Rising starlet: the starlet sea anemone, *Nematostella vectensis*

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Summary

In recent years, a handful of model systems from the basal metazoan phylum Cnidaria have emerged to challenge long-held views on the evolution of animal complexity. The most-recent, and in many ways most-promising addition to this group is the starlet sea anemone, *Nematostella vectensis*. The remarkable amenability of this species to laboratory manipulation has already made it a productive system for exploring cnidarian development, and a proliferation of molecular and genomic tools, including the currently ongoing *Nematostella* genome project, further enhances the promise of this species. In addition, the facility with which *Nematostella* populations can be investigated within their natural ecological context suggests that this model may be profitably expanded to address important questions in molecular and evolutionary ecology. In this review, we explore the traits that make *Nematostella* exceptionally attractive as a model organism, summarize recent research demonstrating the utility of *Nematostella* in several different contexts, and highlight a number of developments likely to further increase that utility in the near future. *BioEssays* 27:211–221, 2005. © 2005 Wiley Periodicals, Inc.

Introduction

Model systems research was the dominant paradigm of twentieth century biology, and its importance is unlikely to wane in the foreseeable future. However, the dawn of genomics and the flourishing of interdisciplinary approaches to biological systems (evo-devo, eco-devo,(1) evolutionary and ecological functional genomics,(2) etc.), has prompted researchers to adopt a more integrative approach to choosing model organisms. Where the model organisms of the past were chosen primarily for their convenience to researchers in one particular discipline, the model organisms of tomorrow will be selected for their ability to address questions that cut across the boundaries of traditional disciplines, integrating molecular, organismal and ecological studies. A premium will also be placed on choosing model systems for their phylogenetic informativeness, so that they might serve as a complement to existing model systems in reconstructing evolutionary history.

One recent reflection of this strategic shift is the growing interest in outgroups to the Bilateria. If we are to understand the origin of developmental processes and genetic architecture that underlie the diversity and complexity of Bilaterian animals, then we must understand the ancestral Bilaterian condition, and this, in turn, requires an appropriate outgroup.(3–6) In response to this challenge, many researchers have turned to the Cnidaria, a basal eumetazoan lineage comprising corals, sea anemones, jellyfish and hydroids.(7,8) The starlet sea anemone *Nematostella vectensis* is one of a number of informative cnidarian model systems, including *Acropora*,(6) *Eleutheria*,(9) *Hydra*,(10) *Hydractinia*(5) and *Podocoryne*,(11) that have already yielded important insights into early metazoan evolution.

Here we outline some of the most-appealing characteristics of *Nematostella* as a model organism, and review the important evolutionary insights that have already been garnered through investigation of this species. We also suggest some promising avenues of further development, and indicate ways in which *Nematostella* can be promoted as a useful model for addressing a number of fundamental biological questions. The summary presented here provides tangible confirmation of a prediction made by Hand and Uhlinger in 1992, that "this sea anemone has the potential to become an important model for research in cnidarian biology."(12)

Why *Nematostella*?

A number of established cnidarian model systems (e.g. *Acropora*,(6) *Eleutheria*,(9) *Hydra*,(10) *Hydractinia*(5) and *Podocoryne*(11)) have provided important general insights into developmental evolution, and have also been employed to address specific biological problems such as pattern formation

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and self-organization,[13,14] coloniality and alloimmunity,[5] and transdifferentiation of striated muscle tissue.[11,15] *Nematostella* is a valuable addition to these existing cnidarian models, not so much for its special utility in addressing a particular biological question, but rather for its general utility for studying embryogenesis and larval development. Furthermore, *Nematostella* is particularly amenable to interdisciplinary approaches because it combines exceptional laboratory convenience with easy access to natural populations in the field (Fig. 1). Finally, this species resides in a phylogenetic position relative to the other cnidarian models that renders it particularly informative about evolution within the phylum.

Ease of culture

Nematostella is exceptionally easy to culture in the laboratory.[12,16] Adult anemones can be maintained indefinitely in very little space and at very little expense. *Nematostella* will thrive and reproduce prolifically in unfiltered, non-circulating, dilute artificial seawater (~12 ppt) maintained at room temperature. Weekly water changes and semi-weekly feedings are sufficient to keep the cultures in a healthy and reproductive state. Routine long-term maintenance of adult anemones (e.g. to preserve living genetic stocks) requires even less frequent (i.e. monthly) water changes and feedings.[12] Furthermore, populations can be maintained at extremely high densities without apparent adverse effects.

Reproductive and developmental plasticity

Nematostella is capable of reproducing via sexual and asexual means (Fig. 2), and it will readily undergo its complete reproductive and developmental cycle under laboratory conditions. The species is dioecious and fertilization is external. The female releases a gelatinous egg mass containing tens to hundreds of eggs. The eggs are large (~200 μm), and they are easily manipulated after the gelatinous coat is removed (by washing in 2% cysteine[8]). The adults remain reproductive

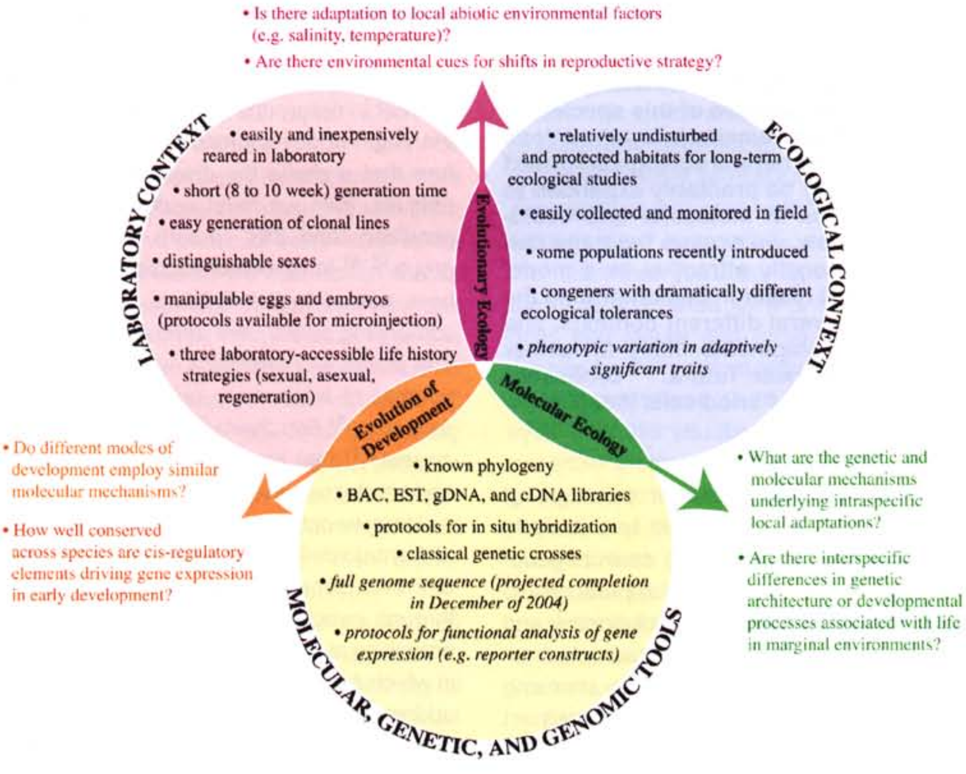

Figure 1. *Nematostella vectensis* as a model system. *Nematostella* possesses a number of characteristics that make it attractive as a model system for a variety of research questions, ranging from dissection of developmental pathways and comparative evolutionary genomics to investigation of ecologically relevant phenotypic traits. *Nematostella's* physiology and life history make it particularly well suited for laboratory manipulation (red circle), its ecological context makes it amenable to field monitoring and population-level studies (blue circle), and the growing availability of molecular and genomic tools (yellow circle) have encouraged a small but expanding research community. At the intersection of these three contexts of accessibility lie important interdisciplinary research questions of broad biological relevance (representatives examples indicated here in purple, green, and orange), many of which cannot be addressed adequately from within each context independently. Items listed in italics are being developed, but are not currently available (note scheduled completion date for full genome sequence). A *Nematostella* BAC library is available at http://bacpac.chori.org/library.php?id=219. More detailed information on the development, evolution and ecology of *Nematostella* may be found at http://www.nematostella.org.

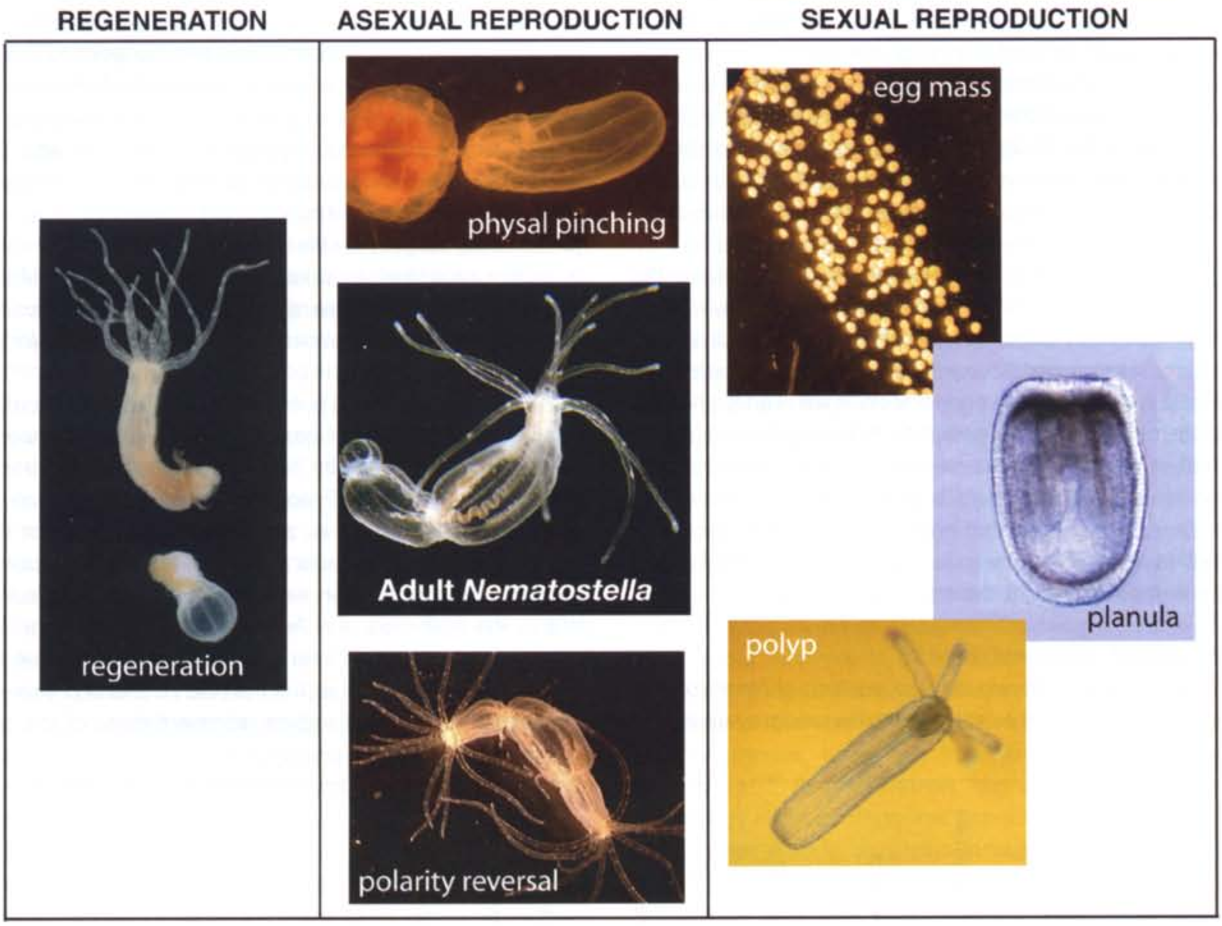

Figure 2. Life history of *Nematostella vectensis*. Regeneration subsequent to injury (left) represents a potential mode of passive asexual reproduction, but it is unknown what role this process may play in natural populations. The primary mode of active asexual reproduction is through transverse fission: contraction in the body column ultimately results in separation of the pedal end of the animal, which then regenerates head structures (top middle). Alternatively (but less frequently), asexual reproduction may occur through "polarity reversals", in which a new head forms, typically at the pedal end of the anemone, and the animal divides at its midpoint and regenerates new pedal structures (bottom middle). Sexual reproduction (right) begins with external fertilization of eggs extruded in large jelly masses (top). Eggs develop into swimming planulae (middle), which settle and form juvenile polyps (bottom). Development from fertilized egg to sexual maturity takes roughly 2–3 months.

throughout the year in laboratory culture, spawning reliably at a frequency of roughly once per week.(17) Approximately two days after successful fertilization, the ciliated planula larva emerges from the gelatinous egg mass. It spends 7–10 days engaged in active swimming. Soon after the first four tentacles have emerged, the larva settles and continues its development into an adult polyp. Unlike many cnidarian larvae, settlement of *Nematostella* planulae is not dependent on any external cue, nor do they require a substratum.(18) The relatively rapid generation time of 2–3 months renders classical genetic analysis feasible.(12)

Among cnidarians, *Nematostella* is remarkable for the ease of studying sexual reproduction.(19) Of the prominent cnidarian model organisms other than *Nematostella*, only *Hydractinia* presents a widely accessible laboratory model for the study of embryogenesis and early development.(5) In laboratory cultures of *Hydra*, sexual reproduction occurs rarely and unpredictably. Individual fertilized embryos spend an indeterminate amount of time in a brood pouch located on the body column of the female. These factors complicate studies of early development and preclude classical genetic studies.(20) The study of *Acropora* embryogenesis is rendered difficult by the inability to reliably spawn this species in the laboratory.(6) Progress in understanding its embryogenesis has relied on annual collections of fertilized eggs during mass spawnings in the Great Barrier Reef.

Asexual reproduction in *Nematostella* occurs via transverse fission (Fig. 2).(21) In the more common mode of transverse fission (physal pinching), the animal undergoes a pronounced circumferential constriction in the aboral portion of the body column, pinching off the distal section of the physa (or foot). In a matter of days, this aboral fragment regenerates the missing body column and oral structures to form a new adult. Far less commonly, a novel head forms at the site of the existing foot (polarity reversal). The central body column then elongates, and a novel foot region forms midway between the two heads. A circumferential constriction in this newly formed foot region then separates two completely formed individuals.

Besides sexual and asexual reproduction, *Nematostella* has extensive capacity for regeneration. It will readily undergo complete bidirectional regeneration following bisection: the oral half will regenerate a new foot and the aboral half will regenerate a new head. The efficiency of regeneration allows clonal lines to be propagated easily by transversely bisecting anemones and allowing them to recover. Regenerated adults can be re-cut within days, depending on their size.

Phylogenetic relationships

From a phylogenetic standpoint, the addition of *Nematostella* to the small collection of existing cnidarian model systems is a strategic advance that will enable better reconstruction of key ancestors within the phylum (Fig. 3). *Nematostella* is a member of the order Actinaria (sea anemones) and the class Anthozoa. Multiple lines of evidence place the Anthozoa as the sister taxon to the Medusozoa, a clade comprising the three other Cnidarian classes: Hydrozoa, Scyphozoa and Cubozoa.(22,23) The major organismal trait that delineates the Medusozoa from the Anthozoa is the possession of a jellyfish (or medusa) stage in the life history of the former. The medusa is widely regarded as a unique invention of the Medusozoa.(24,25) If the medusa was derived in the Medusozoan ancestor, then the Anthozoan life history typified by *Nematostella*, consisting of only the polyp stage, is more representative of the primitive cnidarian condition.(22,23) Although uncertainty persists in the dating of early evolutionary divergences, the Anthozoa are thought by some to have diverged from the Medusozoa by the late Precambrian, some 575 million years ago.(26) If this dating is accurate, then the most-recent common ancestor of *Hydra* and *Nematostella* likely predated the most-recent common ancestor of humans and fruit flies. Within the Anthozoa, the Actinaria diverged from the Scleractinia (hard corals) no later than the mid-Triassic, some 225 million years ago.(27) The fact that the Actinaria preserve the ancestral solitary habit makes representatives of this group,

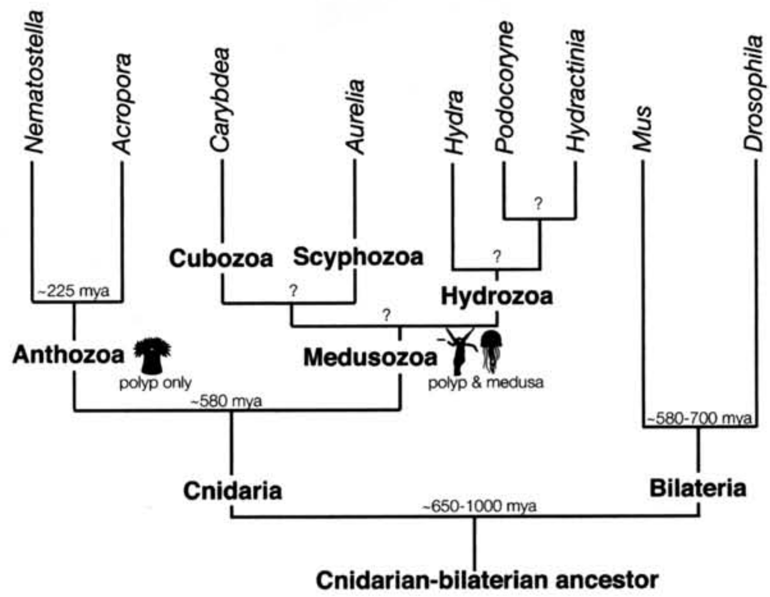

Figure 3. Phylogenetic relationships among cnidarian model systems. Estimated divergence times are given in millions of years before the present.(26,77–79) A great deal of uncertainty surrounds these estimates. The Cnidaria are the probable sister group to the Bilateria. The Cnidarian-Bilaterian divergence occurred perhaps as early as one billion years ago. *Nematostella* belongs to the class Anthozoa (sea anemones, corals, sea pens, etc). The Anthozoa is the sister group to the Medusozoa, a clade that comprises three different classes: Hydrozoa (hydromedusae, hydras, etc), Scyphozoa (true jellyfishes), and Cubozoa (box jellyfishes or sea wasps). Estimated divergence times within the Medusozoa have not been reported in the literature. Representative model systems are shown for each class of Cnidaria. To this point, the class Hydrozoa has received far more study than any of the other classes. Currently, no particular Cubozoan species has emerged as a leading laboratory model system. *Mus* and *Drosophila* are shown as representative Bilaterian models.

including *Nematostella*, important additions to the currently available Anthozoan models (e.g., colonial corals such as *Acropora*). Data from multiple Anthozoa (e.g. *Nematostella*, *Acropora*, and ideally others) and multiple Medusozoa (e.g. *Hydra, Eleutheria, Hydractinia* and *Podocoryne*, and ideally others) are required if we hope to reconstruct with confidence the conditions that existed in key ancestors such as the ancestral Anthozoan, the ancestral Medusozoan and the ancestral Cnidarian. Reliable inferences about the ancestral Cnidarian are critical if we intend to leverage the phylum's status as an outgroup to the Bilateria to root our analyses of Bilaterian evolution.

Ease and relevance of field studies

In the wild, *Nematostella* inhabits salt marshes, saline lagoons and other estuarine habitats.(28) These sites are typically accessible from the landward margin. Thus, it is possible to study *Nematostella* in its natural surroundings without watercraft or specialized equipment. Depending on the topography of a particular site and the local population density, a single researcher on foot may collect hundreds or thousands of *Nematostella* in the span of a few hours. Furthermore, the estuarine habitats inhabited by *Nematostella* are of abiding ecological interest due to their high level of primary productivity, their importance as a breeding ground or nursery for commercially important species, and their proximity to major human population centers.

Nematostella is widely distributed along the Atlantic coast of North America, throughout temperate latitudes from Nova Scotia to the Gulf of Mexico. *Nematostella* is also found along the Pacific coast of the United States and the southeastern coast of England.(28) It may represent a recent introduction in both of these outlying locales.(28–31) The physiological characteristics that make *Nematostella* an ideal organism for laboratory culture (broad thermal tolerance, broad salinity tolerance and starvation tolerance) represent adaptations to the highly variable conditions encountered in coastal estuaries. These same broad environmental tolerances, coupled with the capacity for clonal propagation, may pre-adapt this species for successful colonizations of novel habitat. *Nematostella* is generally abundant in North America, and it may even be acquired commercially through the Marine Biological Laboratories at Woods Hole.(32) The species is relatively rare in England, and it has been listed as a protected species. Samples of English populations are correspondingly more difficult to secure.(31)

***Nematostella* as a tool for discovery—realized and anticipated findings**

Recent years have seen growing interest in *Nematostella*, primarily as a representative cnidarian for the investigation of developmental pathways. These studies have generated an impressive array of molecular tools (Fig. 1), and have helped to solidify, along with research on other cnidarian models, important insights into the developmental evolution of early animals. The sequencing of the *Nematostella* genome will open an important new era in evolutionary genomics, allowing the first genome-wide comparisons between Bilaterian and non-Bilaterian animals. Future studies will apply molecular and genomic tools to address significant questions in the molecular and evolutionary ecology of *Nematostella*.

Insights from comparative molecular studies of development

To date, molecular studies of cnidarian development have emphasized a candidate gene approach, characterizing cnidarian homologs of genes with conserved developmental functions in the Bilateria (Fig. 3).(8,9,33–48) Collectively, these studies have revealed unexpected complexity in the genomic regulatory systems and developmental patterning mechanisms of cnidarians, blurring the boundary between the supposedly "simple" Cnidaria and the "complex" Bilateria. Some traits widely regarded as Bilaterian inventions may actually predate the split between Cnidaria and Bilateria. One compelling example is bilateral symmetry, the hallmark of Bilateria. Despite the textbook characterization of cnidarians as radially symmetrical animals, many cnidarians, including sea anemones, have long been known to exhibit bilateral symmetry.(49) In the Bilateria, the development of bilateral symmetry requires staggered expression of *Hox* genes along the primary body axis (the anterior–posterior axis) and asymmetric expression of the TGF-β gene *dpp* along the secondary axis (the dorsal–ventral axis). Comparable expression of these genes in *Nematostella* implies a conserved role in the development of bilateral symmetry. The expression domains of five *Hox* genes subdivide the *Nematostella* bodyplan into distinct regions along the primary body axis during embryogenesis and larval development.(40) *Dpp* is expressed asymmetrically about the second body axis, the directive axis, which runs orthogonal to the primary body axis.(40) Asymmetric *dpp* expression was observed both in the embryo, along the margin of the blastopore, and in the larva, in the ectodermal lining of the pharynx. The embryonic expression of *dpp* in *Nematostella* resembles the embryonic expression of *dpp* in the coral *Acropora*, results that had suggested a possible ancestral role for *dpp* in axis formation.(50) The larval expression of *dpp* has not been reported in *Acropora*. The comparable *Hox* and *dpp* expression seen in *Nematostella* and Bilateria supports the hypothesis that bilateral symmetry evolved before the divergence of Cnidaria and Bilateria.(51)

Another important evolutionary innovation ascribed to Bilaterian animals is triploblasty, the possession of three organized tissue layers: endoderm, ectoderm and mesoderm. *Nematostella* exhibits diploblasty, possessing only two tissue layers: endoderm and ectoderm. However, *Nematostella* does possess homologs for many genes that are critical for proper specification and patterning of mesodermal cell fates in Bilaterians, including orthologs of *forkhead, snail, twist* and *brachyury*.(35,39) In *Nematostella*, these "mesodermal genes" are expressed predominantly in the endoderm or presumptive endoderm, indicating a role in germ-layer specification and supporting the hypothesis that both the mesoderm and endoderm of Bilaterians are derived from the endoderm of a diploblastic ancestor.(52)

The availability of molecular and genomic tools coupled with the accessibility of *Nematostella* development to laboratory manipulation make possible functional assays of developmental regulatory genes. Recently, the spatial and temporal dynamics of the *β-catenin* protein were monitored in living *Nematostella* by means of a fusion protein combining *Nematostella β-catenin* with green fluorescent protein.(38) This study identified a conserved role for the nuclear localization of β-catenin protein in the development of the embryonic axis and the specification of germ layers. Future functional studies in *Nematostella* will undoubtedly employ techniques for misexpressing genes, for silencing gene expression, and for tracking gene regulation via reporter constructs.

Gene Family	Gene	Bilaterian Homolog (*Dm, Hs/Vert*)	Bilaterian Toolkit Function	Putative Cnidarian Function	Cnidbase Accession #	Reference / Accession #
Homeobox						
HOX	*anthox1*	*abdB, Hox10*	AP Patterning	AP Patterning	Cns13	40
	anthox1a	*abdB, Hox10*	AP Patterning	AP Patterning	Cns18	40
	anthox6	*labial, Hox 1*	AP Patterning	AP Patterning	Cns9	40
	anthox7	*proboscipedia, Hox 2*	AP Patterning	AP Patterning	Cns21	40
	anthox8	*proboscipedia, Hox 2*	AP Patterning	AP Patterning	Cns19	40
ParaHox	*anthox2*	*ind, gsx*	AP Patterning	AP Patterning	Cns28	8
Extended Hox	*antheve*	*even-skipped, Evx*	AP, neural	?	Cns25	U42733
	mox1	*buttonless, mox*	Mesoderm	?	Cns183	AY339866
	mox2				Cns184	AY339867
Paired	*paxB*	*eyeless, pax6*	Eye	?	Cns185	AY339873
	otx	*otd, otx*	AP Patterning	?	Cns186	AY465181
NK	*hbxA*	*Nkx6.1, nk6*		?	Cns32	U42735
	hbxD				Cns26	AF020964
	Nk2	*Tinman, nk2.3*	Heart, Mesoderm	?	Cns12	AY339869
	Nk4				Cns187	AY339871
	Nk1	*Nk1, sax1*		?	Cns188	AY339868
Zinc Finger						
	snail A	*Worniu, snail-1*	Mesoderm	Gastrulation, Endoderm	Cns180	35
	snail B				Cns181	
	GATA	*Grain, GATA1,2,3*	Mesoderm	Endoderm	Cns177	35
TGFβ						
	dpp	*dpp, BMP2,4*	DV patterning	Directive Axis	Cns173	40
	GDF5-like	*GDF5?*	DV patterning	Directive Axis	Cns174	40
OTHERS						
Winged helix	*forkhead*	*forkhead, FOXA2*	Mesoderm	Gastrulation, Oral Endoderm	Cns176	35
bHLH	*twist*	*twist, TWIST1*	Mesoderm	Endoderm	Cns182	35
MADS box	*mef2*	*mef2*	Mesoderm	Oral Ectoderm, Tentacles	Cns178	35
LIM box	*muscle lim*	*Mlp60A/84A, CSRP1-3*	Mesoderm	Endoderm, Tentacles	Cns179	35
T-box	*bra*	*brachyury*	Gastrulation		Cns164	37
	beta-catenin	*beta-catenin*	Wnt path	Polarity, germ layer	Cns162	38
COE	*COE*	*COE*	Neural dev	Chemoreception	Cns175	81

Figure 4. *Nematostella vectensis* developmental regulatory genes. The identification of orthologs of certain developmental regulatory genes across Bilateria led Carroll et al.(53) to propose that all Bilateria possess a common toolkit of developmental regulatory genes. These genes are critical for proper patterning and specification during embryogenesis. The recent identification of orthologs of many of these genes in *Nematostella* implies that this toolkit is ancestral to both Cnidaria and Bilateria. Here we list all developmental regulatory genes identified to date in *Nematostella*. When known, their proposed function in *Nematostella* is listed. Genes are listed generally as "homologs" to indicate uncertainty in the precise assignment of orthology in many cases. Expression patterns are available through Cnidbase (www.cnidbase.org). References are provided when available, otherwise genbank accession numbers are listed. *Dm, Drosophila melanogaster, Hs/Vert, Homo sapiens*/vertebrate; AP, anterior/posterior; DV, dorsal/ventral.

The large number of developmental regulatory genes with comparable expression patterns in Cnidaria and Bilateria suggests that a substantial 'genetic toolkit' existed in the cnidarian-Bilaterian ancestor (Fig. 4).(53) This toolkit represents the shared ancestral legacy upon which two subsequent, independent evolutionary radiations were built. These two putative sister lineages experienced radically different evolutionary fates. The cnidarian radiation was relatively modest, giving rise to two major body plans (polyp and medusa) and some ten thousand extant species. The Bilaterian radiation was far more extensive, giving rise to more than thirty distinct body plans and at least two million extant species (with some estimates placing the number well over ten million). The Cnidaria may be especially informative if indeed cnidarian development has remained relatively unchanged since the late Precambrian some 570 million years ago, as suggested by fossil evidence.(26) If cnidarians have experienced relative developmental and morphological stasis, they may provide unparalleled insights into the genomic regulatory systems of Precambrian metazoans.

The Phylum Cnidaria is a valuable model for developmental biology beyond its status as an outgroup to the Bilateria. Cnidarians provide unique insights into the study of developmental mechanisms since these animals may undergo development in four different contexts: (1) embryogenesis and larval development following sexual reproduction, (2) various modes of asexual reproduction (fission, budding, laceration, etc.), (3) regeneration, and (4) steady-state patterning and morphogenesis in adult animals, which has been well characterized in *Hydra*.(54) The mechanistic similarities and differences among these different developmental contexts are not well understood. Gene expression assays can indicate whether a given gene is performing a conserved developmental role in different developmental contexts and, fortunately, it is extremely easy to compare gene expression during embryogenesis, regeneration and transverse fission in *Nematostella* (Fig. 5). Future research will reveal the extent to which these different developmental contexts employ homologous developmental mechanisms, and how the remarkable developmental flexibility of cnidarians is encoded in the genome.

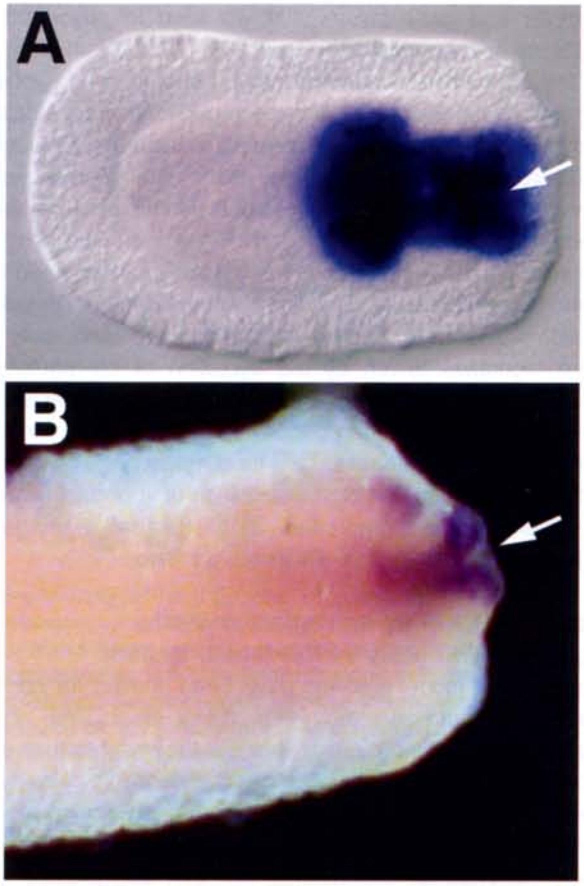

Figure 5. Comparing gene expression by in situ hybridization during embryogenesis and regeneration. The site of the future mouth is indicated by an arrow in both frames. **A:** The *forkhead* transcript is restricted to cells in the region of the blastopore that give rise to the oral endoderm during *Nematostella* embryogenesis.(80) **B:** During regeneration, *forkhead* expression is also restricted to the cells that will give rise to the oral endoderm (Burton, Krone, and Finnerty, unpublished data). The photo depicts the oral portion of a pedal fragment that is in the process of regenerating a head. The fragment comes from an anemone bisected below the pharynx, through the trailing edge of the mesenteries. The animal was fixed approximately 24 hours after the bisection was performed.

Insights from comparative genomics

The genome of *Nematostella* should be sequenced in its entirety near the end of 2004 (Dan Rokshar, Joint Genome Institute, personal communication). The value of a complete Cnidarian genome is foreshadowed by recent cnidarian EST projects, which have revealed that representatives from most gene families and most signaling pathways utilized in Bilateria were present in the common ancestor of Cnidaria and Bilateria.(55–59) These studies have forced us to re-conceive of the Cnidarian–Bilaterian ancestor as an animal of unexpected genomic complexity. Many genes formerly regarded as vertebrate inventions due to their apparent absence from the genomes of *Drosophila melanogaster* and *Caenorhabditis elegans* have been identified in cnidarian genomes, pushing their origin back hundreds of millions of years to the Cnidarian–Bilaterian ancestor.(58) Furthermore, in many cases, vertebrate transcripts show more sequence similarity to cnidarian sequences than to *Drosophila* and/or *C. elegans* sequences.(58) Unpublished analyses of *Nematostella* ESTs in our own laboratory corroborate these findings (J.F.R., M.E.M. and J.R.F., unpublished data). These comparative genomic data cement the realization that the fruit fly and nematode genomes have undergone rapid evolution, including sequence changes and gene losses that will tend to obscure the existence of vertebrate orthologs.

The *Nematostella* genome project should shed light on genomic changes underpinning the remarkable morphological and ecological radiation of Bilaterian metazoans. Cnidarian EST projects and molecular developmental studies have revealed extensive commonalities between the gene complements of cnidarians and Bilaterians. Thus, the origin of novel genes and signaling pathways are not likely to have been the predominant forces driving Bilaterian innovations. Possibly far more important was an increase in the complexity of gene regulation driven by the evolution of *cis*-regulatory modules.(60–64) The *Nematostella* genome appears relatively compact among cnidarian genomes, with estimates ranging from 225–320 megabases.(65,66) Its compact genome will aid in the identification and characterization of important regulatory elements and give clues as to the nature of gene regulation in the cnidarian–Bilaterian ancestor.

Nematostella in its ecological context: expanding the utility of the model system

Most current model systems have been chosen for convenience of laboratory study (e.g. direct life cycles, short generation times etc.), rather than for the insights they can provide into ecological function or adaptation.(2,67) However, the genetic and genomic tools developed for leading model systems (e.g. *Drosophila* and *Arabidopsis*) are increasingly being exploited for the insights that they can provide into questions of ecological import.(68,69) The amenability of *Nematostella* to both field studies and laboratory manipulation (Fig. 1) makes this species particularly well suited to investigating adaptation at multiple levels. Because we can characterize genetic diversity and environmental variability in the field, and we can characterize developmental mechanisms at both organismal and molecular levels in the laboratory, we can investigate how ecology and development interact to affect fitness. In other words, we can study "development in the real world."(1)

Nematostella is a particularly interesting organism in which to study the interplay of ecology and development because it is developmentally plastic, and it routinely encounters a tremendous range of environmental variation. *Nematostella* has been reported at salinities ranging from 2 to 52 parts per thousand,(31) and temperatures ranging from 1.5° to 32.5°C.(70,71) In addition, *Nematostella* is known to inhabit both pristine and highly polluted habitats. A recent study in our laboratory has cataloged a substantial amount of intraspecific genetic diversity in just a fraction of the *Nematostella's* wide geographic range, and we are maintaining living genetic stocks representing all wild caught populations.(29) With this resource in hand, it will be possible to investigate heritable differences in many traits with adaptive significance including salinity tolerance, temperature tolerance and pollution tolerance. For example, the range of *Nematostella* extends across a dramatic thermal cline, and it is likely that natural populations occupying extremes of this range may exhibit metabolic differences in phenotype similar to those observed in other species.(72)

Intraspecific studies on *Nematostella* can be complemented by potentially very informative comparisons with closely related outgroups. *Nematostella* is a member of the family Edwardsiidae, a monophyletic clade of burrowing anemones with global distribution.(73) Where *Nematostella* is a euryhaline, eurythermal, estuarine species, the other members of the family, including its congeners, *N. nathorstii* and *N. polaris*, are primarily arctic species living in marine environments.(74,75) A recent phylogenetic analysis of the family Edwardsiidae suggests that the broad tolerances of *Nematostella* are evolutionarily derived, and along with a benthic egg mass and a predominantly demersal larval stage, these represent adaptations to a novel estuarine habitat.(73) The availability of closely related species with dramatically more limited habitat requirements raises the possibility of comparative studies aimed at determining the genetic and molecular mechanisms underlying transitions to novel habitats.

One area in which *Nematostella* is particularly likely to provide insight is in the role of reproductive plasticity in shaping population structure. The early literature on *Nematostella* is replete with observations of reproductive plasticity at the organismal and physiological level, including studies of the environmental variables influencing reproductive decisions, and tantalizing hints at individual-level variation in rates of asexual reproduction.(12,21,76) More recently, population-level studies have demonstrated that reproductive plasticity plays an important role in shaping regional genetic structures. Analysis of English populations of *Nematostella* have revealed the presence of a widely distributed clonal genotype, extending throughout the entire region and comprising over 60% of the entire English population (Fig. 6).(30) The dominance of clonal propagation in this region may not be surprising, given earlier failures to identify male anemones in England and the growing evidence suggesting that English populations may be recent introductions from the native range in the Western Atlantic.(28–31) The pattern of genetic variation within that native range differs dramatically from the pattern observed in England, with a far greater frequency of sexual reproduction and no single genotype extending over more than one site (Fig. 6).(29)

The reasons for the unusual genotype distribution in English populations are currently unclear. It may be that extreme founder events have resulted in drastic reductions in diversity in introduced English populations. Alternatively, there may be some adaptive advantage enjoyed by the dominant English genotype. What *is* clear is that the ability to both monitor *Nematostella* populations in their natural ecological

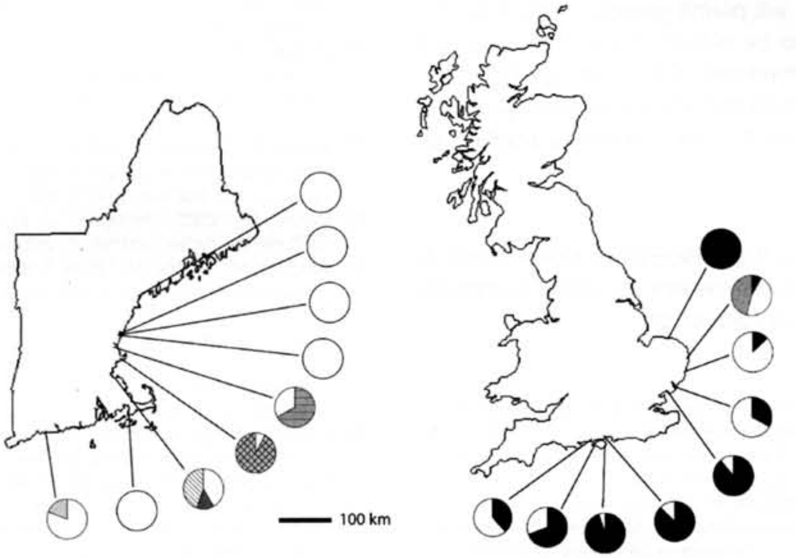

Figure 6. Frequency of clonality in *N. vectensis* populations from New England, US (left) and England, UK (right). Pie charts indicate proportion of individual animals derived by clonal reproduction. White areas represent sums of all individual genotypes comprising less than 1% of the total sample. All shaded and hatched areas are clonal genotypes. In New England, no single genotype appears in more than one population, and the highest frequency genotype (cross-hatched) makes up approximately 13% of the total population. In England, a single widely distributed genotype (black) was found in 60% of all individuals sampled. Only one other clonal genotype was found at significant frequency in English populations (gray). Data for English populations are based on 31 randomly amplified polymorphic DNA (RAPD) markers, while those for US populations are based on 169 amplified fragment length polymorphism (AFLP) markers.(29,30) Since data were generated in two independent studies using two different kinds of molecular markers, the genetic relationship among animals from England and New England cannot directly be assessed.

context and manipulate individuals in the laboratory, in conjunction with the available molecular and genomic tools, presents a singular opportunity to investigate reproductive plasticity at the genetic, molecular, physiological, ecological and evolutionary levels. In spite of dramatic influences on individual fitness, genetic structure and evolutionary potential, reproductive plasticity has received limited mechanistic investigation due to the obvious limitations of most model systems with regard to this trait. *Nematostella* ought to allow unique access, from various perspectives, to this important biological phenomenon.

Conclusions

Nematostella has already proven its utility by providing insights into the evolution of fundamental metazoan traits such as axial patterning, bilateral symmetry and triploblasty. *Nematostella*'s central role in these discoveries, coupled with the obvious advantages of an organism whose entire life history is so amenable to exploitation in the laboratory, has driven its recognition by the broader research community and motivated the ongoing *Nematostella* genome project. The outcome of this project is certain to address a number of pressing issues regarding the evolution of genomic regulatory systems.

Still untapped is the potential of *Nematostella* to address fundamental issues of microevolutionary change relating to life history strategies and the invasion of novel habitats. Greater attention to the ecological context of this species and closely related taxa will allow the expanding list of molecular tools to be employed to address such questions. As suggested in Fig. 1, the increasingly interdisciplinary nature of biological research recommends pursuit of model systems that allow genomic, molecular and developmental studies to be placed within ecological context. Only in such cases will it be possible to conduct studies that bridge genomes with ecology and connect microevolutionary processes to macroevolutionary patterns. It is our hope that with future investment and increased interest within the research community, *Nematostella* may become the type of model organism that facilitates such important work.

In this era of comparative biology, it is widely recognized that the inferences to be gained from any model system have limited generality. Each potential model system must be considered for its unique position on the tree of life, and the likely informativeness of that position relative to established models systems. In this era of integrative biology, we also recognize that the most-important questions in biology span multiple levels in the biological hierarchy, from the molecular, to the organismal, to the environmental. Therefore, the most-valuable model systems will permit genomic, molecular and developmental studies to be placed within their real-world ecological context. *Nematostella* is a model system tailor-made for the explicitly comparative and integrative approach of modern biology. This "rising starlet" merits a position on Biology's center stage.

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