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The Evolution and Development of Coloniality in Hydrozoans

Running title: Hydrozoan Coloniality

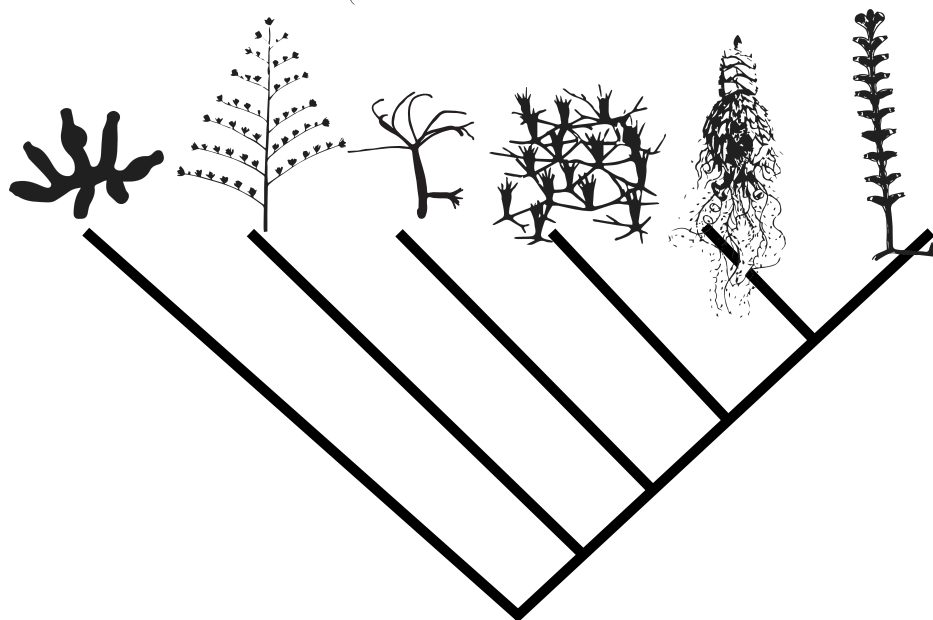
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Abstract

Hydrozoan colonies display a variety of shapes and sizes including encrusting, upright and pelagic forms. Phylogenetic patterns reveal a complex evolutionary history of these distinct colony forms, as well as colony loss. Within a species, phenotypic variation in colonies as a response to changing environmental cues and resources has been documented. The patterns of branching of colony specific tissue, called stolons in encrusting colonies and stalks in upright colonies, are likely under the control of signaling mechanisms whose changing expression in evolution and development are responsible for the diversity of hydrozoan colony forms.

Although mechanisms of polyp development have been well studied, little research has focused on colony development and patterning. In the few studies that investigated mechanisms governing colony patterning, the Wnt signaling pathway has been implicated. The diversity of colony form, evolutionary patterns and mechanisms of colony variation in Hydrozoa are reviewed here.

Keywords: Hydrozoa, coloniality, evolution, Wnt pathway

Introduction

Hydrozoans, like all cnidarians, comprise a relatively simple construction, with just two epithelial layers and a handful of cell types. Paradoxically, despite their simplicity in component parts, hydrozoans display some of the most complex and integrated colony forms amongst all animals. The life cycle of the hydrozoan *Hydractinia* is shown in Figure 1. A primary polyp is formed through metamorphosis of a planula larva. This polyp then undergoes asexual budding to

form a colony, where polyps remain connected through continuous epithelia and a shared gastrovascular cavity. This interconnectivity enables the colony to function as a physiologically integrated unit. In some hydrozoans, the polyps will bud off free-swimming sexual medusa (Fig. 2A). Thus, in the life cycle of a hydrozoan several distinct structures are patterned sequentially; that of planula larva, the polyp, the colony, and when present, the medusa. The hydrozoan polyp has been the primary focus of modern developmental studies. And while morphogenetic, ecological, and physiological factors have been identified to influence colony form, little is known of the molecular genetic signals that underlie colony development (but see Bagaeva et al., 2019, Hensel et al., 2014 and Sanders et al., 2020 on Wnt pathway genes discussed below).

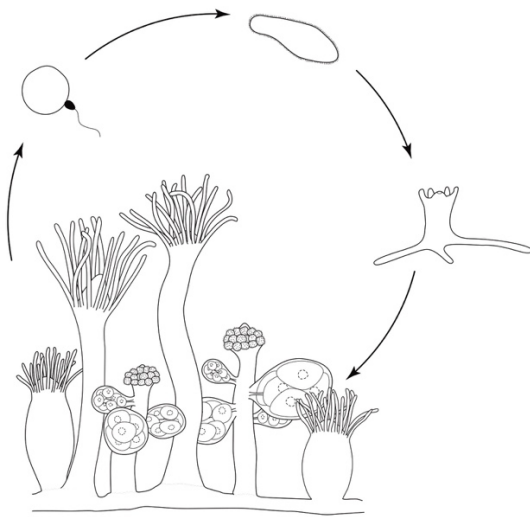


Figure 1. Life cycle of the hydrozoan *Hydractinia symbiolongicarpus*. Sexual reproduction occurs through external fertilization after release of gametes from sporosacs. The zygote develops into a free-living planula larva that eventually settles onto a gastropod shell inhabited by a hermit crab, and metamorphoses into a primary polyp. This polyp will asexually produce other polyps to form a colony and the cycle repeats. Shown here are both feeding and reproductive polyps of *Hydractinia*.

The evolution of coloniality within Hydrozoa was a key innovation that enabled them to better compete for substrate in the benthos (Coates and Jackson, 1985; Jackson, 1977), elaborate their colonial organization through a division of labor (Berrill, 1953; Burnett et al., 1967; Müller, 1964), and even enter the pelagic realm to function effectively as an integrated individual, as in siphonophores and porpitiids (Mackie, 1963). Here we review the diversity of hydrozoan colonies, provide a brief discussion of some of the experimental and descriptive studies of

colony morphogenesis, and discuss evidence for the Wnt signaling pathway playing a key role in hydrozoan colony development and evolution.

Hydrozoan colony diversity

The individual unit of a hydrozoan is the polyp, which can exist as a solitary organism such as *Hydra* (Fig. 2D). In colonial species, colony form is dictated by the branching pattern of tube-like structures, called stolons in encrusting forms and stalks in upright forms, and by the budding pattern of polyps along the stolons and stalks. The initiation of stolon/stalk branching and polyp budding during colony development appear to be the main drivers of colony patterning (Kosevich, 2005) and it is this combination of processes that are responsible for the three-dimensional shaping of hydrozoan colonies. This is underscored by the findings of Mayorova et al. (2015) that reported the shoot tip of the colony has organizing properties in that when transplanted, it can initiate a new axis.

Depending on the species, benthic hydrozoan colonies can be either stolonal and encrust the substrate, or grow upright. In stolonal colonies, such as *Clytia hemisphaerica* (Fig. 2B) and *Hydractinia echinata* (Fig. 2C), the stolons are encrusting and do not exhibit any upright branching patterns. In upright colonies, such as *Bougainvillea muscus* (Fig. 2A) and *Hartlaubella gelatinosa* (Fig. 2E), the creeping stolon buds stalks which can branch. Upright colonies differ in the pattern of branching (monopodial or sympodial), whether branching is regular or irregular, and whether or not each branch terminally differentiates into a polyp (Berking et al., 2002; Kosevich, 2013). While nearly all hydrozoan colonies are benthic, hydrozoan colonies belonging to Siphonophorae (Fig. 2F) and Porpitidae are pelagic and navigate the water column through coordinated behavior of their component parts, called zooids

which are modified polyps and medusae. In benthic species, polyps are the modular unit of the colony and each polyp typically displays tentacles and a mouth to capture prey for feeding. Some hydrozoan colonies display a division of labor, called polyp polymorphism, with subsets of polyps specialized for feeding, reproduction and defense (Fig. 2C, F) (Mackie 1986). While it is easy to identify the modular components (polyps) of a simple hydrozoan colony (Fig. 2A-C), this modularity becomes blurred in more integrated colonies where the zooids lose their individualized identity and instead are structured to perform a particular function for the colony as a whole. The pinnacle of colonial integration through division of labor is found in the pelagic siphonophores (Dunn, 2009) (Fig. 2F).

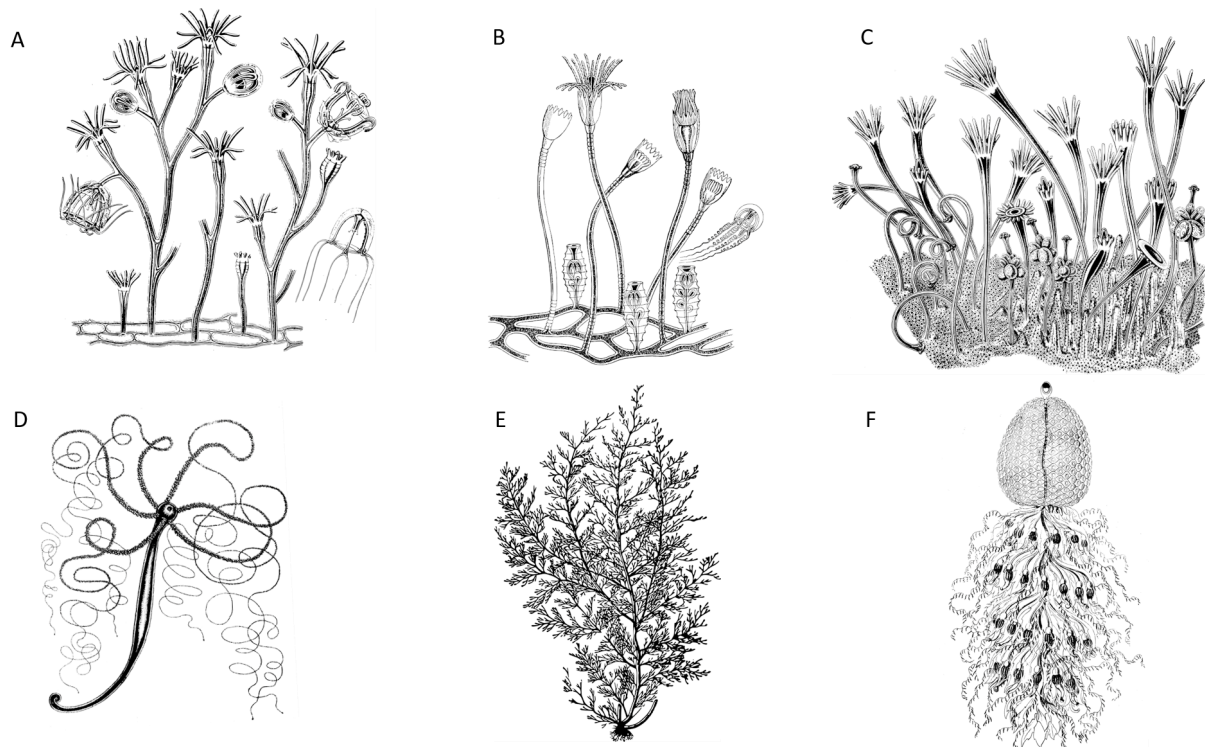


Figure 2. Species of hydrozoans displaying distinct colonial forms. (A) Erect and branched colony of *Bougainvillia muscus*. (B) Stolonal colony of *Clytia hemisphaerica*. (C) Encrusting polymorphic colony of *Hydractinia echinata*. (D) Solitary polyp of *Hydra oligactis*. (E) Erect and highly branched colony of *Hartlaubella gelatinosa*. (F) Pelagic colony of the siphonophore "*Strobalia cupola*". Modified from Allman (1871) Cuvier (1834) and Haeckel (1889).

Evolutionary patterns of coloniality within Hydrozoa

Given that asexual reproduction is a nearly ubiquitous feature amongst hydrozoans, coloniality likely evolved through disruption of physical separation from asexually budding individuals. The evolutionary history of coloniality is depicted in the phylogeny modified from Cartwright and Nawrocki (2010) (Fig. 3). Hydrozoans are divided into two main groups, Trachylina and Hydroidolina. Coloniality evolved at the base of Hydroidolina following the divergence of Trachylina. Trachylina comprise primarily solitary taxa, although some small colonies are found in trachyline Limnomedusae, which likely evolved independently from coloniality in Hydroidolina. Within Hydroidolina, coloniality was lost or greatly reduced multiple times (Fig. 3). Within the Hydroidolina order Leptothecata, two main colony morphologies can be found, upright and stolonal. Leclère et al. (2019) have shown that while the upright morphology evolved once and was maintained in the leptothecate clade Macrocolonia and at least 10 transitions from stolonal to upright morphologies occurred in the other major leptothecate clade, Statocysta. The hydrozoan clade Aplanulata, which includes the model system *Hydra*, comprises primarily solitary species, suggesting that the ancestor of Aplanulata lost the ability to form colonies. Surprisingly, within Aplanulata, coloniality appears to have re-evolved in species of *Ectopleura* (Cartwright et al., 2008; Nawrocki et al., 2013). However, *E. larynx* colonies are not a product of asexual budding, and instead result primarily from fusion of sexually reproduced juvenile polyps (Chang et al., 2018; Nawrocki and Cartwright, 2012).

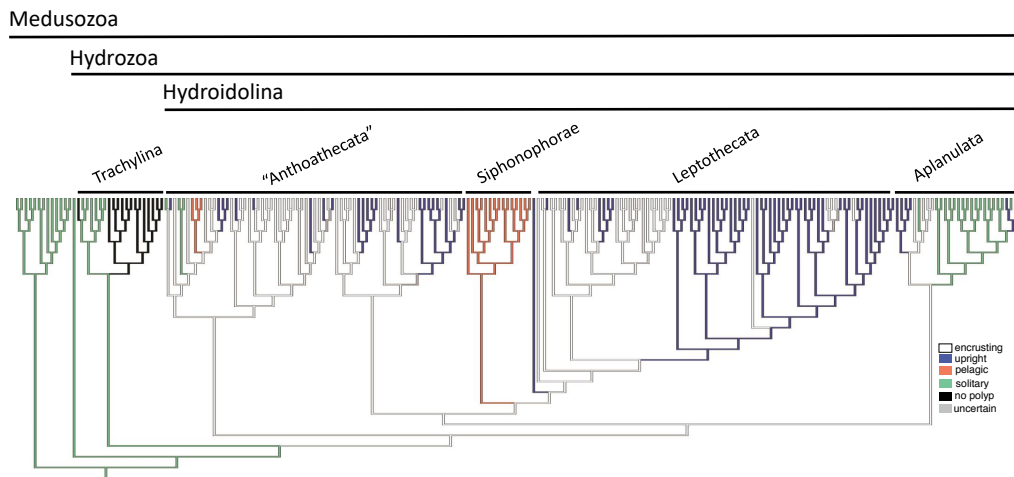


Figure 3. Phylogeny of Medusozoa depicting the evolution of coloniality. Adapted from Cartwright and Nawrocki (2010). Terminal taxa were coded for no colony (holopelagic), no coloniality (solitary benthic polyp), encrusting colony, upright colony and pelagic colony. Ancestral state character reconstruction reveals that coloniality evolved at the base of Hydrozoa and that different forms of coloniality evolved multiple times.

Intra-specific phenotypic variation in colony form

Many hydrozoan colonies exhibit developmental plasticity in response to environmental cues (Dudgeon and Buss, 1996). It has been shown that the uncoupler of oxidative phosphorylation, 2, 4-dinitrophenol (DNP) decreases gastrovascular fluid circulation which produces colonies with denser polyps in both *Podocoryna carnea* and *Hydractinia symbiolongicarpus* (Blackstone 1999; Blackstone and Buss, 1992, 1993). In addition, experimental manipulation of gastrovascular flow in *Hydractinia* drastically alters overall colony morphology (Dudgeon and Buss, 1996). The changes observed in colony form mirror heterochronic changes that occur between distinct life history strategies (i.e. fast growing runner-like colonies in *Podocoryna* vs. dense sheet-like colony forms in *Hydractinia*), suggesting adaptive plasticity in colony morphology in response to changing environments (Buss and Blackstone 1991). This phenotypic plasticity can extend to an alteration of the overall colony organization by changes in polyp composition as well as branching pattern across the colony.

Indeed, upon environmental cues, *Bougainvillea muscus* colonies exhibit a change in the polyp density and morphology as well as in their stolon network (Griffith and Newberry, 2008) while the fire coral species *Millepora dichotoma*, canonically an erect branching grower, becomes encrusting (Meroz-Fine et al, 2003). Given that environmentally induced changes in colony form mimic morphological differences between hydrozoan species, it is likely that the downstream signaling mechanisms that dictate branching and budding are conserved in evolution.

The development and placement of polymorphic polyps within the colony can also be influenced by environmental cues. Most species of *Hydractinia* grow on the gastropod shells inhabited by hermit crabs. Feeding polyps (gastrozooids) develop initially, with reproductive polyps (gonozooids) typically appearing towards the center of the colony when the colony has reached the limits of its substrates (Berrill, 1953). In addition, the hunting polyps (dactylozooids) only appear at the aperture of the gastropod shell when it is inhabited by hermit crabs, presumably induced by flow created by the crab. A fourth polyp type, the tentaculozoid, only appears as a defense when another organism (non-hydractiniid) settles near the colony. A specialized type of stolon, called hyperblastic stolons develop as a defense against a conspecific colony (Ivker, 1972; Lange et al., 1992).

In contrast, siphonophore colony development appears to be less affected by environmental cues. Colonial integration is achieved in siphonophores by stereotypical budding of zooids from a growth zone (Dunn and Wagner, 2006), such that the organization of the polymorphic zooids is largely invariant between individual colonies (Mackie, 1986). This type of canalized development enables siphonophore colonies to function efficiently in the water column as a highly integrated individual.

Developmental mechanisms dictating colonial morphogenesis

As discussed above, much has been uncovered regarding the diversity, evolution and environmental factors that dictate colony form in Hydrozoa. By contrast, little is known about the molecular genetic mechanisms underlying colony growth and patterning. Studies on morphogens identified tissues-specific inhibitors and inducers, emanating from the polyps and stolon tips, that appears to dictate spatial patterning of polyps and stolon branches of colonies (Lange and Müller 1991; Müller et al. 1987; Müller and Plickert, 1982). The homeobox gene *Cnox2* displays a spatially restricted expression pattern at the tips of stolons in *Hydractinia* (Cartwright et al. 2006) that is correlated the distinct histological features that characterize the growing developmental tip (Belousov, 1973; Kosevich, 2005; Wytenbach, 1968).

Characterization of developmental signaling pathways that operate in colony development is a critical step to understand the mechanisms underlying the evolutionary patterns of colony diversity in Hydrozoa, the phenotypic plasticity of colony form within species, and the role of spatially restricted morphogens and transcription factors in colony-specific tissues. The Wnt signaling pathway is a good candidate given that it plays a prominent role in patterning the polyp (Broun et al., 2005; Duffy et al., 2010; Hobmayer et al., 2000; Plickert et al., 2006,) and medusa (Condamine et al., 2019; Khalturin et al., 2019; Nawrocki and Cartwright, 2013; Sanders Cartwright 2015a; Sanders and Cartwright 2015b).

In addition, the non-canonical Wnt ligand Wnt5a has been shown to be expressed in the posterior tip of the *Hydractinia* planula larvae and may be involved in organizing the future oral axis of the polyp (Stumpf et al, 2010). Transplantation experiments of this organizer region in *Gonothyrea lovenii*, *Dynamena pumila*, *Clava multicornis* and *Hydractinia echinata* show a conserved role initiating a new axis (Stumpf et al, 2010, Kraus 2011, Mayorova et al 2015).

During metamorphosis *Wnt5a* expression is maintained and is later involved in the patterning of the primary polyp (Stumpf et al, 2010).

A recent study by Bagaeva et al. (2019) documented the expression of the canonical Wnt pathway (*Wnt3* and downstream components) in the leptothecate hydrozoan *Dynamena pumila*. *D. pumila* displays monopodial upright growth with symmetrical budding of polyps along a growing shoot. Thus, in *D. pumila*, the patterning of the colony is intricately tied to the patterning of polyp buds. Bagaeva et al. (2019) found that the canonical Wnt pathway is involved in dictating the position of polyp buds, and that upregulation of *Wnt3* resulted in decreased polyp budding along the upright stalk and increased encrusting stolon formation. Strikingly, the phenotypic effects of *Wnt3* upregulation mimics colony form in other leptothecate species (Bagaeva et al., 2019).

Hensel et al. (2014) identified several Wnt genes to be expressed in the stolons of *Hydractinia echinata*, with the ligand *Wnt11a* appearing to be specific to this particular structure. Sanders et al., 2020 found that the expression of the putative *Wnt 11a* receptor, *Frizzled3*, is spatially restricted to colony-specific tissues, the stolonial mat and stolons, in the hydractiniid hydrozoans *Hydractinia* and *Podocoryna*.

These studies of canonical and non-canonical Wnt ligands suggest that the Wnt pathway plays a role in the patterning throughout hydrozoan ontogeny, including dictating the morphology of the adult colony. Future investigations on the function of Wnt pathway genes in colony specific tissue should help to provide insight into the role of the Wnt pathway in colony development and evolution. For example, if the role of *Frizzled3* is to signal colony-specific tissue through binding with *Wnt11a*, then blocking the expression of *Frizzled3* should disrupt colony development. Functional experiments in *Hydractinia* is now possible through the

successful application of CRISPR (Sanders et al., 2018) and shRNA (Quiroga-Artigas et al., 2020), and thus further insight into the functional role of the Wnt pathway in hydrozoan colony development should be forthcoming.

Conclusions

Our review of the diversity of hydrozoan colonial forms in evolution, their phylogenetic patterns, and their ecological plasticity within species, illustrate that distinct colony forms are in large part achieved through the signaling of branching and growth of colony specific tissues. Although coloniality is a prominent feature of hydrozoans, little is understood about the molecular mechanisms underlying its patterning. Confirmation that the Wnt signaling pathway may play a key role in hydrozoan colony evolution and development awaits further investigations.

Acknowledgments

We thank the editors for the invitation to submit this manuscript and three reviewers for providing helpful suggestions. We would also like to thank Neil Blackstone and Leo Buss for lab cultures of *P. carnea* and Kirsten Jensen for the illustration in Figure 1. This work was supported by NSF grants EF-0531779 and DEB-095357 to PC.

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