SHORT COMMUNICATION

Shift from widespread symbiont infection of host tissues to specific colonization of gills in juvenile deep-sea mussels

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The deep-sea mussel Bathymodiolus harbors chemosynthetic bacteria in its gills that provide it with nutrition. Symbiont colonization is assumed to occur in early life stages by uptake from the environment, but little is known about this process. In this study, we used fluorescence in situ hybridization to examine symbiont distribution and the specificity of the infection process in juvenile B. azoricus and B. puteoserpentis (4–21 mm). In the smallest juveniles, we observed symbionts, but no other bacteria, in a wide range of epithelial tissues. This suggests that despite the widespread distribution of symbionts in many different juvenile organs, the infection process is highly specific and limited to the symbiotic bacteria. Juveniles >= 9 mm only had symbionts in their gills, indicating an ontogenetic shift in symbiont colonization from indiscriminate infection of almost all epithelia in early life stages to spatially restricted colonization of gills in later developmental stages.

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Introduction

Associations with chemosynthetic bacteria have evolved independently in at least four lineages of marine bivalves (Dubilier et al., 2008). In all these associations, the symbionts are generally restricted to the gills in adult bivalves (Taylor and Glover, 2010), except hosts that transfer their symbionts directly to their offspring and harbor symbionts in their gonads (Endow and Ohta, 1990; Cary and Giovannoni, 1993). In adult Bathymodiolus mussels, symbionts have only been observed in the gills (Distel et al., 1994; Duperron, 2010). These mussels occur worldwide at hydrothermal vents and cold seeps and harbor their intracellular sulfur- and methane-oxidizing symbionts in gill cells called bacteriocytes. Bathymodiolus is assumed to acquire its symbionts from the environment, but the developmental stage at which this occurs is not known (Won et al., 2003; Duperron et al., 2007). Post-larval Bathymodiolus azoricus and B. heckerae as small as 0.12 mm appear to already harbor symbionts in their gills based on transmission electron microscopy (TEM) observations of bacteria morphologically similar to the sulfur- and methane-oxidizing symbionts (Salerno et al., 2005). These bacterial morphotypes were also found in the mantle epithelia of post-larval and juvenile B. azoricus and B. heckerae (0.12–8.4 mm). Similarly, in B. childressi juveniles (4–8 mm) TEM revealed bacteria that looked like the methane-oxidizing symbionts in gills as well as mantle and foot epithelia, and labeling experiments showed that these bacteria fixed carbon from methane (Streams et al., 1997). These studies suggest that Bathymodiolus is colonized by its symbionts at a very early stage and that other organs besides the gills also contain symbionts.

In this study, we focused on the following questions: (1) Do the symbionts indiscriminately infect all host tissues of juvenile Bathymodiolus? (2) Are the symbionts the only bacteria that colonize juvenile mussels? (3) Given that adult mussels are assumed to only have symbionts in their gills, is there a developmental stage at which the broad
colonization of host tissues ends? To answer these questions, we analyzed eight B. puteoserpentis and five B. azoricus juveniles (4–21 mm) from two vent sites on the Mid-Atlantic Ridge (Supplementary Table S1) by fluorescence in situ hybridization (FISH). Semi-thin sections of whole juveniles were examined with symbiont-specific probes as well as a general eubacterial and a negative probe (see Supplement for more details about methods).

Results

Symbionts broadly colonize tissues of smallest juvenile mussels

We observed symbiont-specific FISH signals indicating the presence of both the sulfur- and the methane-oxidizing symbionts in gill bacteriocytes of all 13 Bathymodiolus juveniles (Figure 1). In the smallest individuals (4–7 mm), we also found...
A symbiont-specific FISH signals in the epithelial cells of the mantle, foot and retractor muscles (Table 1, Figure 1), but not in other tissues or organs. The distribution and abundance of both symbions was comparable in all organs except for the much denser colonized gills, based on microscopic observations.

The symbions are the only bacteria that colonize juvenile host tissues
Extensive FISH analyses of all 13 juveniles showed overlap between the symbiont-specific and general eubacterial probes (as well as with DAPI staining) in all colonized host tissues (Figure 1). This indicates that the sulfur- and methane-oxidizing symbions are the only bacteria that colonize juvenile mussels.

Larger Bathymodiolus mussels only have symbions in their gill tissues
In contrast to B. azoricus and B. puteoserpentis juveniles ≤7 mm, larger juveniles of both species (9–29 mm) had symbiont-specific FISH signals only in their gills (Supplementary Figure S1). To examine if this spatial restriction of colonization to the gills is also maintained in adult Bathymodiolus, we examined mantle tissues attached to gills dissected from B. azoricus (n = 6, 55–100 mm). We only observed the symbions in the gills but never in the mantle.

Discussion and Conclusions
Our study provides the first FISH-based evidence that the symbions of early-stage B. azoricus and B. puteoserpentis ≤7 mm colonize a wide range of epithelial tissues. Together with earlier studies on juvenile B. azoricus, B. heckerae and B. childressi (Streams et al., 1997; Salerno et al., 2005), these results indicate a consistent pattern of indiscriminate symbiont colonization of a wide range of epithelial tissues in Bathymodiolus ≤8.4 mm (Table 1). This is remarkable given that in most symbioses examined to date, infection sites are spatially limited to specific tissues or areas, even in early life stages of the host (Bright and Bulgheresi, 2010). The ontogeny of symbiont colonization has been thoroughly examined in only a few of the many bacteria–eukarya symbioses, and it is possible that wide-spread colonization of many different tissues in early life stages is not restricted to Bathymodiolus but may also occur in other hosts. Despite the pervasive infection of Bathymodiolus juvenile epithelia, our study shows that colonization is only achieved by the symbions (although we did not check for the intranuclear bacterial parasite that can also infect these hosts (Zielinski et al., 2009). Clearly, the infection process must be highly regulated and specific to ensure that only the symbions colonize host cells.

The most obvious explanation for the widespread colonization of host epithelia in juvenile Bathymodiolus is that it provides the host with additional nutrition. In bivalves, the gills typically develop after the mantle and foot (Raven, 1958; Streams et al., 1997), and symbions in mantle and foot epithelia could thus provide the host with nutrients before the appearance of its gills. However, filter-feeding is assumed to be common in Bathymodiolus, and symbions in mantle and foot epithelia could thus provide the host with nutrients before the appearance of its gills. However, filter-feeding is assumed to be common in Bathymodiolus (Pile and Young, 1999; Kiou et al., 2010) and could provide juveniles with enough food to make the nutritional contribution from the small numbers of symbions in mantle and foot epithelia irrelevant.

In both Bathymodiolus species investigated in this study from two vent sites separated by almost 3000 km, we observed that juveniles ≥9 mm only had symbions in their gills. This indicates an ontogenetic shift in symbiont colonization from indiscriminate infection of different epithelia to a highly restricted spatial limitation to gill bacterioocytes at a developmental stage between 8.4–9 mm. To our knowledge, such an ontogenetic shift in symbiont distribution patterns has not been previously described in Bathymodiolus or any other symbioses. Bathymodiolus gills with their greatly enlarged surface areas and ciliary ventilation are by far the most efficient tissues for providing the symbions with the oxygen and reduced compounds they need to gain energy. However, the thin outer layer of non-gill epithelia apparently has sufficient numbers of symbions in mantle and foot epithelia to provide the host with nutrients. Despite the pervasive infection of Bathymodiolus juvenile epithelia, our study shows that colonization is only achieved by the symbions (although we did not check for the intranuclear bacterial parasite that can also infect these hosts (Zielinski et al., 2009). Clearly, the infection process must be highly regulated and specific to ensure that only the symbions colonize host cells.

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symbiont-containing cells in these tissues are not immediately adjacent to the hemolymph lacuna as are the gill bacteriocytes. The cost of harboring symbionts in non-gill tissues might therefore outweigh their nutritional benefit. Alternatively, the immune system of early stage Bathymodiolus might not be sufficiently developed to prevent indiscriminate infection (Luna-Gonzalez et al., 2004; Huan et al., 2012). Teasing apart the roles that nutrition and the host immune system have in this ontogenetic shift will provide a better understanding of how bacterial colonization patterns are established, maintained and regulated in animal symbioses.

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