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The gastropod foregut – evolution viewed through a developmental lens¹

by

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Abstract

Comparative data on the developing gastropod foregut suggest that this multi-component feeding complex consists of two developmental modules. Modularity is revealed by delayed development of the buccal cavity and radular sac ('ventral module') relative to the dorsal food channel ('dorsal module') in gastropods with feeding larvae compared to those that may have never had a feeding larval stage. If non-feeding larvae like those of extant patellogastropods and vetigastropods are ancestral for gastropods, then the uncoupling and heterochronic offset of dorsal and ventral foregut modules allowed the post-metamorphic dorsal food channel to be co-opted as a simple but functional esophagus for feeding larvae. Furthermore, by reducing energy cost per ovum, the heterochronic offset may have given mothers the evolutionary option of increasing fecundity or investing in protective egg encapsulation material. A second developmental innovation was spatial separation of the dorsal and ventral foregut modules, as illustrated by distal foregut development in buccinid neogastropods and venom gland development in cone snails. Spatial uncoupling may have enhanced the evolvability of gastropod foreguts by allowing phenotypic variants of ventral module components to be selected within post-metamorphic ecological settings, without needing to be first tested for compatibility with larval feeding. Finally, we describe a case in which foregut modularity has helped facilitate a highly derived life history in which encapsulated embryos ingest nurse eggs.

Keywords: modularity, evolvability, life history evolution, heterochrony, metamorphosis, nurse eggs

Developmental modules and evolvability

A major consequence of re-incorporating developmental biology into evolutionary theory has been recognition of the importance of modular developmental organization for organismal evolution (Raff 1996; Hall 1999; Schlosser and Wagner 2004; Klingenberg 2008). A developmental module is a set of traits that develop together, presumably under control of a discrete gene regulatory network, and mostly independent of other modules (see Wagner 1996; Raff and Raff 2000; Friedman and Williams 2003; Wagner et al. 2007; Lacquaniti et al. 2013). The relative autonomy of individual developmental modules means they can spin off phenotypic variants, for example by changes to time of onset or termination of the module's development or changes in patterns of cell proliferation within the module, without necessarily disrupting development as a whole. As a result, the embryo need not be fatally compromised and the novel phenotypes can be tested within a selective post-embryonic environment. Developmental modularity has therefore been interpreted as a major contributor to "evolvability", defined as the capacity of a developmental system to generate heritable phenotypic variation (Wagner and Altenberg 1996; Kirschner and Gerhart 1998; Hendrikse et al. 2007; Pigliucci, 2008).

Evolvability as an emergent property of modular developmental organization complements the explanation of asymmetric sister group diversification provided by the Modern Synthesis. Under the Modern Synthesis, differing rates of diversification among related lineages are explained by differences in ecological opportunity (see Losos and Mahler 2010), but mechanisms that might generate the phenotypic differences needed to exploit these opportunities are given scant attention. Evolvability by virtue of developmental modules may be an important precondition for clade diversification by enhancing production of heritable phenotypic variants offered to new environmental selective regimes (Gilbert et al. 1996; Erwin 2015). In the words

of Brakefield (2011, p. 2072) “evolvability must be at the heart of explanations of patterns in evolution, especially with respect to how radiations of species explore morphospace.”

If developmental modules connect genotype to phenotype (Alberch 1991; Wagner and Altenberg 1996; Mezey et al. 2000; Hall 2003; Pigliucci 2010), then links should exist between the hierarchical levels of genetic networks, developmental modules, morphofunctional units, and ultimately evolutionary change within and between clades. Investigating these linkages among diverse animals and plants is a major goal of EvoDevo research (Wagner et al. 2007; Hendrikse et al. 2007). However, recognizing developmental modules and their intermediary role between genetic networks and morphological evolution may be challenging (Klingenberg 2008). To date, important insights have come from studies on serial homologues, such as segmental body appendages of arthropods (e.g. Ronshaugen et al. 2002; Liubicich et al. 2009) and their individual podomeres (Claverie and Patek 2013), skeletal elements of the branchial arches in vertebrates (e.g. Wake and Larsen 1987), fins and limbs of vertebrates (e.g. Ruvinsky and Gibson-Brown 2000; Hall 2007), and the teeth of mammals (e.g. Jernvall 2000; Kavenagh et al. 2007; Salazar-Ciudad and Jernvall 2010). Serial homologues within and between species share many elements of developmental regulation and organization, but also display many examples of derived differences. Young et al. (2010) have argued that serial homologues must first overcome pervasive overlap in genetic control of their development (“integration”) before they can respond to differential selection and evolve along different paths of phenotypic specialization.

A different approach to the challenge of identifying developmental modules and their significance for clade diversification might come from a top down perspective. If developmental modules facilitate evolvability, then highly diverse clades might be expected to possess modular developmental organization for the morphofunctional systems that show marked differences

among lineages. A first step would be to target these systems for comparative developmental analysis within a phylogenetic context.

The Mollusca is a huge clade consisting of eight Classes and possibly 200,000 extant species (Ponder and Lindberg 2008), of which eighty percent are gastropods (Bieler 1992; Haszprunar et al. 2008). Except for the Bivalvia, the other Classes of molluscs are tiny in terms of number of species compared to the Gastropoda. However, species are not evenly distributed across the major groups of gastropods; the Caenogastropoda accounts for 60% of gastropod species and its sister group, the Heterobranchia, is the next most species-rich lineage.

A major evolutionary theme among caenogastropods and to a lesser extent the heterobranchs has been diversification of feeding systems (Ponder and Lindberg 1997). The ancestral feeding system for caenogastropods and indeed gastropods as a whole involved the use of a rasping radula to graze detrital, bacterial, or algal films; a feeding mode retained by many extant species. However, a host of other feeding strategies have evolved, including: grazing on sessile animal colonies, suspension feeding, deposit feeding, scavenging, parasitism and predatory feeding on prey that are armoured or active. Most species of predatory caenogastropods are contained within the Neogastropoda, which originated in the early Cretaceous (Tracey et al. 1993) and underwent rapid diversification during the Mesozoic (Ponder 1973; Taylor et al. 1980) to produce an estimated 16,000 living species (Bouchet 1990). Predatory feeding by neogastropods involves use of a proboscis and various highly derived but diverse morphological changes to the foregut (Ponder 1973; Kantor 1996).

Our objective is to consolidate previously published and new data indicating that the gastropod foregut consists of two developmental modules, a dorsal and ventral module. We suggest that this modularity may have helped facilitate not only the evolution of derived feeding

systems among lineages of adult caenogastropods, notably among predators, but also evolutionary change within different stages of the multi-stage life histories of these molluscs.

Morphology and development of the gastropod foregut (herbivorous grazers)

Members of the caenogastropod family Littorinidae, such as *Lacuna vineta* (MONTAGU, 1803), are herbivorous grazers during the juvenile/adult stage and can serve to illustrate basic components of the gastropod feeding system and its development. As described by Fretter and Graham (1994), the foregut of littorinid gastropods begins with a short buccal tube that immediately expands into the buccal cavity, followed by an esophagus that is subdivided into anterior, middle, and posterior regions (Fig. 1A,B). A long outpocketing from the ventral side of the back of the buccal cavity is the radular sac, which secretes a ribbon of many rows of recurved, chitinous teeth that extends anteriorly onto the floor of the buccal cavity. The radula is supported below the floor of the buccal cavity by a set of cartilage-like elements. During feeding, the cartilages and radular ribbon are protruded out of the mouth and the teeth rasp diatoms, algae or detritus from rocks or scrape the surface of macroalgae. The repetitive cycle of radular protrusion, sweep of radular teeth, and radular retraction is accomplished by a complex set of muscles (Fretter and Graham 1994).

An additional important component of the foregut of littorinids and other herbivorous gastropods is revealed by transverse sections through the anterior or mid-esophagus (Fig. 1C). The lateral walls are thickened and infolded (called “dorsal folds”) to delineate a ciliated dorsal food channel, which runs along the dorsal midline of the buccal cavity and down through the anterior and mid-esophagus. The dorsal folds also delineate a ventral channel. The wall of the

ventral channel along the length of the mid-esophagus becomes expanded with glandular cells; this is the mid-esophageal gland (Fretter and Graham 1994).

Most species of gastropods, including most littorines such as *Lacuna vincta*, begin their life as free-swimming larvae that feed on planktonic microalgae, which are captured by bands of cilia of the larval velar lobes (Fig. 2A). When these larvae hatch from the egg mass their foregut is a simple ciliated tube that conducts ingested microalgae from mouth to stomach – this is the larval esophagus (Fig. 2B). As described by Page (2000), an outpocketing develops from the ventral side of the distal end of the larval esophagus after approximately one-third of completed larval development. The outpocketing subsequently differentiates into the future, post-metamorphic buccal cavity, salivary glands, and radular sac with secreted radular teeth, and radular cartilages and musculature differentiate beneath the floor of the buccal cavity (Fig. 2C). Therefore, the post-metamorphic foregut achieves an advanced state of differentiation within late stage larvae, although it does not become functional until after metamorphosis. At metamorphosis, the larvae settle onto a benthic substrate, the velar lobes are discarded or ingested, and young juveniles begin using the radula for feeding within 3 days of losing the velar lobes. Notably, the larval esophagus is retained through metamorphosis as the dorsal food channel of the juvenile foregut, but its diameter is reduced by selective loss of some of its cells (compare profile of larval esophagus [le] in Fig. 2C with profile of the dorsal food channel [asterisk] in Fig. 2D).

Foregut modules: temporal dissociation and life history transition

An indirect life cycle, in which the first functional product of embryogenesis is a swimming larval stage, has been widely acknowledged as plesiomorphic for gastropods and the

majority of extant marine gastropods retain this life history pattern (Haszprunar et al. 1995; Ponder and Lindberg 1997). However, gastropod larvae fall into two broad groups according to whether the swimming larval stage must feed before becoming competent to metamorphose. Members of the Patellogastropoda and Vetigastropoda typically broadcast spawn their gametes and fertilized eggs develop rapidly into swimming but *non-feeding* larvae (see Hickman [1992] for deviations from this pattern among trochoidean vetigastropods, although none have feeding larvae). In these clades, energy fueling offspring development through metamorphosis is provided by maternal provisioning of ‘yolk’ to eggs and the larvae are described as lecithotrophic. However, most members of the Caenogastropoda, Heterobranchia, and Neritimorpha deposit fertilized eggs within benthic egg capsules and offspring usually hatch as swimming larvae that must feed to achieve metamorphic competence and to successfully complete metamorphosis (planktotrophic larvae). This is the larval type described above for the caenogastropod, *Lacuna vincta*. Although feeding larvae have been secondarily lost multiple times within the Caenogastropoda, Heterobranchia, and Neritimorpha, there is no evidence that a feeding larval stage occurred at any time during the clade history of either the patellogastropods or vetigastropods. We therefore refer to the lecithotrophic larvae of patellogastropods and vetigastropods as primary non-feeding larvae (to distinguish these from larvae that are non-feeding because capacity for feeding has been secondarily lost). The question of which larval type, feeding or primary non-feeding, is plesiomorphic for gastropods has been controversial. The bulk of current evidence supports the view that the ancestral larval type for gastropods was planktonic but non-feeding. This evidence includes: 1) sequences of cell lineage originations during embryogenesis, with polarity of change predicted by outgroup comparison within a phylogenetic framework (Lindberg and Guralnick 2003), 2) chronological sequence of fossil

protoconchs and steinkerns of protoconchs (the shell secreted prior to metamorphosis) characteristic of either feeding or non-feeding larvae (Nützel et al. 2006; Nützel 2014; Runnegar 2007; but see Freeman and Lundelius 2007), 3) inferences from some phylogenetic reconstructions (e.g. Haszprunar et al. 1995; McArthur and Harasewych 2003; Aktipis et al. 2008), and interpretations of comparative patterns of morphogenesis (Page 2009). If non-feeding larvae are ancestral for gastropods, then the primary non-feeding larvae of living patellogastropods and vetigastropods are the best extant approximation of this ancient larval type and feeding gastropod larvae were derived from the non-feeding type. Nevertheless, the most recent molecular-based phylogenetic reconstructions of relationships among major gastropod clades (Zapata et al. 2014) and major molluscan clades (Smith et al. 2011; Kocot et al. 2011; Vinther et al. 2012), have yielded ambiguous results regarding ancestral larval type for gastropods.

Regardless of the ancestral condition for larvae of gastropods (either a feeding type or a non-feeding type like that of extant vetigastropods and patellogastropods), comparative observations on the developing foregut within gastropod embryos and larvae suggest that the dorsal and ventral halves of the foregut constitute separate developmental modules. Specifically, the dorsal module begins as a ciliated tube extending inward from the larval mouth and eventually becomes the dorsal food channel of the post-metamorphic foregut. The ventral module originates as a ventral outpocketing from the dorsal module but becomes the buccal cavity with salivary glands and the radular sac of the post-metamorphic stage. Initial evidence for this modularity was provided by Fretter (1969), who noted that the developing radular apparatus of patellogastropods and vetigastropods, as described by Smith (1935) and Crofts (1937), respectively, seemed to begin formation at an earlier stage of overall development than

the radular apparatus of caenogastropods with feeding larvae. This type of temporal offset during developmental evolution is often a hallmark of developmental modules (Raff 1996). Given that evodevo research during the years since Fretter's (1969) observation has brought to light the significance of developmental modularity for evolvability, we now recognize the importance of further investigating the possibility of a modular developmental organization for the gastropod foregut.

Comparing developmental timing for subcomponents of the gastropod foregut among different clades is complicated by the fact that overall rate of development from fertilized egg to onset of metamorphic competence for the non-feeding larvae of patellogastropods and vetigastropods is much more rapid than for feeding larvae of caenogastropods, heterobranchs, and neritimorphs. Furthermore, rate of development for foregut components within feeding and non-feeding larvae must be compared to other ectodermally-derived components and not to endodermal components. This is because ectodermal derivatives, including the foregut, arise from the first three tiers of micromeres produced during early embryonic cleavage, whereas the endodermal midgut is derived from the fourth tier of micromeres and from macromeres (Verdonk and van den Biggelaar 1983; Render 1997; Hejnol et al. 2007; Lyons et al. 2015). Studies by Pernet and colleagues (Pernet and McHugh 2010; Pernet et al. 2012; Jones et al. 2016) have shown that cytoplasm is preferentially sequestered to macromeres during cleavage of annelid and gastropod embryos. A greater amount of cytoplasmic yolk in macromeres (which is typical of lecithotrophic larvae) is correlated with a slowdown in rate of development of midgut structures arising from these macromeres. Therefore, a method to demonstrate a temporal shift in time of development of dorsal and ventral foregut components among clades of gastropods with feeding and primary non-feeding larvae needs to control for differences in overall rate of larval

development and must avoid comparisons that would be influenced by differing amounts of yolk in embryonic cells.

We therefore recorded time of initial development of the buccal cavity and radular sac, recognized as a ventral outpocketing from the ciliated tube of the embryonic foregut, to time of development for other head structures derived from the first three tiers of micromeres. These cephalic ectodermal structures had the same developmental sequence in members of all five clades that we examined. The sequence was: ciliated stomodeum – statocysts (when first apparent as hollow epithelial spheres) – eyespots (when pigment was first apparent) – cephalic tentacles or rhinophores (when first visible as obvious protrusions from the cephalic plates). Our data come from observations on live embryos and larvae and histological sections through sequential developmental stages of representatives of all five major clades of gastropods and includes: the patellogastropod *Lottia (Tectura) scutum* (RATHKE, 1853), the vetigastropod *Haliotis kamtschatkana* JONAS, 1845, the caenogastropods *Lacuna vincta* (MONTAGU, 1803), *Trichotropis cancellata* HINDS, 1843, and *Marsenina stearnsii* (DALL, 1871), the heterobranchs *Berthella californica* (DALL, 1900), *Corambe (Doridella) steinbergae* (LANGE, 1962) and *Haminoea vesicula* (GOULD, 1855) and the neritimorph *Nerita melanotragus* E.A. SMITH, 1884.

As shown in Fig. 3, the outpocketing of the buccal cavity/radular rudiment appeared *before* the statocysts, eyespots, and cephalic tentacles in the patellogastropod and vetigastropod that we examined (primary non-feeding larvae) (Fig. 4A,B), whereas the outpocketing of the future buccal cavity/radular sac formed *after* appearance of the statocysts and eyespots in all three caenogastropods (Fig. 4C,D), one of the heterobranchs, and the neritimorph. In two other species of heterobranchs, the outpocketing appeared coincident with the eyespots. Among the seven species with feeding larvae that we examined, the time at which the outpocketing appeared

ranged from approximately the time of hatching in the caenogastropod *Trichotropis cancellata* to $\frac{1}{4}$ to $\frac{1}{3}$ of completed larval development after hatching in the remaining species. These planktotrophic larval species required from 5 to 7 weeks to complete larval development when cultured in the laboratory at 10-15 °C (Bickell and Chia 1979; Page 2000, 2002, Page - unpublished data on *Haminoea vesicula*; Parries and Page 2003; LaForge and Page 2007) or at 20-23 °C in the case of *Nerita melanotragus* (Page and Ferguson 2013). In all species of feeding larvae, the larval esophagus became the dorsal food channel of the post-metamorphic foregut.

A shift in developmental timing between two or more structures is evidence of developmental modules because a shift demonstrates dissociability. Dissociability is only possible when development of a structural complex is entirely or mostly independent of other structures (Raff 1996). If feeding larvae are a derived larval type within the Gastropoda, as supported by existing interpretations of comparative embryological and paleontological data, then we conclude that the dorsal food channel of the gastropod foregut has been co-opted as a larval esophagus in feeding gastropod larvae and constitutes a dorsal module for the developing gastropod foregut, whereas the developing buccal cavity/radular sac is a separate, ventral module, that is at least partially dissociable from the dorsal module. The ventral module arises from a nest of stem cells along the ventral surface of the dorsal module, so these two modules are not *completely* separate.

What might be the evolutionary significance of the offset in time of development of the ventral module (buccal mass) of herbivorous grazers relative to the dorsal module? The offset is not necessary for allowing unimpeded larval feeding, because late stage larvae of gastropods that will become herbivorous grazers after metamorphosis have well-developed ventral module components, but are still able to feed on phytoplankton. However, if the non-feeding larval type

produced by patellogastropods and vetigastropods is plesiomorphic for gastropods, then shifting the morphogenesis of the complex assembly of post-metamorphic buccal mass structures to the feeding larval stage would have allowed mothers to off-load the cost of its construction to feeding larvae. Under this hypothesis, the heterochronic shift in time of development of the ventral module removes a major constraint on reducing energy cost per egg. Cheaper eggs would mean that mothers could either produce more eggs (increase fecundity) or the additional energy could be invested in encapsulating material to provide protection for embryos during the period of embryogenesis.

The costs and benefits of feeding versus non-feeding larvae within the life history of marine invertebrates have been extensively debated by larval biologists (reviewed by Strathmann 1993), but speculation about the possible advantages of one over the other is compromised by uncertainty concerning environmental conditions when long ago transitions in life history took place. Nützel et al. (2006), for example, correlated the presumed initial appearance of planktotrophic-type gastropod protoconchs at the Cambrian-Ordovician boundary with “an explosive radiation of benthic suspension feeders” and inferred that predation pressure on small hatchlings together with increased density of phytoplankton favoured larval planktotrophy at this time. Alternatively, if increased density of benthic suspension feeders were the critical threat to planktonic larvae, then the emergence of feeding larvae may have been a side effect of selection to increase fecundity – enhancing the probability that at least some larvae might escape benthic suspension feeders when first released into the plankton. Whatever the selective factors favouring increased fecundity and larval planktotrophy, we suggest that modularity of the gastropod foregut and delayed formation of the ventral module was a developmental modification that helped facilitate the transition.

Spatial dissociation of modules and ventral module specialization

Wagner and Altenberg (1996, p. 967) suggested that modularity can promote evolvability because there are “few pleiotropic effects among characters serving different functions.” For this same reason, we could add that developmental modularity may provide options for circumventing constraints to specialization when a structural complex is faced with competing requirements during different life history stages. The developing foregut of a predatory neogastropod illustrates how subdivision of the foregut into dorsal and ventral modules may have facilitated evolution of a specialized adult foregut within a life cycle where the initial larva requires a foregut to simply propel microalgae from mouth to stomach.

Neogastropods feed with a proboscis and the foregut has been highly modified in various ways among different lineages of neogastropods (Ponder 1973; Kantor 1996). In muricoidean and buccinoidean neogastropods, the extra length of foregut needed to extend down the protruded proboscis during feeding is provided by a very long and muscularized anterior esophagus. Furthermore, a novel structure known as the valve of Leiblein is placed at the junction between anterior and mid-esophagus, presumably to prevent regurgitation of food during feeding (Graham 1941).

The foregut of a buccinid neogastropod, *Nassarius mendicus* (GOULD, 1850) begins development in the feeding larval stage much like the foregut of the herbivorous caenogastropod, *Lacuna vincta*. The larval esophagus in young larvae of both these species is a simple ciliated tube extending between mouth and stomach, but eventually the post-metamorphic buccal mass begins to form as a ventral outpocketing from the wall of the larval esophagus. However, subsequent morphogenesis of this outpocketing is radically different in these two

caenogastropods (Page 2000, 2005). In *L. vincta*, the wall of the developing buccal cavity remains confluent with the wall of the overlying larval esophagus (Fig. 2C) and the larval esophagus is carried through metamorphosis to become the dorsal food channel, although its cross-sectional profile becomes much reduced at metamorphosis (Fig. 2D). By contrast, in the predatory neogastropod *N. mendicus* the buccal cavity that develops from the ventral outpocketing is completely separate from the overlying larval esophagus and, most remarkably, the original ventral outpocketing also generates the entire, post-metamorphic anterior esophagus and valve of Leiblein (Fig. 5A). During metamorphosis of *N. mendicus*, the larval esophagus anterior to the valve of Leiblein is completely destroyed by cell dissociation (Fig. 5B). Furthermore, the larval mouth of *N. mendicus* is sealed shut and a new post-metamorphic mouth is formed when the wall of the buccal cavity ruptures through the end of the developing proboscis (Page 2005).

As summarized in the schematic sketches shown in Fig. 6, the comparative data reveal two innovations in the evolution of foregut development among caenogastropods: a first innovation was temporal uncoupling of dorsal and ventral foregut modules relative to the pattern in primary non-feeding larvae, whereas a second innovation seen in a buccinid neogastropod is a spatial uncoupling between these two modules. The second innovation may have facilitated the evolution of a highly derived type of post-metamorphic foregut because the larval and post-metamorphic foreguts can develop in almost complete isolation (Page 2000, 2005). The semi-isolation means that the ventral module can acquire additional specialized components (e.g. muscular ‘anterior esophagus’) prior to metamorphosis without compromising larval feeding. The advanced differentiation of this specialized post-metamorphic foregut prior to metamorphosis, together with rapid elongation of the proboscis during metamorphosis, explains

why juveniles are able to feed on animal matter within only 3 days of metamorphic loss of velar cilia.

A second example of spatial uncoupling between dorsal and ventral foregut modules that has facilitated a highly derived neogastropod feeding system comes from developmental study of a species of cone snail. Many members of the hyperdiverse neogastropod superfamily Conoidea, have an extraordinary feeding system that involves envenomation of prey with a cocktail of over a thousand peptide toxins (“conotoxins”) that rapidly immobilize prey by mainly targeting ligand and voltage-gated ion channels of the neuromuscular system (see Olivera 2006; Davis et al. 2009). The venom delivery system consists of highly derived foregut components together with a specialized proboscis (Sheridan et al. 1973; Marsh 1977; Taylor et al. 1993; Kohn et al. 1999; Kantor and Taylor 2000; Marshall et al. 2002; Holford et al. 2009; Schulz et al. 2004; Salisbury et al. 2010; Castelin et al. 2012). The radular sac of *Conus* secretes hollow, harpoon-shaped teeth that are homologous to marginal radular teeth of other gastropods (Ponder 1973; Kantor and Taylor 2000). The teeth are stored in a specialized subcompartment of the radular sac, which is probably a homologue of the subradular pouch of other gastropods (Taylor et al. 1993). During an attack on prey, a single tooth is placed within the distal end of the proboscis, the proboscis is extended to touch the prey, and the tooth is driven into the prey using a ballistic mechanism (Schulz et al. 2004; Salisbury et al. 2010). Simultaneously, a burst of peptide toxins shoots down the buccal tube of the proboscis and is hypodermically injected into the prey through the hollow radular tooth. The venom is produced by a long, narrow, convoluted gland that enters the foregut immediately behind the buccal cavity and its distal end is capped by a very large muscular bulb. The mechanism by which toxins from the venom gland are propelled down the proboscis is controversial (see Marshall et al. 2002; Salisbury et al. 2010). However, recent evidence showing

that muscle fibers of the muscular bulb contain a large amount of arginine kinase, an enzyme that facilitates rapid release of high energy phosphate to fuel burst muscle contraction, was interpreted to suggest that brief but forceful contraction of the muscular bulb may propel toxins into the buccal cavity and possibly even down the length of the proboscis during a predatory attack (Safavi-Hemami et al. 2010). Alternatively, Salisbury et al. (2010) suggested that toxins may be propelled down the proboscis by contraction of the muscular buccal cavity. Whatever the mechanism that drives conotoxins down the buccal tube within the proboscis during a predatory cone snail attack, it is apparent that the hypodermic delivery system requires that toxins be delivered directly into the buccal cavity and indeed the venom gland discharges into the back of this cavity.

A developmental study of *Conus lividus* indicated that the venom gland is a homologue of the mid-esophageal gland of other caenogastropods (Page 2012), which confirmed a hypothesis proposed many years ago by Amaudrut (1898). In *C. lividus* and in other caenogastropods (e.g. *Euspira lewisii*; *Lacuna vincta*, *Marsenina stearnsii*), the mid-esophageal gland develops in late-stage larvae when secretory cells differentiate within the walls of the ventral channel of the mid-esophagus. This region can be interpreted as the posterior region of the developing foregut's ventral module. In juveniles and adults of most non-neogastropods, the hypertrophied glandular epithelium extends down the entire length of the mid-esophagus so that secretory product is discharged throughout this portion of the esophagus. However, during metamorphosis of *C. lividus*, the glandular wall of the ventral channel peels away from the dorsal channel (former larval esophagus) to generate a tube of glandular epithelium that remains connected to the foregut only at a site immediately behind the buccal cavity. The muscular bulb subsequently differentiates at the distal end of this almost completely detached homologue of the

mid-esophageal gland. In this instance, spatial uncoupling between the dorsal and ventral developmental modules of the foregut occurs as an abrupt morphogenetic remodeling of an epithelial tube to subdivide it into two parallel tubes; the wall of one of these tubes is glandular and secretes conotoxins. By enabling delivery of conotoxins directly to the buccal cavity, rather than diffuse release down the entire length of the mid-esophagus, the spatial uncoupling between dorsal and ventral modules of the mid-esophagus is a developmental remodeling that is critically important for the functioning of the envenomation apparatus in cone snails.

Dorsal module specialization

The foregoing examples of developmental and evolutionary change involving the gastropod foregut have emphasized changes to the ventral module. Nevertheless, it should be apparent from the previous description of foregut development in *Lacuna vincta* and *Nassarius mendicus* that the dorsal module has a larger cross-sectional profile in the feeding larval stage relative to its size after metamorphosis, when it serves as the dorsal food channel or is destroyed altogether in the anterior region of the foregut. If non-feeding larvae were ancestral for gastropods, then the esophagus of feeding larvae was not only co-opted from the dorsal food channel of the juvenile, but the co-option also involved enlargement of this epithelial channel.

A second example of evolutionary change to the dorsal module involves its novel remodeling to accommodate a derived life history strategy in members of the neogastropod genus *Nucella*. *Nucella* is a north temperate genus of neogastropods that includes eight valid species. None of these extant species has a swimming, feeding (planktotrophic) larval stage (Marko et al. 2014), presumably because a swimming, feeding larva was secondarily lost one or more times among ancestors within this genus. Fertilized eggs deposited in benthic egg capsules

develop into veliger larvae with ciliated velar lobes, but these undergo metamorphosis within the egg capsule shortly before the crawling juveniles hatch from the capsule. For six of the eight species of *Nucella*, mothers deposit both viable and non-viable eggs within the capsules and viable embryos eat the non-viable, so-called nurse eggs (Marko et al., 2014). Viable embryos of *Nucella ostrina* (GOULD, 1852) ingest all nurse eggs within the egg capsule at a relatively early stage of embryogenesis, before enlargement of the velar lobes or formation of pigmented eyespots (Fig. 7A).

Transverse sections through embryos of *N. ostrina* during the phase of nurse egg ingestion revealed that ingested nurse eggs are temporarily stored within a spacious, epithelium-lined sac just inside the mouth (Hookham and Page 2016). This sac could be identified as a homologue of the larval esophagus of feeding neogastropod larvae (i.e. the dorsal module) because the ventral module of the future foregut had already begun to form as an out-pocketing from its ventral side (Fig. 7B). Eventually, ingested nurse eggs disappear from the dorsal module, presumably because they move to the midgut for assimilation of nutrients, and the still large but now empty bag of the dorsal module collapses (Fig. 7C). The dorsal module subsequently loses a substantial portion of its epithelial cells and during intracapsular metamorphosis the dorsal module is completely destroyed (Hookham and Page 2016). The fact that the dorsal module has a greatly increased surface area of epithelium to accommodate a large mass of ingested nurse eggs is evidence that it has undergone evolutionary change to accommodate this derived life history strategy. Nevertheless, the process has not compromised the morphogenesis of the ventral module to form the derived post-metamorphic foregut of these muricid neogastropods.

Conclusions and Future Directions

The existence of two foregut modules is not apparent from the morphology of juvenile and adult gastropods, where the dorsal food channel, buccal cavity, salivary glands, and ribbon of radular teeth function together as a multi-component functional complex with the single purpose of acquiring and ingesting food. For molluscs such as vetigastropods and patellogastropods that produce primary non-feeding larvae, the larval body is essentially the developing juvenile body that is embellished with a ciliated velum to allow a transient planktonic lifestyle before settlement on the benthos (Page 2009). Viewed in this light, it is perhaps not surprising that the prospective buccal mass (buccal cavity + radular sac + cartilages and muscles) of these gastropods develops relatively synchronously with the dorsal food channel, because the first and only functional role for both requires their cooperative action for feeding. We would predict that members of other molluscan classes (with the exception of bivalves because a radular apparatus is absent from all bivalve species) would also show simultaneous development of the dorsal and ventral halves of the distal foregut, given that molluscs other than most gastropods and bivalves all have a non-feeding larval stage.

Our interpretation of comparative data on gastropod foregut development proceeds from the premise that feeding gastropod larvae are a derived larval type. Under this hypothesis, the data suggest that an uncoupling of dorsal and ventral foregut modules occurred, possibly through reduction of pleiotropic interactions between these two (see Young et al. 2010), and a heterochronic shift in time of onset of the ventral module correlates with the production of feeding larvae. It appears that only the dorsal module of the post-metamorphic stage has been recruited and enlarged to serve as the esophagus of planktotrophic larvae, which feed on microalgae without using a radular apparatus. This selective recruitment of the dorsal food

channel as the esophagus of larvae, achieved by delayed development of the ventral module, was a developmental innovation because it required uncoupling of a pre-existing and possibly tight developmental integration of the dorsal and ventral components of the foregut.

A host of studies, mostly on various species of echinoderm larvae, have examined effects of experimental manipulation of egg or embryo size or direct changes to egg nutrient content (literature reviewed by Pernet et al. 2012). These have generally found that “planktotrophs [use] most of their maternally provided energy to construct the larval body, and lecithotrophs [use] most of their maternally provided energy to provision the post-metamorphic body” (Pernet et al. 2012, p.83). Selective allocation of maternal resources to either the larval or post-metamorphic body is an option for animals like echinoderms, where the larval and post-metamorphic bodies are almost completely separate entities. However, the larval body of gastropods (both primary non-feeding and feeding forms) is essentially the juvenile body under construction, except for the velar lobes and larval apical sensory organ (Page 2009). The buccal mass is a highly complex structure that occupies a large proportion of overall tissue mass when it first becomes functional in young juvenile gastropods. As long as development of dorsal and ventral modules of the post-metamorphic buccal mass was tightly integrated, the need to provide nutrient resources for the entire construction cost of a buccal mass might be expected to limit the quantity of resources that mothers could withhold from eggs. Gastropods that produce feeding larvae have made a heterochronic adjustment to morphogenesis so that the energetically expensive buccal mass, not needed for larval feeding, has been delayed. In essence, the uncoupling may have given mothers ‘permission’ to substantially reduce provisioning to individual eggs because self-nourishing larvae can feed without a radular apparatus and can acquire the energy for building a radular apparatus without a maternal subsidy. Future studies comparing egg size with relative time of

ventral module morphogenesis among gastropod sibling species that differ in larval nutritional mode might reveal a reversal of delayed ventral module morphogenesis in species that have secondarily lost the feeding larval stage.

The innovation that uncoupled developmental integration between dorsal and ventral foregut modules may have been profoundly important for facilitating the transition from non-feeding to feeding larvae within the Gastropoda, but there is currently little compelling evidence that this innovation of itself directly enhanced evolvability of adult gastropods. Certainly a feeding larval stage is ancestral for caenogastropods and heterobranchs and these clades collectively include by far the majority of extant gastropods. However, a feeding larva is also the probable ancestral condition for neritimorphs (Nützel et al. 2007) and *Nerita melanotragus* shows delayed development of the ventral module. Nevertheless, neritimorphs constitute a relatively small clade of gastropods and most members are herbivorous grazers (Lindberg 2008).

The second innovation of foregut development was spatial uncoupling of the dorsal and ventral modules, as evidenced by development of the distal foregut in a buccinid neogastropod (Fig. 6) and development of the mid-esophagus in a conid neogastropod. Spatial uncoupling, riding on the back of temporal uncoupling, may have truly been a key innovation leading to enhanced evolvability within the Gastropoda; certainly the neogastropods have undergone very rapid speciation and diversification since their origin in the early Cretaceous. We have suggested that the spatial uncoupling freed the post-metamorphic foregut from the functional constraint of the feeding larval stage, which allowed “exploration of morphospace” by the ventral module (prospective post-metamorphic foregut) within the post-metamorphic environment. Specifically, phenotypic variants of the post-metamorphic foregut could develop and be tested within post-metamorphic environments without being subject to an initial filter of compatibility with larval

functional needs. If this is the case, similar developmental mechanisms to isolate the larval foregut from highly derived post-metamorphic foreguts might be expected in other clades of gastropods with feeding larvae. For example, the Pyramidellida is a species-rich clade of heterobranch gastropods that feed with a proboscis and have highly derived foregut structures (Fretter and Graham 1949; Wise 1993). These structures evolved entirely independently from those of any caenogastropod group.

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Draft

Figure Captions

Fig. 1. Sketches illustrating foregut anatomy of a herbivorous grazing caenogastropod. (A) Mid-sagittal view of head showing major foregut components. (B) Dorsal view showing subdivisions of esophagus and mid-esophageal gland along ventral wall of the mid-esophagus. (C) Transverse section through anterior esophagus showing folds delineating dorsal food channel and ventral channel, circular profiles are ducts of salivary glands.

Fig. 2. Whole mount of a feeding larva of a caenogastropod in ventral view showing ciliated velar lobes (A) and histological transverse sections through the distal foregut of the herbivorous caenogastropod *Lacuna vincta* during young larval stage (B), late larval stage (C), and 2 days after metamorphic loss of velar lobes (D); dorsal food channel indicated by asterisk. Abbreviations: bc, buccal cavity, ca, cartilage-like elements; le, larval esophagus; s, statocysts; vl, velar lobe. Arrowheads indicate ducts of salivary glands. Scale bars, 25 μm .

Fig. 3. Sequence of developing ectodermal structures of head during gastropod development. Position within this sequence for initial formation of buccal cavity/radular sac rudiment in nine gastropods is indicated by red arrowheads. Clade affiliations of named genera are colour coded as: red, Vetigastropoda; dark blue, Patellogastropoda; brown, Heterobranchia; green, Caenogastropoda; purple, Neritimorpha. NF, primary non-feeding larvae; F, feeding larvae. Neither cephalic tentacles nor rhinophores develop in *Haminoea vesicula*.

Fig. 4. Development of ectodermal head structures in gastropod larvae. (A) Live non-feeding larva of the patellogastropod *Lottia scutum* at 60 h after fertilization; neither eyes nor

statocysts have developed. (B) Mid-sagittal section of *L. scutum* at 60 h after fertilization showing buccal cavity (bc) and radular sac rudiment (large arrowhead); arrows indicate more posterior portion of esophagus. (C) Live feeding larva of *Lacuna vincta* within 1 day of hatching showing left statocyst (arrowhead) and pigmented eye (arrow). (D) Mid-sagittal section of *L. vincta* within 1 day of hatching showing larval esophagus (le) as simple ciliated tube; ventral outpocketing of future buccal cavity/radular sac has not yet formed. Other abbreviations: f, foot; pr, protoconch; st, stomach; v, velum.

Fig. 5. Histological transverse sections through distal foregut of the predatory neogastropod *Nassarius mendicus* during (A) late larval stage, and (B) 3 days after metamorphic loss of velar lobes. In (A), the larval esophagus (le) is completely separate from the post-metamorphic anterior esophagus (ae) and in (B) the larval esophagus has been destroyed. Abbreviations: ae, anterior esophagus; le, larval esophagus. rs, radular sac. Scale bars, 25 μm .

Fig. 6. Schematic sketches illustrating changes to the developing foregut of patellogastropods and vetigastropods with primary non-feeding larvae, the herbivorous caenogastropod *Lacuna vincta* with feeding larvae, and the predatory neogastropod *Nassarius mendicus* with feeding larvae; the mouth lies to the left for all sketches. The foregut of young larvae of the primary non-feeding type consists of both a dorsal and ventral modules (dm, vm); the dorsal module becomes the dorsal food channel (dfc) and the ventral module becomes the buccal cavity (bc) and radular sac (rs). In gastropods with a feeding larva, the tube diameter of the dorsal module is enlarged as the larval esophagus (le) and a 1st innovation is delayed initiation of the ventral module during development. A second innovation exhibited by the predatory neogastropod *Nassarius mendicus* is spatial separation of the

developing dorsal and ventral modules and full destruction of the distal larval esophagus at metamorphosis. Abbreviations: ae, anterior esophagus; bc, buccal cavity; dfc, dorsal food channel; dm, dorsal module; le, larval esophagus; mg, mid-esophageal gland; rs, radular sac; vm, ventral module.

Fig. 7. Nurse egg feeding by young embryos of the neogastropod *Nucella ostrina* removed from egg capsule. (A) Viable embryos (e) ingesting a central mass of nurse eggs (ne); broken line indicates level of histological section shown in B. (B) Transverse section through embryo showing ingested nurse eggs packed into enlarged homologue of larval esophagus (wall indicated by arrows); note initial formation of ventral module (vm). (C) Transverse section through embryo after all nurse eggs have been eaten showing spacious but now empty homologue of larval esophagus (le) with developing ventral module (vm). Scale bars, 50 μm .

Fig. 1. Sketches illustrating foregut anatomy of a herbivorous grazing caenogastropod. (A) Mid-sagittal view of head showing major foregut components. (B) Dorsal view showing subdivisions of esophagus and mid-esophageal gland along ventral wall of the mid-esophagus. (C) Transverse section through anterior esophagus showing folds delineating dorsal food channel and ventral channel, circular profiles are ducts of salivary glands.

Fig. 1

95x105mm (300 x 300 DPI)

Fig. 2. Whole mount of a feeding larva of a caenogastropod showing ciliated velar lobes (A) and histological transverse sections through the distal foregut of the herbivorous caenogastropod *Lacuna vincta* during young larval stage (B), late larval stage (C), and 2 days after metamorphic loss of velar lobes (D); dorsal food channel indicated by asterisk. Abbreviations: bc, buccal cavity, ca, cartilage-like elements; le, larval esophagus; s, statocysts; vl, velar lobe. Arrowheads indicate ducts of salivary glands. Scale bars, 25 μ m.

Fig. 2
76x155mm (300 x 300 DPI)

Fig. 3. Sequence of developing ectodermal structures of head during gastropod development. Position within this sequence for initial formation of buccal cavity/radular sac rudiment in nine gastropods is indicated by red arrowheads. Clade affiliations of named genera are colour coded as: red, Vetigastropoda; dark blue, Patellogastropoda; brown, Heterobranchia; green, Caenogastropoda; purple, Neritimorpha. NF, primary non-feeding larvae; F, feeding larvae. Neither cephalic tentacles nor rhinophores develop in *Haminoea* vesicula.

Fig. 3

32x12mm (300 x 300 DPI)

Fig. 4. Development of ectodermal head structures in gastropod larvae. (A) Live non-feeding larva of the patellogastropod *Lottia scutum* at 60 h after fertilization; neither eyes nor statocysts have developed. (B) Mid-sagittal section of *L. scutum* at 60 h after fertilization showing buccal cavity (bc) and radular sac rudiment (large arrowhead); arrows indicate more posterior portion of esophagus. (C) Live feeding larva of *Lacuna vincta* within 1 day of hatching showing left statocyst (arrowhead) and pigmented eye (arrow). (D) Mid-sagittal section of *L. vincta* within 1 day of hatching showing larval esophagus (le) as simple ciliated tube; ventral outpocketing of future buccal cavity/radular sac has not yet formed. Other abbreviations: f, foot; pr, protoconch; st, stomach; v, velum.

Fig. 4
157x175mm (300 x 300 DPI)

Fig. 5. Histological transverse sections through distal foregut of the predatory neogastropod *Nassarius mendicus* during (A) late larval stage, and (B) 3 days after metamorphic loss of velar lobes. In (A), the larval esophagus (le) is completely separate from the post-metamorphic anterior esophagus (ae) and in (B) the larval esophagus has been destroyed. Abbreviations: ae, anterior esophagus; le, larval esophagus. rs, radular sac. Scale bars, 25 μ m.

Fig. 5

66x128mm (300 x 300 DPI)

Fig. 6. Schematic sketches illustrating changes to the developing foregut of patellogastropods and vetigastropods with primary non-feeding larvae, the herbivorous caenogastropod *Lacuna vincta* with feeding larvae, and the predatory neogastropod *Nassarius mendicus* with feeding larvae; the mouth lies to the left for all sketches. The foregut of young larvae of the primary non-feeding type consists of both a dorsal and ventral modules (dm, vm); the dorsal module becomes the dorsal food channel (dfc) and the ventral module becomes the buccal cavity (bc) and radular sac (rs). In gastropods with a feeding larva, the tube diameter of the dorsal module is enlarged as the larval esophagus (le) and a 1st innovation is delayed initiation of the ventral module during development. A second innovation exhibited by the predatory neogastropod *Nassarius mendicus* is spatial separation of the developing dorsal and ventral modules and full destruction of the distal larval esophagus at metamorphosis. Abbreviations: ae, anterior esophagus; bc, buccal cavity; dfc, dorsal food channel; dm, dorsal module; le, larval esophagus; mg, mid-esophageal gland; rs, radular sac; vm, ventral module.

Fig. 6
157x139mm (300 x 300 DPI)

Fig. 7. Nurse egg feeding by young embryos of the neogastropod *Nucella ostrina* removed from egg capsule. (A) Viable embryos (e) ingesting a central mass of nurse eggs (ne); broken line indicates level of histological section shown in B. (B) Transverse section through embryo showing ingested nurse eggs packed into enlarged homologue of larval esophagus (wall indicated by arrows); note initial formation of ventral module (vm). (C) Transverse section through embryo after all nurse eggs have been eaten showing spacious but now empty homologue of larval esophagus (le) with developing ventral module (vm). Scale bars, 50 μ m.

Fig. 7

182x60mm (300 x 300 DPI)