# Comparison of developmental trajectories in the starlet sea anemone Nematostella vectensis: embryogenesis, regeneration, and two forms of asexual fission

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Abstract. The starlet sea anemone, *Nematostella vectensis*, is a small burrowing estuarine animal, native to the Atlantic coast of North America. In recent years, this anemone has emerged as a model system in cnidarian developmental biology. Molecular studies of embryology and larval development in *N. vectensis* have provided important insights into the evolution of key metazoan traits. However, the adult body plan of *N. vectensis* may arise via four distinct developmental trajectories: (1) embryogenesis following sexual reproduction, (2) asexual reproduction via physal pinching, (3) asexual reproduction via polarity reversal, and (4) regeneration following bisection through the body column. Here, we compare the ontogenetic sequences underlying alternate developmental trajectories. Additionally, we describe the predictable generation of anomalous phenotypes that can occur following localized injuries to the body column. These studies suggest testable hypotheses on the molecular mechanisms underlying alternate developmental trajectories, and they provoke new questions about the evolution of novel developmental trajectories and their initiation via environmental cues.

Additional key words: Nematostella, planula, regeneration, transverse fission

Nematostella vectensis STEPHENSON 1935 is a euryhaline, eurythermal, burrowing sea anemone (Anthozoa, Actiniaria) living in coastal estuarine habitats throughout North America and Southern England (Stephenson 1935; Crowell 1946; Hand 1957). Nematostella vectensis is a member of the family Edwardsiidae, a monophyletic clade of burrowing sea anemones with a global distribution (Daly 2002; Daly et al. 2002). Like many cnidarians, N. vectensis can undergo multiple developmental trajectories, i.e., the adult polyp may develop via embryogenesis, asexual fission, or regeneration (Hand & Uhlinger 1992). Unlike the majority of cnidarians, N. vectensis is a relatively well-studied species with a rich literature describing its natural history (Hand & Uhlinger 1994; Sheader et al. 1997), geographic distribution (Hand & Uhlinger 1994 and references therein; Pearson et al. 2002; Darling et al. 2004), population genetic structure (Pearson et al. 2002; Darling et al. 2004), and conservation (Sheader et al. 1997).

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Nematostella vectensis is the only sea anemone species whose members have been cultured throughout its entire life cycle in the laboratory (Fautin 2002), and recently, it has emerged as an important basal metazoan system for research in development and genomics (reviewed in Darling et al. 2005). Studies utilizing N. vectensis have led to a better molecular understanding of anthozoan development while illuminating critical events in the early evolution of animals such as the origin of bilateral symmetry (Finnerty et al. 2004), anterior-posterior patterning (Finnerty & Martindale 1999), and triploblasty (Scholz & Technau 2003; Martindale et al. 2004). With completion of its genome sequence and the characterization of 150,000 expressed sequence tags, N. vectensis's utility as a genomic model system will increase dramatically (Sullivan et al. 2006).

Despite the increasing prominence of *N. vectensis* as a laboratory model for development, its complete developmental repertoire has not yet been thoroughly explored. The specific developmental sequences of embryogenesis, asexual reproduction, and regeneration have not been explicitly compared in one source. Portions of each process have been described in previously published papers. Frank & Bleakney (1976)

and Hand & Uhlinger (1992), and later Martin (1997), discussed the general features of embryogenesis, the planula larva, and early juvenile morphology. More detailed observations of early embryogenesis and larval/juvenile development have been reported in recent papers addressing the expression of developmental regulatory genes (e.g., Finnerty et al. 2004; Martindale et al. 2004). A number of authors (Lindsay 1975; Frank & Bleakney 1978; Hand & Uhlinger 1992, 1995) have described one type of asexual reproduction in N. vectensis that we have termed "physal pinching" (Darling et al. 2005). Herein, we describe this process in greater detail, and we also describe a second, distinct form of asexual reproduction that we have termed "polarity reversal." Complete bidirectional regeneration following bisection (referred hereafter as just regeneration) in adults of N. vectensis has been discussed largely as an explanation for naturally occurring and laboratory-generated "anomalies" (Frank & Bleakney 1978). Adults are frequently collected in the field with multiple oral crowns sharing a common physa (Frank & Bleakney 1978). Hand & Uhlinger (1995) reported a range of regeneration variants, including a four-crowned anemone, by administering specific local injuries to the body wall.

Members of N. vectensis comprise only one among the thousands of cnidarian species that are capable of developing via alternate developmental trajectories. Understanding the evolution of these trajectories and the developmental mechanisms will also be strengthened by comparisons with previous work on development for other species with multiple developmental pathways. Within the Cnidaria, a large body of work has investigated alternate developmental trajectories, especially regeneration and asexual reproduction by budding, and to a lesser extent, embryogenesis. Most of this work has been carried out with the hydrozoan Hydra, which has become a model system for studying asexual reproduction and regeneration (Bode & Bode 1984; Bode 2003). Extensive work manipulating adults with excision and tissue grafts resulted in models for tissue dynamics and pattern formation in Hydra (Meinhardt 1993). Adults of Hydra that undergo steady-state morphogenesis continually produce cells in the body column that migrate to the oral and aboral poles, where they differentiate into region-specific cells. Recent work has begun characterizing the specific genes and peptides involved in cell differentiation and tissue patterning during this process (Steele 2002). Individuals of Hydra can regenerate after bisection or even dissociation into single cells, resulting in a number of abnormal adult phenotypes including multiple oral regions (Gierer et al. 1972). However, the Cnidaria have a large diversity of developmental and reproductive mechanisms, making investigations with many species across the phylum necessary for a broader, comparative picture (Collins et al. 2005). Comparisons among additional species would help elucidate underlying similarities and differences in developmental processes across the Cnidaria.

Nematostella vectensis provides an opportunity to elucidate the molecular mechanisms underlying multiple developmental trajectories because members will undergo four distinct trajectories in the laboratory (embryogenesis, regeneration, asexual reproduction via physal pinching, and asexual reproduction via polarity reversal). Understanding the various developmental trajectories at the morphological level is a necessary prerequisite for framing reasonable hypotheses about the underlying molecular mechanisms. Previous works with Hydra (Fröbius et al. 2003) and an oligochaete, Pristina leidvi SMITH 1896 (Bely & Wray 2001), have hypothesized that the molecular mechanisms underlying alternate developmental trajectories are likely to be highly similar, as the final phenotype resulting from each process is identical. However, differences among developmental trajectories in N. vectensis in both temporal sequence and spatial relationship of particular developmental events suggest that the underlying molecular mechanisms for alternate developmental trajectories may differ in important ways. For example, embryogenesis proceeds from a single cell, while regeneration and fission occur in fully differentiated adults. Likewise, while regeneration is initiated by external forces, fission is initiated by endogenous signals. Here, we compare and contrast four alternate developmental trajectories in N. vectensis at the morphological level, with the long-term goal of understanding how such developmental flexibility may be encoded in the genome, and how it may evolve.

## Methods

#### Animal care

Adults of *Nematostella vectensis* were maintained in glass or plastic dishes in 33% strength artificial seawater ( $\sim$ 12‰) at room temperature (21°–22°C). Adults were fed freshly hatched nauplii (*Artemia* sp.) daily, and water was changed weekly.

# **Developmental observations**

Observations were performed by eye or with the aid of a dissecting microscope (model SZX9, Olympus, Melville, NY, USA). Images were recorded on a digital camera (Nikon CoolPix 900, Tokyo, Japan) affixed to the dissecting microscope. Scanning electron micrographs were taken for developmental stages between the gastrula and the metamorphosed juvenile. Individuals were prepared for scanning electron microscope SEM by fixation in 2.5% glutaraldehyde/0.2 mol L<sup>-1</sup> phosphate buffer. After fixation, specimens were dehydrated in a graded series of ethanol steps, critical point dried in a Tousimis Samdri 780a critical point drier, coated with gold–pallidium using a Tousimis Samsputter 2A sputter coater (Rockville, MD, USA), and observed with a JEOL JSM-840 SEM (JEOL, Peabody, MA, USA) at 15 kV.

**Embryogenesis and larval development.** Egg masses were isolated from laboratory populations derived from individuals of *N. vectensis* originally collected from the Rhodes River estuary in Maryland (K. Uhlinger, pers. comm.). Embryos were removed from the egg jelly as described previously (Finnerty et al. 2004). Developing embryos and larvae were provided with the same general culture conditions as adults. However, they were not provided with food. Larvae readily metamorphose into benthic juvenile polyps and settle in culture dishes without addition of a settlement cue.

Asexual reproduction by physal pinching. Physal fragments were collected from 44 individuals that had recently undergone asexual reproduction via physal pinching. Well-fed adults will frequently and spontaneously undergo physal pinching in laboratory culture. The number of parent mesenteries in each physal fragment, the time to tentacle bud eruption, and the number of tentacles present at the time of tentacle eruption were recorded for each fissioned physa. Possible correlations between the number of parent mesenteries and the time to tentacle eruption and the number of tentacles at tentacle eruption were statistically analyzed by linear regression using Minitab 12 for Windows (Minitab Inc., State College, PA, USA). Because asexual reproduction by polarity reversal is a far less frequent event in our laboratory cultures, compiling a similar data set was not feasible.

**Regeneration.** To examine regeneration, adults of *N. vectensis* were manipulated with partial and complete bisections at three locations along the primary body axis: (1) immediately aboral of the pharynx, (2) midway along the primary axis in the region containing the mesenteries, and (3) midway within the physa, aboral to all mesenteries. One hundred animals were completely bisected at each of these locations. The resulting portions were observed until regeneration was complete. Fifty animals were cut 50% through the body column at each of these locations

and similarly observed until regeneration was complete.

# Results

#### Sexual reproduction

Nematostella vectensis is a dioecious species. No evidence of sex reversal has been documented. We have maintained single-sex populations in the lab for >2 years. Oocytes developed in small outpockets of the macronemes (thicker strands of mesentery tissue). Oocytes were pooled with nematosomes (small spheres composed of nematocysts and flagellated cells, Williams 1979) into a gelatinous egg mass (Fig. 1A–C). The egg mass was then released from the female's mouth. The jelly is produced by secretory cells of the mesenteries. Fertilization is presumably external.

Cleavage in embryos of N. vectensis was erratic and varied both within a single clutch and within an embryo (Fig. 1D,E). The zygote's initial cleavages were typically radial and holoblastic, but the timing was not always synchronized. During early cleavage, we observed many embryos with odd numbers of blastomeres (e.g., three and five cells). In such embryos, cytokinesis may have been delayed relative to karyokinesis, or blastomeres may have undergone fusion. In many cases, we observed that these oddnumbered embryos are the result of delayed cleavage after karyokinesis (Fig. 1D). These odd-numbered embryos progressed through development with no apparent adverse effects on later stages. The blastula was spherical, hollow, and uniformly ciliated. Gastrulation occurred through unipolar ingression at the site of the future oral end (Fig. 1F,G). At the gastrula stage, clear ectodermal and endodermal germ layers were evident (Fig. 1H).

After ~2 d, planula larvae emerged from the gelatinous mass. The planulae were free swimming for 5–10 d, although larvae are typically observed at the bottom of culture dishes with little movement unless disturbed. The early planula was roughly spherical with a diameter of ~ 200  $\mu$ m. The future-adult aboral end of the planula was marked by a prominent apical tuft of cilia (Fig. 2A). As the planula continued to develop, it elongated and assumed a pyramidal shape while losing the apical tuft (Fig. 2B,C). At this stage, longer cilia surrounded the future adult oral end. Before settling, planulae developed two to four tentacle buds at the future oral end (Fig. 2D). A mouth, mesenteries, and a rudimentary but functional pharynx had usually formed by the time settlement occurred.



**Fig. 1.** Stages in the sexual development in *Nematostella vectensis*, from egg mass to gastrula. **A.** Egg mass. **B.** Ovum. **C.** Nematosomes. **D.** Embryo with asynchronous second cleavage; arrows indicate two nuclei in one blastomere. **E.** Same embryo as in D after delayed cytokinesis. **F.** Light micrograph of gastrulating embryo (asterisk indicates blastopore). **G.** SEM of gastrulating embryo. **H.** Two stages in gastrulation where the embryo on the left is at an earlier stage before qthe ectoderm (ecto) and endoderm (endo) abut one another (asterisk indicates blastopore). Scale bars: A = 2.5 mm; B, D-H = 50 µm; C = 10 µm.

At the time of settlement, the juvenile polyp possessed four tentacles and measured  $\sim 400-500 \,\mu\text{m}$ along its primary (oral-aboral) axis (Fig. 2E). After settlement, the polyp elongated and formed 8–16 additional tentacles. Mesenteries, epithelial lamellae derived from the endodermal gastrodermis, developed along the inner surface of the body wall, extending from the pharyngeal region through the body column and terminating short of the physa. Mesentery formation, typically initiated



**Fig. 2.** Stages of sexual development from planula to metamorphosed juvenile in *Nematostella vectensis*. **A.** Early planula with a prominent apical tuft (at) of cilia. **B.** Later-stage pyramid-shaped planula that has lost the apical tuft but has extensive ciliation around the mouth (m). **C.** Aboral view of planula in **B** showing loss of apical tuft by this stage. **D.** Planula–juvenile transition with four tentacle buds. **E.** Early polyp with mouth (m), mesenteries (mes), pharynx (p), and tentacles. Scale bars =  $50 \,\mu$ m.

before settlement, resulted in two or four mesenteries that were clearly visible at the juvenile polyp stage.

# Asexual reproduction via transverse fission

Asexual reproduction in *N. vectensis* occurred via two distinct forms of transverse fission. We refer to these distinct types as physal pinching and polarity reversal.

Physal pinching. During physal pinching, a constriction arose in the aboral portion of the body column, resulting in the formation of a small, isolated aboral compartment (Fig. 3A,B). At the site of the constriction, the animal cleaved in two, leaving a visible fission scar on both the larger oral fragment and the smaller aboral fragment (Fig. 3C). Depending on the location of the constriction, this isolated aboral compartment frequently contained portions of one or more parent mesenteries. The number of partial mesenteries in the physal fragment varied from none to eight, with the modal category being four (Fig. 4). After separation, the aboral fragment then developed eight complete mesenteries, a pharynx, a mouth, and 12-20 tentacles (Fig. 3D). These structures did not develop in a strict sequence, e.g., the pharynx appeared before or after the tentacles emerged.

The number of partial mesenteries present in the physal fragment after pinching did not appear to affect the subsequent course of development. We observed a non-significant but negative correlation between the number of partial mesenteries enclosed within the physal fragment and the timing of tentacle formation (r = 0.166, p = 0.06, Fig. 5A). Tentacle buds typically developed 3–4d post-fission. When tentacles first developed in the physal fission fragment, the number varied from 3–12. The number of tentacle buds at the onset of tentacle eruption also did not correlate with the number of partial mesenteries in the physal fragment (r = 0.01, p = 0.513, Fig. 5B).

**Polarity reversal.** In a second type of transverse fission, polarity reversal, a new oral crown developed at the site of the existing physa. This form of asexual reproduction has been described previously as a developmental anomaly (Frank & Bleakney 1978). However, polarity reversal merits designation as a second form of asexual propagation because the anemones undergo a predictable constellation of developmental events; these events are triggered endogenously, and the eventual result is clonal reproduction—two offspring from a single parent individual. The first morphological manifestations of polarity reversal were the eruption of new tentacles and the formation of a novel mouth and pharynx at

the aboral pole. The relative order of tentacle eruption and mouth formation varies. The existing mesenteries then expanded into this second oral region, eventually joining the newly formed pharynx. At this point, the mesenteries were continuous from one end of the animal to the other (Fig. 3E,F). The second oral region developed a functional crown of tentacles and was able to feed. The two oral ends behave independently in both feeding as well as more general behaviors like tentacle retraction and response to tactile stimuli. Eventually, the midportion of the mesenteries degenerated as a common physa forms in the midsection of the adult (Fig. 3G,H). Weeks or even months after the mesenteries degenerated, a constriction formed in the common physa and transverse fission occurred, producing two complete adult polyps.

# Regeneration

Following complete bisection, regeneration typically produced two complete adults with a normal phenotype regardless of cut position (Fig. 6). Regeneration was similar to transverse fission via physal pinching in that missing structures were reproduced following a bisection of the adult body column. However, physal pinching was preceded by a pronounced endogenously triggered constriction where regeneration commenced with the wounding of the animal by an external factor.

Depending on the nature of the injury, regeneration also generated anemones with multiple oral or physal ends. Individuals of *N. vectensis* were occasionally observed in field collections and laboratory cultures with multiple oral regions on opposite sides of the body column (Fig. 7A). As discussed above, this phenotype will arise via asexual reproduction by polarity reversal. However, we have also observed that this phenotype arose following bisection immediately proximal to the pharynx (Fig. 6). Such a transverse section through the body column in the pharyngeal region generated a two-oral-ended anemone in 8% of individuals studied.

Regeneration following a focal injury to the body column, one that failed to bisect the animal, also produced Y-shaped animals with two oral crowns. In 4% of cases, a local injury to the body wall immediately proximal to the pharynx generated a Y-shaped individual with two complete and functional oral regions, sharing a single physa. Individuals with this phenotype have been commonly collected at low frequencies from field populations (A.M. Reitzel, unpubl. data) At the site of the injury, a lateral outgrowth developed (Fig. 7B), and one or two of



**Fig. 3.** Two processes for asexual reproduction in *Nematostella vectensis*: physal pinching (A–D) and polarity reversal (E–H). **A.** Initial stage of tissue constriction at site transverse fission (cz, constriction zone). **B.** Later stage in tissue constriction where a small filament of tissue remains between oral and physal ends (cz, constriction zone). **C.** Newly "pinched" physal end with remaining tissue termed fission scar (fs). **D.** Developing physal end with regenerating tentacles (rt). **E.** Individual that has undergone polarity reversal, resulting in two oral ends on opposite sides of body column. **F.** Magnified view of the central portion of the individual in E showing that mesenteries are continuous between individual oral regions. **G.** Later stage of polarity reversal where mesenteries have receded centrally, resulting in two separate sets of mesenteries, one for each oral region. **H.** Close-up of the individual in G showing the separation of mesenteries.



**Fig. 4.** Frequency histogram of individuals resulting from physal pinching with parent mesenteries. The highest percentage of individuals were observed with four parent mesenteries, although we observed individuals containing from zero to all eight parent mesenteries.

the adult mesenteries expanded into the outgrowth. Tentacles and pharynx developed at the distal tip of the outgrowth, appearing roughly simultaneously. The number of initial tentacles varied two to six. Within  $\sim$ 2 weeks, the second oral region developed the same number of tentacles and mesenteries as the original oral region (Fig. 7C,D).

Y-shaped individuals with two physae connected to a single oral crown (Fig. 7E) were only generated through incomplete bisection in the midcolumn of the adult (4%, Fig. 6). As in the Y-shaped individuals with two oral crowns discussed above, the animal initiated lateral outgrowth at the site of injury, but a second physa formed rather than a second oral region. Unlike the Y-shaped double oral individuals discussed above, which form only when the injury occurs immediately proximal to the pharynx, Yshaped double physa anemones formed at the same frequency when the injury occurred within the midportion of the body column. Focal injuries in the aboral region of the body column never resulted in a Y-shaped animal. Injuries at the extreme oral end always resulted in tissue healing.

In general laboratory cultures, two individuals were observed that contained one oral region positioned between two physa, one at each end of the primary body axis (Fig. 7F). The phenotype was not observed in any of the experimental cuts and it is not known how these individuals develop to this phenotype. These two individuals were observed from bowls of laboratory animals that had undergone successive transverse cuts. This phenotype is clearly dysfunctional as the animals were not able to ingest food. We suggest that this may represent a true



**Fig. 5.** Relationships of parent mesenteries with two measures of differentiation: **A.** days to tentacle bud eruption and **B.** number of initial tentacles. There was a non-significant negative correlation between number of parent mesenteries and days to tentacles erupting (r = 0.166, p = 0.06). Secondly, there was no correlation (r = 0.01, p = 0.513) between the number of parent mesenteries and the number of initial tentacle eruption in fissioned individuals.

case of a developmental anomaly. Both individuals remained in this state for months with no fission. In one case, the individual began to fission one physa but never completely removed the physa. The individual expelled tentacles from the partially fissioned end but was unable to feed when nauplii were introduced.

#### Discussion

Our goal in this study was to describe more fully the complex developmental repertoire in *Nematostella vectensis* and to document the sequences of developmental events that comprise each developmental trajectory. A better understanding of these developmental sequences at the organismal level is a



Fig. 6. Relationship of cut location and phenotypic outcome from 100 adults of Nematostella vectensis. Adults were cut either partially or completely at three different locations along the oral-aboral axis. The left column shows results for complete bisections. The right column displays results for localized injuries ( $\sim 50\%$ bisections). Percentage values in the left and right columns display the frequency of individuals observed with the represented phenotype. Wild-type adults indicate that no alternate phenotypes were observed after regeneration. For complete bisections, both resulting anemones were examined throughout the regenerative process. With the exception of bisection near the oral regions, all individuals regenerated normally. The result shown for the oral bisections indicates the percentage of oral fragments resulting in double anemones. The aboral portions of orally bisected adults regenerated normally.

prerequisite to future molecular and genomic studies comparing alternate developmental trajectories in *N. vectensis* and comparing this species' developmental repertoire with that of other Cnidaria. Such studies will allow us to understand how the evolution of developmental mechanisms might underlie the diversity of cnidarian life-history strategies.

Overall, embryogenesis in N. vectensis appears to be typical for an anthozoan (Byrum & Martindale 2004). Our observations of sexual reproduction and embryogenesis in N. vectensis reinforce the specific findings of Wilcox (2001). During oogenesis, each gonadal cyst on the macronemes contains up to seven oocytes that develop asynchronously such that variable sizes and stages occur within a single cyst (Wilcox 2001). Interestingly, no ovulating cysts expel more than one mature oocyte, leaving open the question as to whether oocytes accumulate yolk through oocyte fusion in N. vectensis, similar to some Hydrozoans including Hydra (Aizenschtadt 1980; Miller et al. 2000). Embryos in N. vectensis exhibit regulative development through at least the third cleavage. Single blastomeres from the eight-cell embryo retain the capacity to generate viable larvae that successfully metamorphose into juvenile polyps (Wilcox 2001).

With respect to asexual reproduction, individuals of N. vectensis do not appear to be typical for an anthozoan cnidarian. Transverse fission is not a common mode of asexual reproduction among anthozoans. Only a handful of anthozoan species utilize transverse fission for asexual reproduction (Fautin 2002): ceriantharians (Cerfontaine 1909), some solitary scleractinians (Cairns 1988), one zoanthidean (Sphenopus marsupialis GMELIN 1827: Soong et al. 1999), and a few actinarians (Shick 1991; Hand & Uhlinger 1995; Pearse 2001). Both forms of asexual reproduction that have been observed in N. vectensis-physal pinching and polarity reversal-involve transverse fission, but these two modes of reproduction are markedly different with respect to the sequence of developmental events and the axial relationship of the offspring. Tentacle development occurs at the outset of polarity reversal but at the end of physal pinching. In physal pinching, both of the offspring inherit the original primary body axis, but in polarity reversal, a new primary body axis must develop that is opposite to the original primary body axis. Transverse fission in the sea anemone Anthopleura stellula EHRENBERG 1834 begins with the formation of tentacles, but unlike polarity reversal in N. vectensis, the tentacles form in the middle of the body



**Fig. 7.** Unique phenotypes of *Nematostella vectensis* resulting from atypical regeneration and local injury. **A.** Individual with two oral regions on opposing sides of the body column resulting from development of oral structures after bisection. **B.** Developing oral region at the site of local injury to the central portion of the body column. **C.** Y-shaped individual with two oral regions, the smaller of which developed from local injury similar to that seen in **B. D.** Transmission light image of the individual from C showing the arrangement of mesenteries in oral regions. **E.** Y-shaped individual with two physal (p) regions. **F.** Individual with two physal regions (p) on opposite sides of the body column with an oral (o) region central.

column and so there is no reversal of the primary body axis (Schmidt 1970).

Asexual reproduction in actiniarians is typically by longitudinal fission or pedal laceration (Chia 1976; Fautin 2002). However, transverse fission may be the rule rather than the exception in the family Edwardsiidae. In addition to N. vectensis, transverse fission has also been described in the edwardsiid anemone Edwardsiella lineata VERRILL 1873 by Crowell & Oates (1980). The sequence of developmental events is similar to physal pinching in N. vectensis; however, unlike N. vectensis, the small aboral fission fragment produces all 16 tentacle buds simultaneously in E. lineata. Recently, we have observed that the parasitic stage in E. lineata, which resides in the host ctenophore (Mnemiopsis leidyi AGASSIZ 1865), also undergoes asexual reproduction via physal pinching (A.M. Reitzel, unpubl. data). In addition, we have noted that settling juveniles will undergo a polarity reversal (A.M. Reitzel, unpubl. data), suggesting that this mode of asexual reproduction may also be more common in edwardsiid anemones than suspected. Other members of the family Edwardsiidae are reported to achieve extremely high adult densities (e.g., Edwardsia meridionalis WILLIAMS 1981: 23,500 m<sup>-</sup> Williams 1981; *Edwardsia* sp.:  $1200 \text{ m}^{-2}$ , Kumar 2001). Such extreme densities could be the result of local recruitment of larvae, but high densities in populations of N. vectensis  $(12,572 \text{ m}^{-2}, \text{Williams 1983})$ have been recorded in isolated single-sex populations, indicating that asexual reproduction may contribute to the establishment of dense populations in other edwardsiid anemones.

Environmental influences on asexual reproduction in N. vectensis have received very little study. Twoheaded individuals have been reported to exhibit seasonal peaks in abundance in a population from Kingsport, Nova Scotia, with peak occurrence in the autumn (Frank & Bleakney 1978). The seasonality of polarity reversal suggests that appropriate environmental cues may be required to induce this otherwise rare form of asexual reproduction. Standard laboratory culture conditions (Hand & Uhlinger 1992) may be inappropriate for inducing polarity reversal. However, genetic factors may also explain the relatively high rate of polarity reversal in the Nova Scotia populations of N. vectensis. We have collected hundreds of individuals from 32 sites throughout the range of N. vectensis, and the Kingsport, Nova Scotia population is the only site with a high frequency of two-headed individuals (collection in 2004, eight of 81 individuals), despite the apparently similar environments inhabited by other populations. The frequency of asexual reproduction is likely to be influenced by food availability. With respect to the more common mode of asexual reproduction, physal pinching, Hand & Uhlinger (1995) report that particular feeding regimens can increase the rate of fission. We have induced physal pinching through excessive feeding of adults, although the reliability of this method depends on the population, suggesting, again, that genetic factors may influence the frequency of asexual reproduction.

Nematostella vectensis is one of a few sea anemones that are known to exhibit multiple modes of asexual reproduction. In the genus Anthopleura, different modes of clonal reproduction appear to have been lost and regained repeatedly (Geller & Walton 2001). Boloceroidid sea anemones also display a vast array of asexual reproduction processes, including tentacular shedding, longitudinal fission, and pedal laceration (Pearse 2001). Similarly, studies by Minasian (1976) and Atoda (1973) have shown that different populations of the anemone Haliplanella luciae VER-RILL 1898 exhibit different modes of asexual reproduction (most populations have only longitudinal fission while one Japanese population utilizes pedal laceration), potentially from an ancestral condition of having both. If modes of clonal reproduction are highly plastic evolutionarily, then the two distinct mechanisms of asexual reproduction in N. vectensis might be maintained by natural selection.

The selection pressures that might favor two types of transverse fission in N. vectensis are unknown. There appear to be obvious trade-offs between the two strategies, although the ecological significance of these trade-offs has not been studied. Physal pinching generates a small, relatively inexpensive propagule that must regenerate all of its oral structures before it is able to feed on its own. This strategy would cost relatively little for the "parent" individual (the larger oral fission fragment), which does not lose much biomass and whose ability to feed is never interrupted. A large number of propagules may be produced relatively rapidly. However, the survival of the propagules is likely to be low because of their small size and relatively prolonged development. The physal fragment must develop for up to a week before it is able to begin feeding, and when it achieves adulthood, it remains relatively small compared with the parent individual, potentially limiting the type of prey it can ingest. Conversely, asexual propagation via polarity reversal initially results in an adult with two functional oral ends that are both of similar size. Before undergoing fission, this two-headed individual can presumably ingest twice as many prey. At the same time, burrowing ability must be impaired, and the two-headed animal may be effectively epifaunal,

not infaunal, potentially subjecting the individual to greater predation or movement via tidal currents. When two-headed individuals undergo fission, the resulting progeny are equivalently large and capable of feeding on larger prey. With their larger body size, they may also be better prepared to withstand prolonged starvation. Further studies in the field and laboratory could address the potential trade-offs inherent in each reproductive strategy.

In response to a wide range of bisection or point injuries, individuals of N. vectensis typically regenerate the wild-type adult phenotype. However, at low frequencies, in response to injuries at particular locales, phenotypes comprising multiple oral regions or multiple physal regions did develop. These results imply that the adult animals maintain an oral-aboral gradient for axis determination. Previous studies have revealed a complex molecular gradient of developmental regulatory gene expression that persists throughout larval development and likely patterns the oral-aboral axis (Finnerty et al. 2004; Kusserow et al. 2005). At present, we have little information on the extent of developmental regulatory gene expression in adults of N. vectensis and how these genes are expressed during regeneration events (P.M. Burton, unpubl. data).

However, numerous studies have characterized gene expression in adult Hydra during steady-state morphogenesis, regeneration, and budding (e.g., Martinez et al. 1997; Broun et al. 1999; Smith et al. 1999; Hobmayer et al. 2000; Scholz & Technau 2003). In adults of Hydra sp., oral and aboral cell fates are continually specified via the expression of various developmental regulatory genes and small peptides (reviewed by Steele 2002). Many of the same developmental regulatory genes known to be active in adults of Hydra have been studied in embryogenesis in N. vectensis. However, relatively little is known about expression in adults of N. vectensis, and it is unclear whether gene expression patterns observed in adults of Hydra sp. would be similar to gene expression patterns in adults of N. vectensis because the cellular dynamics are so different. In Hydra, the epithelial cells of the central body column are constantly undergoing cell division, even as the animal maintains a constant body size. Presumptive cells for the oral crown are displaced orally and presumptive pedal cells are displaced aborally. In N. vectensis, mitotically active cells are not concentrated in the central body column but are dispersed throughout the animal, so that presumptive oral cells and presumptive physa cells would develop in situ (M.Q. Martindale, pers. comm.). This fundamental difference in the cellular dynamics should be manifest at the level of gene expression. It is perhaps telling that several genes that are known to be expressed during regeneration, fission, and embryogenesis in N. *vectensis* do not appear to be expressed in non-fissioning, non-regenerating adult animals (P.M. Burton, unpubl. data).

Because every model system exhibits inherent peculiarities, a general understanding of the developmental mechanisms that underlie regenerative ability will require comparisons among numerous taxa (Sánchez Alvarado 2000; Collins et al. 2005). Only by comparing multiple systems that exhibit complex developmental repertoires, such as in N. vectensis, and by contrasting them with more developmentally rigid taxa, will we come to understand how multiple developmental trajectories may be encoded by individual genomes. With the emergence of more invertebrate systems where regeneration can be experimentally addressed (e.g., Pristina leidyi, Bely 1999; Bely & Wray 2001; Schmidtea mediterranea BENAZZI 1975, Sánchez Alvarado 2004), further research may provide for conclusions as to generality, if any, of regenerative processes.

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