis.

3.1. SYMBIOTIC ORIGIN OF PLASTIDS

Eukaryotes comprise a half dozen supergroups and algae distribute polyphyletically across these different supergroups (Keeling et al., 2005 and references therein). The algal diversity and polyphyly are explained by multiple endosymbioses (Bhattacharya et al., 2004 and references therein). It is assumed that all the plastids have originated from an ancestral cyanobacterium taken up by a heterotrophic eukaryote. This symbiosis is referred to as the “primary endosymbiosis.” Glaucophytes, rhodophytes, and Viridiplantae (green algae and land plants) are considered to have been derived from a single primary endosymbiosis. Subsequently, the “secondary endosymbioses” in which these photosynthetic algae are engulfed by different heterotrophic eukaryotes took place. Through the secondary endosymbioses, four algal groups (heterokontophytes, haptophytes, cryptophytes, and dinoflagellates) plus a parasitic group (Apicomplexa) acquired red algal plastids, and two algal groups (euglenophytes and chlorarachniophytes) acquired green algal plastids. Thus understanding the plastid acquisition process via endosymbiosis is essential to understanding algal evolution.

One approach to understanding this aspect of algal evolution is to investigate the organisms that are undergoing the process in real time. Recently we reported a potential candidate, *H. arenicola* from Isonoura beach in Japan (Okamoto and Inouye, 2005; Okamoto and Inouye, 2006).

3.2. *H. ARENICOLA* FROM A SANDY BEACH

Isonoura beach is a moderately sheltered sandy shore and the tidal range is around 2 m at spring tide. It has a well-developed seepage face. Microalgae often form noticeable patchy blooms around the upper edge of the seepage face (the exit point). The most abundant bloom-forming algae are diatoms, cryptophytes (some *Chroomonas* species), prasinophytes (*Pyramimonas* and *Tetraselmis* algae), and euglenophytes. As the blooms are visible only for several hours after the surface is above sea level, they are likely migrating vertically.
*H. arenicola* is distributed at the upper zone of the seepage face as are many other microalgae. Although this organism is constantly present except during the winter, it is not abundant and we have never seen a visible bloom of this alga since we discovered it in 2000.

*H. arenicola* appeared to be a new green algal taxon when first encountered. A chlorophyll a-/b- containing plastid-like structure and a red conspicuous eyespot is always at the cell apex. However, the division of the “plastid” is not coupled with cell division and the structure is inherited by only one of two daughter cells. Therefore, the structure is not a “plastid” but a temporary symbiont.

Molecular phylogenetic analysis showed that the symbiont is a *Nephroselmis* (Prasinophyceae, Chlorophyta). Electron microscopy revealed that the symbiont retains the cytoplasm as well as the plastid, though the cell morphology is greatly different from that of the ordinary free-living *Nephroselmis* cell (Fig. 2; Okamoto and Inouye, 2005; Okamoto and Inouye, 2006). The plastid of the symbiont is selectively enlarged up to more than ten-fold. In contrast, the cytoplasm is greatly

![Figure 2. *Hatena arenicola*.](image)
reduced. It only retains the nucleus, a mitochondrion that is often degraded and occasionally a vestigial Golgi body-like structure. Other structures such as the cell covering, the cytoskeleton and the endomembrane system are absent. Such a reduced cytoplasm seems to be insufficient to sustain the enlarged plastid, so the host may compensate for some of the symbiont’s lost metabolism.

We occasionally found symbiont-lacking cells in the environment. Interestingly, the symbiont-lacking cell has a complex feeding apparatus at the cell apex and used it for taking up algal cells (Fig. 2). As the feeding apparatus is absent in the symbiont-bearing cell, it would be decomposed after the uptake of *Nephroselmis* symbiont. In this sense, the symbiotic association in *H. arenicola* causes a drastic change of the morphology of both the host and the symbiont.

This association between the host and symbiont in *H. arenicola* provides us with a new view on this type of symbiosis. The symbiosis is not a mere enslavement of the symbiont; rather it is a process that unites two alien cells into a single organism, that is both morphologically and functionally different.

### 3.3. EYESPOT: A POSSIBLE FUNCTIONAL ASSOCIATION

The eyespot region is on of the places where the host–symbiont association is significantly established. Four different membranes, i.e., the host’s plasma membrane, a symbiont-enveloping membrane of an unknown origin, and the symbiont’s outer and inner plastid membrane are closely layered together, then the eyespot granules are attached to the inner most side of the membrane complex.

Although we do not have enough evidence, it is still worth asking whether the membrane-eyespot complex is functioning for the host’s phototaxis. The algal eyespot is part of photo-sensing machinery (Foster and Smyth, 1980; Melkonian, 1984; Gualtieri, 2001). It shades a photoreceptor in a near-by membrane, helping to detect the light. The arrangement of the membrane-eyespot complex seems reasonable for this purpose. In a preliminary observation, *H. arenicola* cells with the symbiont show a negative response to laterally projected light. This particular photoreaction would be appropriate for this cell’s type of motility. That is, *H. arenicola* cell does not swim but crawls two-dimensionally on the substratum using two flagella, so only the lateral incident illumination matters for the movement of *H. arenicola*. We also observed that *H. arenicola* could not survive under a 14–10 h light–dark cycle with incident illumination of ca. 10 mol photons m$^{-2}$ sec$^{-1}$. Under this light condition, the symbiont becomes swollen and the host cell dies. On the other hand, under the complete dark condition, the symbiont become pale and was eventually lost. To maintain a stable partnership, *H. arenicola* needs some phototactic ability to choose the proper light conditions, weak enough to avoid symbiont’s outgrowth but strong enough to maintain its photosynthesis. This is all the more critical at the intertidal sandy shore where the light intensity drastically declines within several millimeters, and light-induced vertical migration is advantageous. Therefore, it would be quite intriguing to determine, in the
future, if the symbiont’s eyespot can contribute to, or even make changes in the host’s phototaxis.

3.4. HALF PLANT AND HALF PREDATOR MODEL

Based on the observation of the nonsynchronized cell divisions and ultrastructural features, we proposed a hypothetical life cycle, in which *H. arenicola* possibly switches its trophic strategy between heterotroph and phototroph (Okamoto and Inouye, 2005; Okamoto and Inouye, 2006). The symbiosis between these organisms is unique in the suggestion that the plastid acquisition is a more mutual process than expected. It was assumed that the plastid acquisition is a process of “enslaving” an endosymbiont to utilize it as an organelle (Douglas et al., 2001; Cavalier-Smith, 2002; Cavalier-Smith, 2003). This view is compatible with the observation that the plastid structure and genome in any extant plant is greatly reduced and the host controls the much of its function. However, that is the result of evolution and does not really tell us about trials and errors through which the host and the symbiont went during the integration process. That is the reason we need to investigate such symbioses at intermediate steps of the plastid acquisition.

3.5. EVOLUTIONARY IMPLICATIONS AND OTHER EXAMPLES

The process of endosymbiosis is essential to understanding plant evolution. In addition to the case of *H. arenicola*, comparable endosymbioses have been reported from some dinoflagellates. The plastid of most photosynthetic dinoflagellates is of red algal origin acquired via secondary endosymbiosis. Some dinoflagellates are known to replace the original plastid through an additional endosymbiosis with a cryptophyte, diatom, prasinophyte, or haptophyte symbiont (Schnepf and Elbrächter, 1999; Morden and Sherwood, 2002; Hackett et al., 2004). The extent of associations vary, so that these relationships are thought to represent different intermediate stages of integration.

Fig. 3 shows the hypothesized integration process and corresponding examples. First, a phagotrophic flagellate would start to retain a certain prey in the cytoplasm and use it as a temporary symbiont (Stage I; *Amphidinium latum, Amphidinium poecilochroum*, and *Gymnodinium acidotum*). At this stage, the symbiont retains the plastid as well as the nucleus, mitochondrion, endoplasmic reticulum, etc. The host–symbiont association is still temporary and the symbiont would be either digested or lost upon the host’s cell division. The host would repeatedly take up the partner, while feeding on the other prey cells. *H. arenicola* represents Stage I, in that (i) the host and symbiont have an intimate association, but (ii) their cell cycles are not coupled.

The next stage (Stage II, *Durinskia baltica* (formerly *Peridinium balticum*) and *Kryptoperidinium foliaceum* (formerly *Peridinium foliaceum*)) is synchronization of
the cell cycles of the host and the symbiont. This ensures an even inheritance of the daughter symbionts to the daughter host cells, and allows the host to behave as an alga. Then the symbiont continues to lose most of the cell content such as ER and a mitochondrion. The following stage (Stage III) is complete reduction of the symbiont’s cytoplasm. The nucleus persists to the last. We can still see the remnant of the nucleus as the nucleomorph in some algae in the cryptophytes and chlorarachniophytes (Douglas et al., 2001; Gilson and McFadden, 2002). Finally, the nucleomorph disappears, and the process of integration of the plastid is complete (Stage IV; \textit{Lepidodinium viride}, \textit{Gymnodinium chlorophorum}, \textit{Karenia brevis}, \textit{K. mikimotoi}, \textit{Karlodinium micrum}).

At some point of the process, gene transfer from the symbiont to the host’s nucleus would happen (e.g., Gilson and McFadden, 2002; Archibald, 2005). This is accompanied with by the invention of protein import machinery from the host to the symbiont. The host gradually compensates the symbiont’s metabolism. This eventually leads to the reduction of the symbiont’s genome and organelles and the loss of its autonomy.

Interestingly, some of the examples mentioned above are reported from the intertidal sandy beach habitat. \textit{A. latum} (Horiguchi and Pienaar, 1992) and \textit{A. poecilochroum} (Larsen, 1988) are from the intertidal sandy beach and represent early stage I. Both have a symbiont of cryptophyte origin but their partnership is not exclusive. \textit{A. latum} was observed to retain two different cryptophytes species in one cell. Other examples are \textit{Gymnodinium quadrirlotbatum} (Horiguchi and Pienaar, 1994) and \textit{Galeidinium rugatum} (Tamura et al., 2005). They have a

![Figure 3. Hypothetical intermediate steps of plastid integration.](image-url)
diatom symbiont and would represent stage II, although many of the studies about this stage have been performed on *D. baltica* and *K. foliaceum*, especially studies on the cell division process.

It is unclear why the intertidal sandy shore can incubate symbioses that would represent the plastid acquisition process. Of course, examples of plastid acquisition are not restricted to sandy beaches, but also occur in open waters. However, sandy beach habitats seem to be one of most important habitats wherein evolution of plastid acquisition via secondary endosymbioses are ongoing. Because the habitat has not been explored enough, our understanding is far less than needed to reveal the hidden diversity of the sandy shore. The sandy shore is dynamic and heterogeneous at many levels. This extreme condition could be a key for hunting for organisms like *H. arenicola* that are in the process of plastid acquisition. To fully understand this habitat, we need to perform extensive research across different types of beaches with careful consideration of the variation of physio-chemical conditions including those discussed above.

### 4. Conclusion

The intertidal sandy shore is often close to human activity, yet it is an unexplored frontier. Its dynamic and heterogenetic nature allows various organisms to accumulate to a dense community within a small space. However, the very nature and patchy distribution of microalgae, responding to the microenvironments make it difficult to draw a clear picture of the diversity of interstitial microbes. The endosymbiosis of *H. arenicola* and some dinoflagellates are good examples of the hidden treasures in these habitats for studies of plant evolution.

New algal species as well as undescribed heterotrophic protists, that could be candidates for emerging symbioses, are still constantly being described (e.g., Larsen and Patterson, 1990; Lee and Patterson, 2000; Yoshimatsu et al., 2000; Tamura et al., 2005). In addition to *H. arenicola*, we have recognized at least a new lineage of heterokontophyta, a new *Nephroselmis* species, and a marine *Malomonas* species. We have also found a dinoflagellate, *Amphidinium* sp. from the same location that is possibly undergoing the Stage I endosymbiosis with cryptophyte symbiont.

Many sandy shores are always close to us and no specialized vehicles or equipment are needed to explore them. And, these habitats certainly seem to another treasure, one that opens up new possibilities for our understanding of the evolution of algae and related organisms.

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