

A Starlet in the Mud:

Population Genetics, Environmental Stressors, and
Local Adaptation in the Estuarine Anemone
Nematostella vectensis



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Nematostella vectensis

Ecology

- Benthic, infaunal inhabitant of coastal estuaries, particularly in tidally restricted pools
- Easy field collection from diverse range

Utility in the Lab

Easy to culture including breeding and asexual reproduction for generation of clonal genotypes

Molecular Resources

Sequenced genome (JGI)


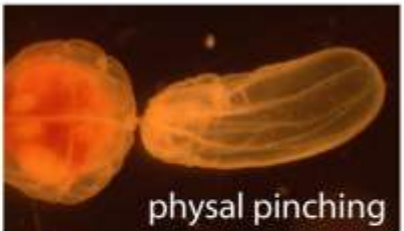

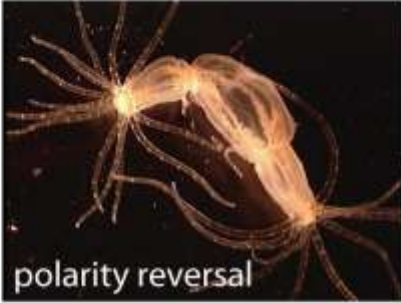

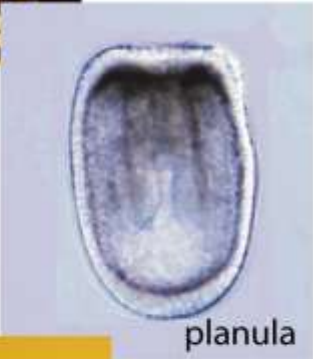
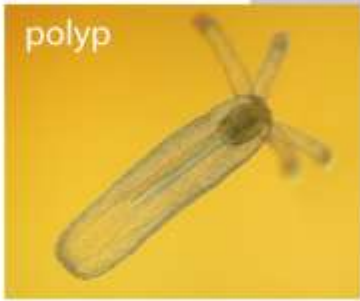
Extensive molecular tools and approaches

Evolutionary Position

Member of Cnidaria, sister group to Bilateria



Complex Life History of *Nematostella*

REGENERATION	ASEXUAL REPRODUCTION	SEXUAL REPRODUCTION
 <p>regeneration</p> <p>The image shows two stages of regeneration. The top stage is a long, thin, translucent polyp with many long, thin tentacles extending from its head. The bottom stage is a smaller, more rounded, translucent polyp with fewer tentacles, representing a regenerating individual.</p>	 <p>physal pinching</p>  <p>Adult <i>Nematostella</i></p>  <p>polarity reversal</p> <p>The top image shows a polyp with a large, rounded, orange-colored structure (the physa) at one end, which is being pinched off. The middle image shows a fully developed adult polyp with a long body and many tentacles. The bottom image shows two polyps, one of which is in the process of reversing its polarity, with the physa moving from one end to the other.</p>	 <p>egg mass</p>  <p>planula</p>  <p>polyp</p> <p>The top image shows a large, dense cluster of small, yellowish, spherical eggs. The middle image shows a single, oval-shaped, translucent planula larva with a distinct head and tail. The bottom image shows a polyp with a long, cylindrical body and several tentacles, set against a yellow background.</p>

Outline of Presentation

- I. Population genetic structure of *Nematostella*: anthropogenic introduction, limited gene flow, and the frequency of clonality
- II. Search for functional polymorphisms: identification and distribution
- III. Local adaptation and plasticity to temperature along a latitudinal cline
- IV. *Nematostella* as an environmental informant: a model for stress-response gene expression

I. Population Genetics: Global Anthropogenic Dispersal of *Nematostella vectensis*

Observation: *Nematostella*'s current range includes three disparate coastlines despite apparently limited dispersal potential



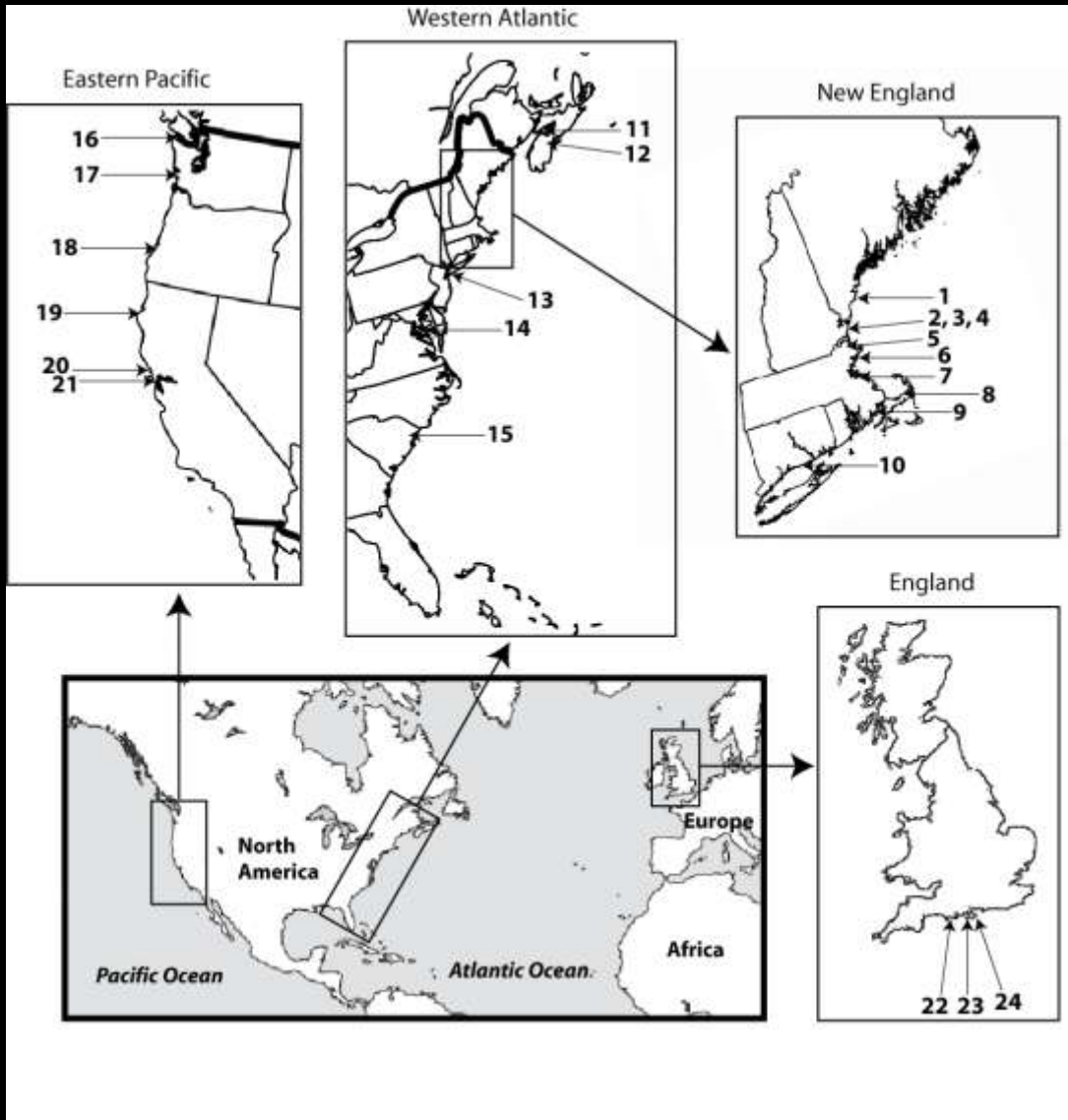
Nematostella is a 'threatened' species in England and receives protection

Hypothesis: *Nematostella* has been introduced to two of these three coasts via human-mediated dispersal

Testing for Native vs. Introduced Range

- Molecular markers provide powerful approach to identify cases of introduction in cryptogenic species
- Expectations: Introduced populations will:
 - A. Be more closely related to populations in native range than are some native populations to each other
 - B. Have reduced genetic diversity
 - C. Have higher frequency of asexual reproduction

Collections of *Nematostella*



Collected Animals

Populations from 24 separate estuaries

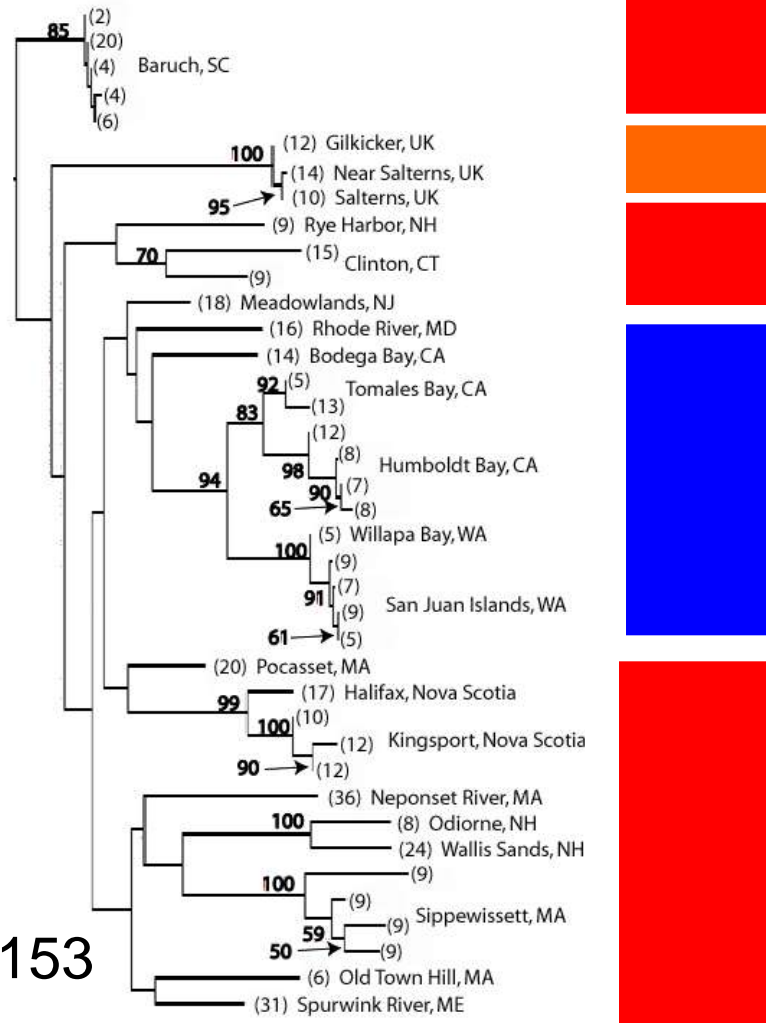
(n = 516)

Molecular Data

Generated genetic fingerprints (AFLPs) for all individuals (169 loci)



A. Populations from England and Pacific coast nest within west Atlantic



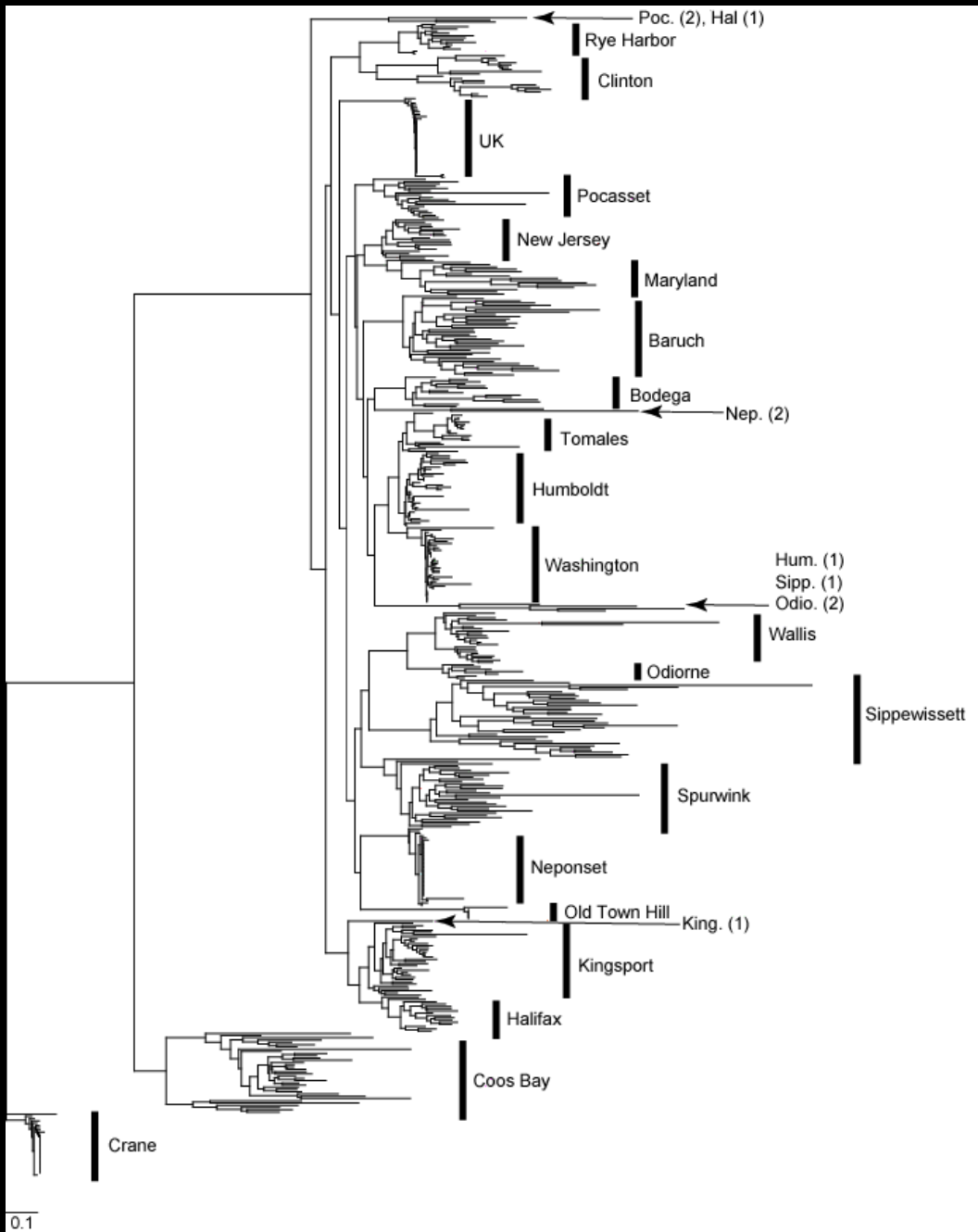
In addition, if this was a natural distribution, we would expect deep divergences among coastlines

- west Atlantic
- Pacific
- east Atlantic

N = 516

AFLP Markers = 153

0.1



98% of Individuals
Cluster With Their
Location of Origin

... suggests very
little gene flow
between estuaries,
even close ones.

B. Higher genetic diversity along west Atlantic compared with other 2 coasts

- **West Atlantic vs. US Pacific**

PPL:	(39.484 vs. 12.715)	$p < 0.0001$
H_E :	(0.139 vs. 0.0728)	$p < 0.001$

- **West Atlantic vs. England**

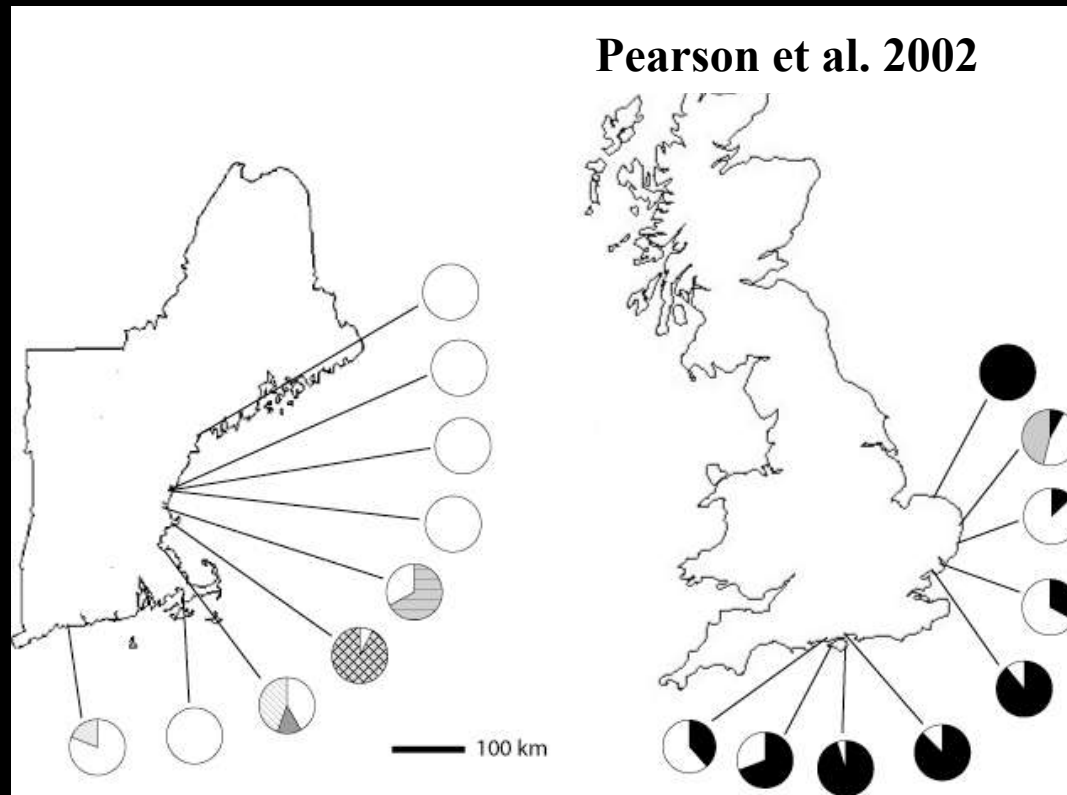
PPL:	(39.484 vs. 2.600)	$p < 0.0001$
H_E :	(0.139 vs. 0.0125)	$p < 0.0001$

PPL = percent polymorphic loci

H_E = expected heterozygosity

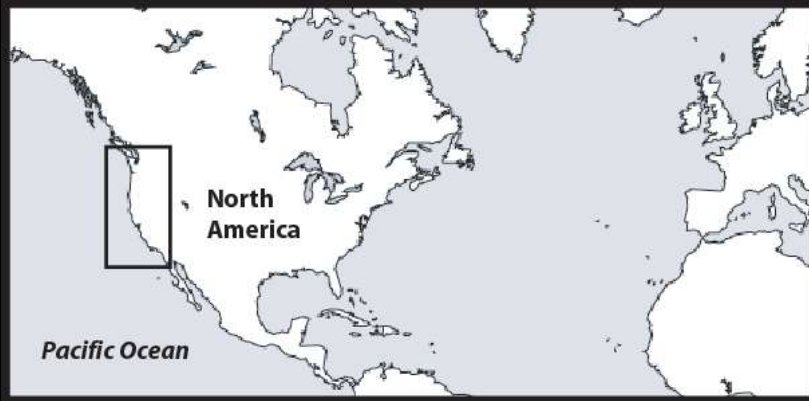
In addition, I have sequenced 6 loci and found no unique polymorphisms in US Pacific or England

C. Clonality more common in English and Pacific Populations



- In England, one genotype represented 60% of individuals and was collected at all locations
- In New England, no genotype was found in more than one location, clonal individuals only found at 4 sites, diversity high (91% polymorphic markers)

C. West Coast *Nematostella*

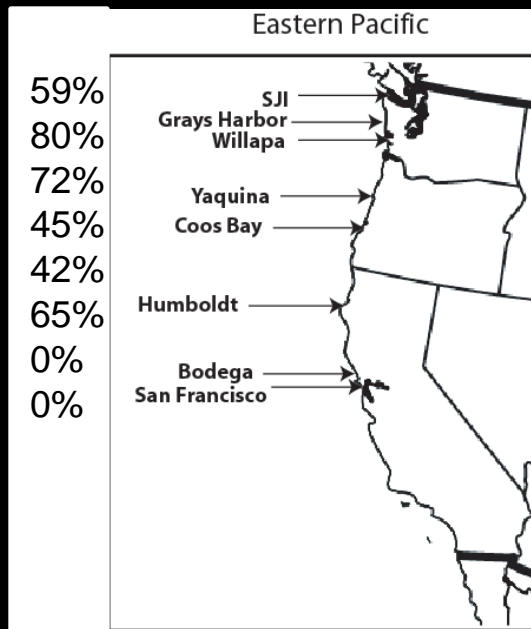
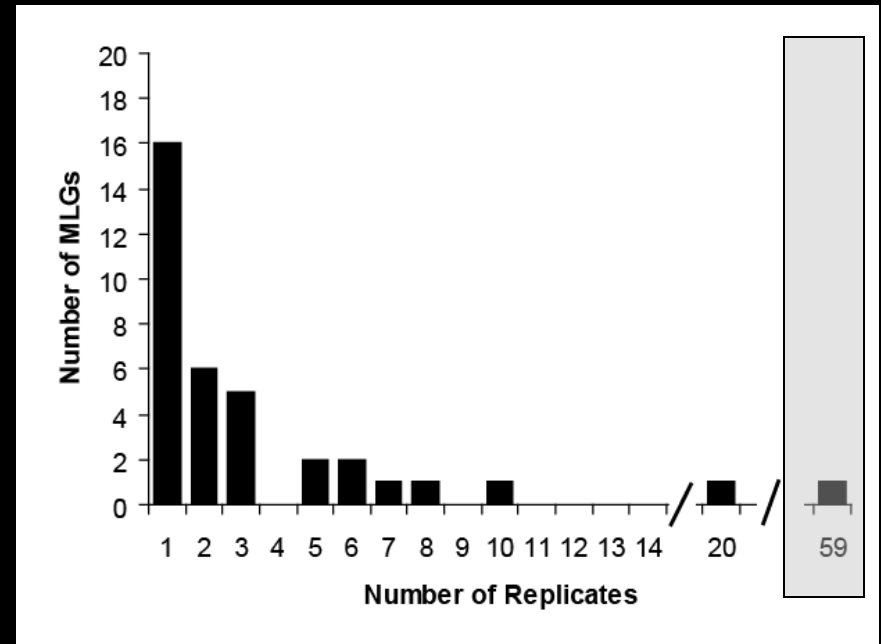
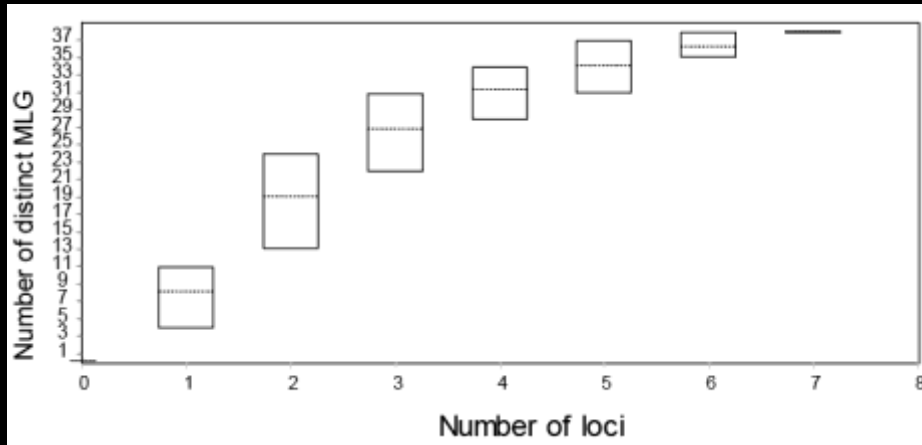


Data

- 7 microsatellite loci
- 231 individuals from 8 estuaries



Clonality: West Coast Style



Results

- Only 29 unique multi-locus genotypes (87% clonality)
- MLGs shared among distinct locations

Summary: Introduction of *Nematostella*

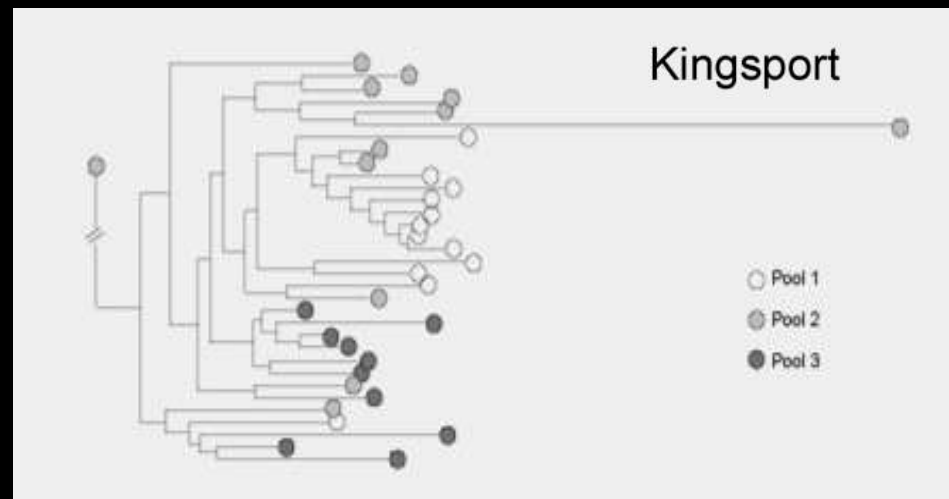
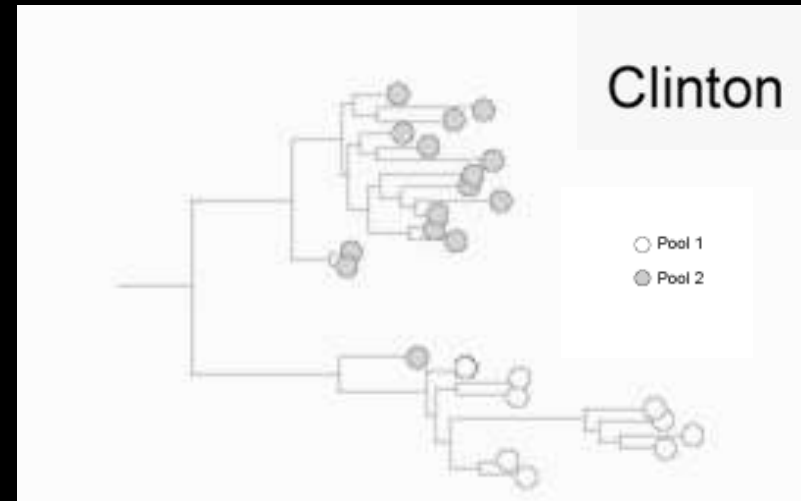
- Genetic evidence supports a hypothesis that *Nematostella* was introduced to the Pacific and to England from Atlantic coast of North America
- For the Pacific coast, two separate introductions
- Clonal reproduction is present in many populations, but more common in the England and Pacific and accounts for a majority of individuals
- Gene flow from natural dispersal appears to be very limited between estuaries

Genetic Structure within Estuaries

Significant F_{ST} values within marshes

- Clinton, CT = 0.492
- Kingsport, NS = 0.083
- Humboldt, CA = 0.142
- Sippewissett, MA = 0.205
- But not all.....
- Baruch, SC = 0.00

Clustering of Individuals From Pools



II. Spatio-temporal Distribution and Population Genetic Structure in a Single Estuary

- Observation: *Nematostella* has significant genetic structure within salt marshes suggesting limited gene flow, extinction-colonizations, and/or population bottlenecks



Hypothesis: *Nematostella* has frequent changes in distribution within pools, genetic composition of subpopulations changes little over time, and colonizations are rare due to limited gene flow

Gene Flow in 'Ecological' Time

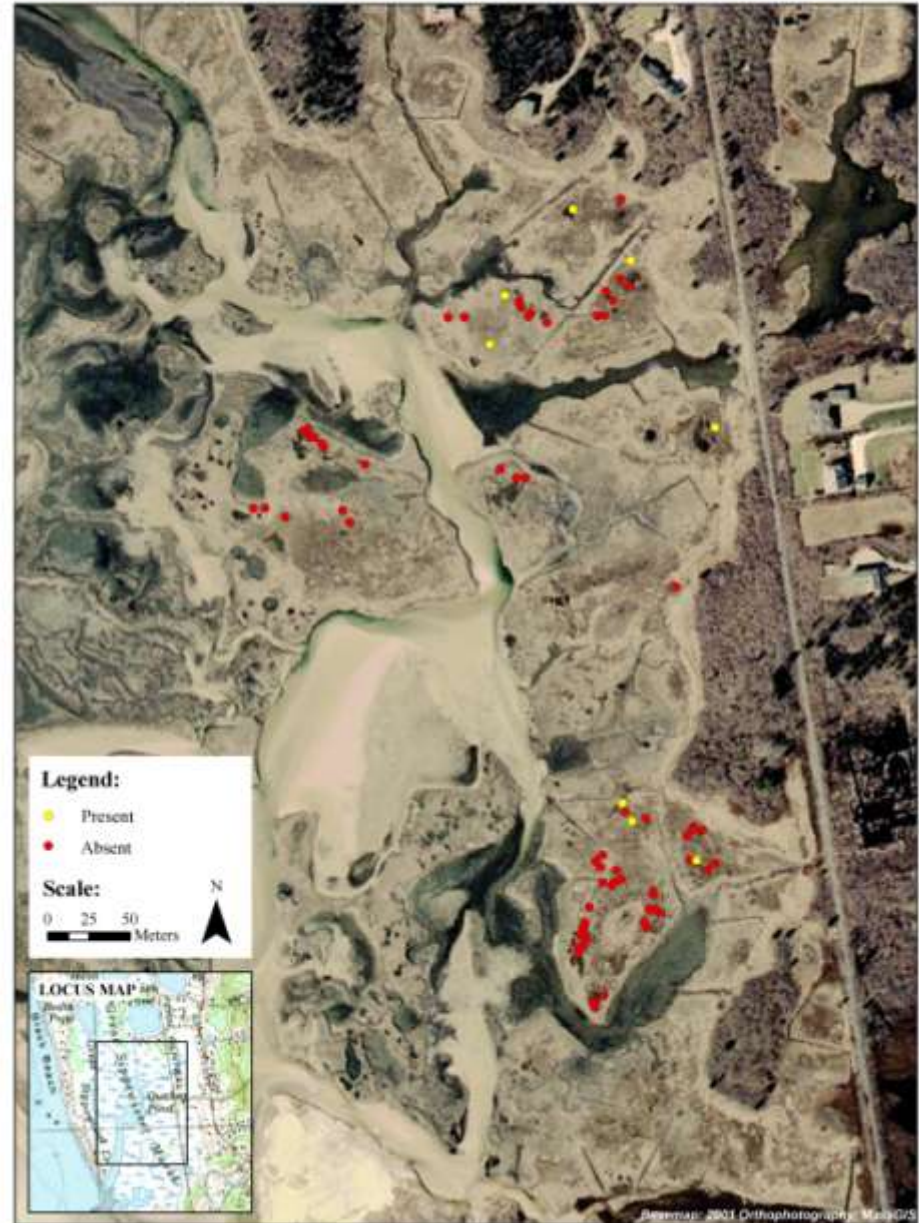
- Single estuaries appear to be the spatial scale for studying ecologically-relevant population genetic processes in *Nematostella*
- Many previous studies have shown that benthic invertebrates with little dispersal potential colonize restored salt marshes slowly (8 – 25 yrs) compared with species with pelagic larval periods (1 – 3 yrs)
- For these slower colonizers, adult dispersal can be important for establishment in novel environments

Great Sippewissett Marsh, MA

- Reasonably natural marsh with complex topology, similar to other higher latitude estuaries



Great Sippewissett Marsh, MA

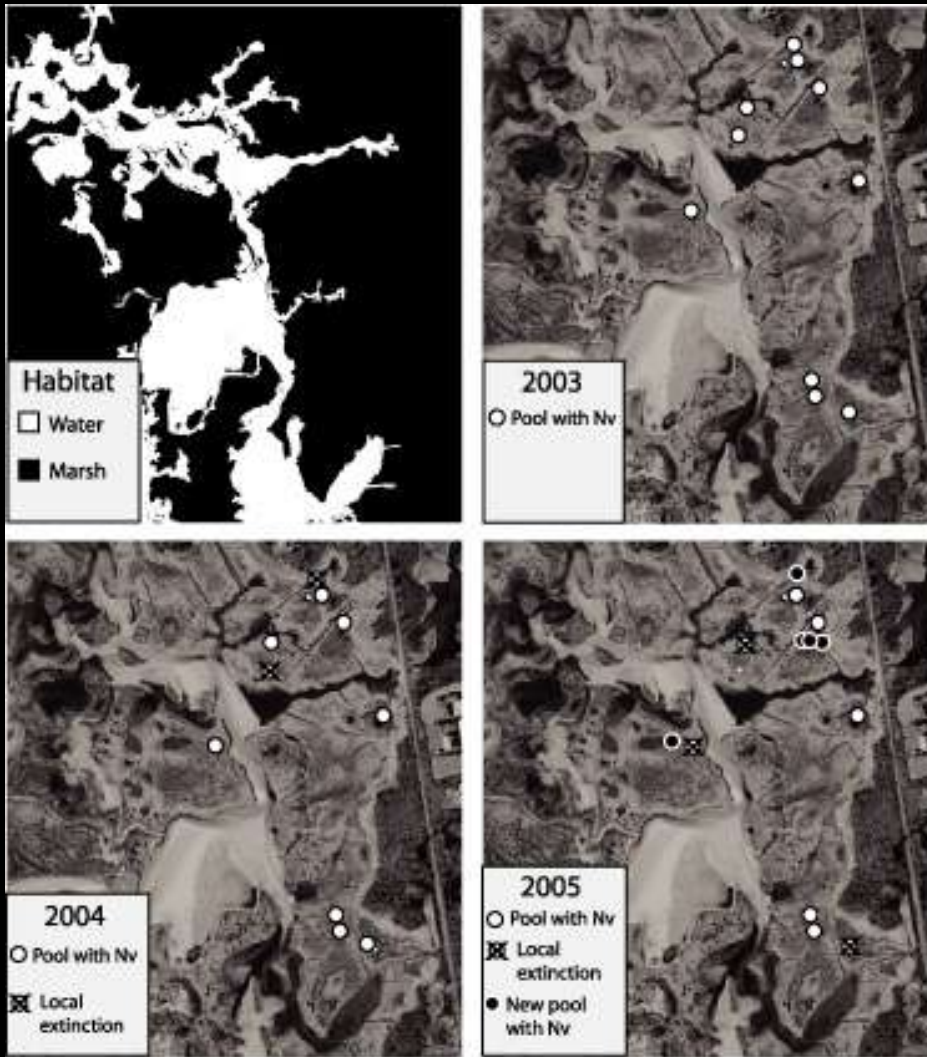


Approach

- A. Surveyed tidally restricted pools and presence/absence of *Nematostella* from 2003 – 2005

- B. Utilized 8 microsatellite loci from 282 individuals to address:
 - 1. Spatial genetic structure
 - 2. Temporal genetic structure within pools
 - 3. Characterizing colonization events
 - 4. Contribution of asexual reproduction to *Nematostella* populations in space-time

A. Habitat and Distribution of *Nematostella*



2003 to 2004

Nematostella: 2 local extinctions

2004 to 2005

Nematostella: 3 local extinctions,
5 colonizations

Over all years, *Nematostella*
occupied less than 20% of
tidally restricted pools

B1. Genetic Data and Spatial Structure.

Summary of 8 microsatellite loci for 282 individuals

- 59 alleles (range 2 – 19 per locus)
- Mean allelic diversity = 3.69 alleles per locus per population

Spatial Genetic Structure

- Overall $F_{ST} = 0.2479$ for 2005 collection
 - Similar to value from AFLPs (0.205)
 - Significant pairwise F_{ST} between most pools
- Private alleles in many pools
 - Up to 5 unique alleles found in only one pool

B2. Colonization Events

- Five pools were colonized between 2004 and 2005
 - 1) No difference in adult size
 - 2) No significant difference in genetic diversity based on the 8 microsatellite loci
 - 3) Most individuals had identical genotypes with individuals from previously established pools, usually nearest neighbor

Summary: Sippewissett study

- Habitat and *Nematostella* populations are variable over short time periods
- Colonization appears to be a relatively rare event, but when it occurs, can occur by larval or adult dispersal
- Asexual reproduction appears to be common and a significant contributor to the number of individuals at Sippewissett
- Evidence from genetic structure supporting restricted gene flow across the marsh

Factors for Limited Larval Dispersal

Potential Explanations

Potential Effect

- Benthic egg mass



Reduced dispersal period

- Largely benthic larvae



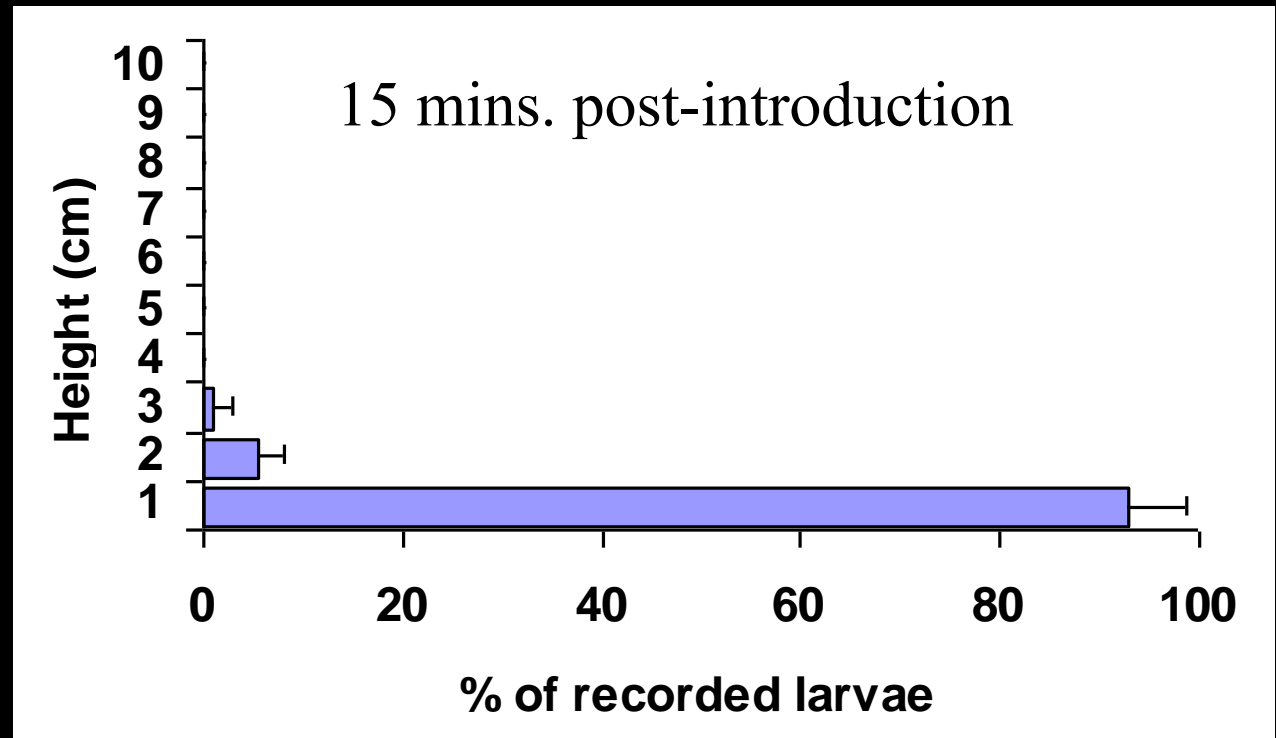
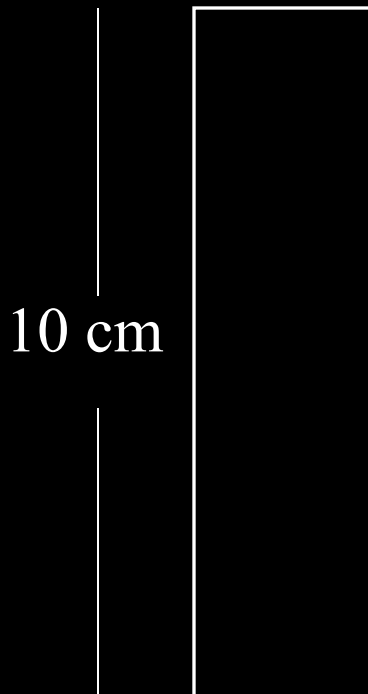
Reduced dispersal potential

- Potentially poor settlers



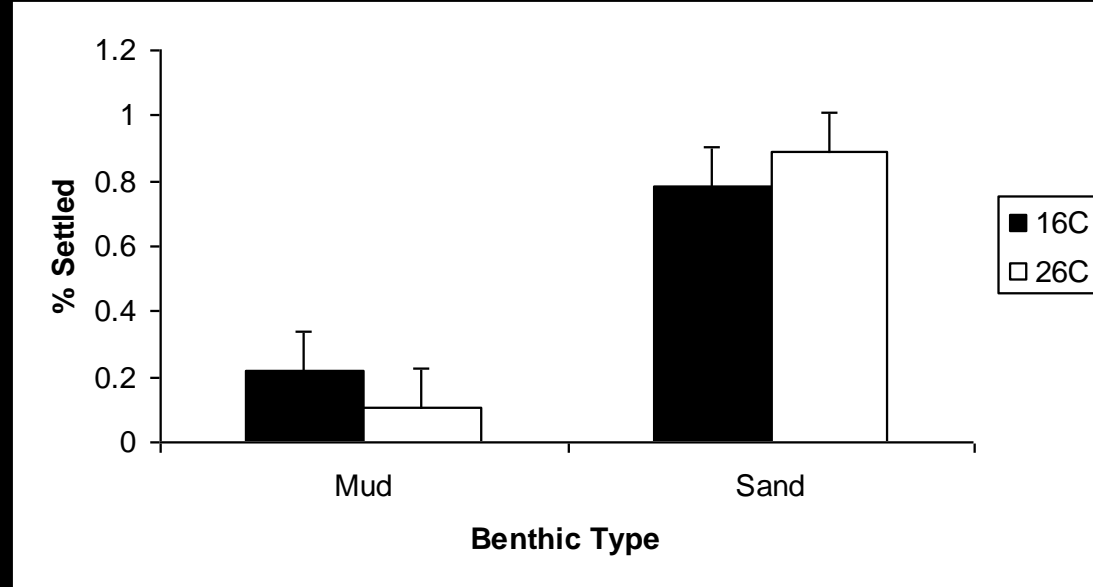
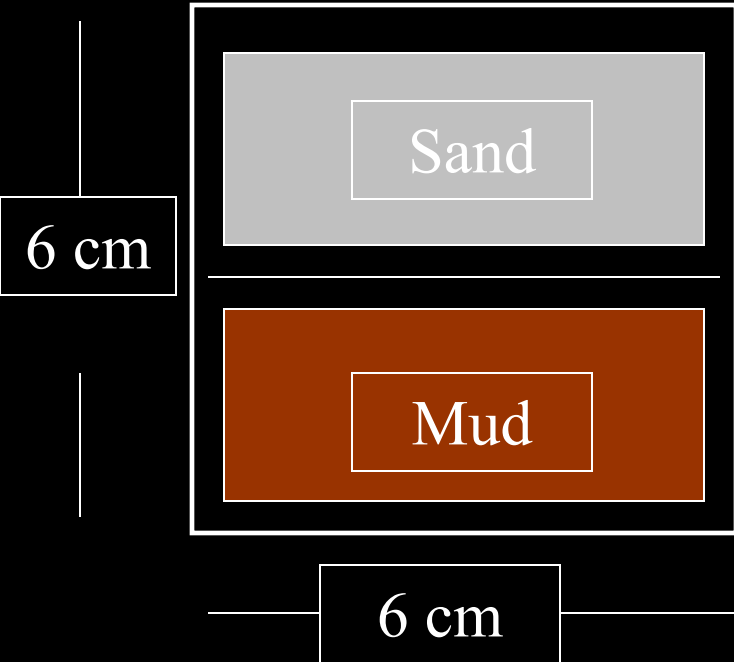
Reduced recruitment success

Vertical distribution of larvae

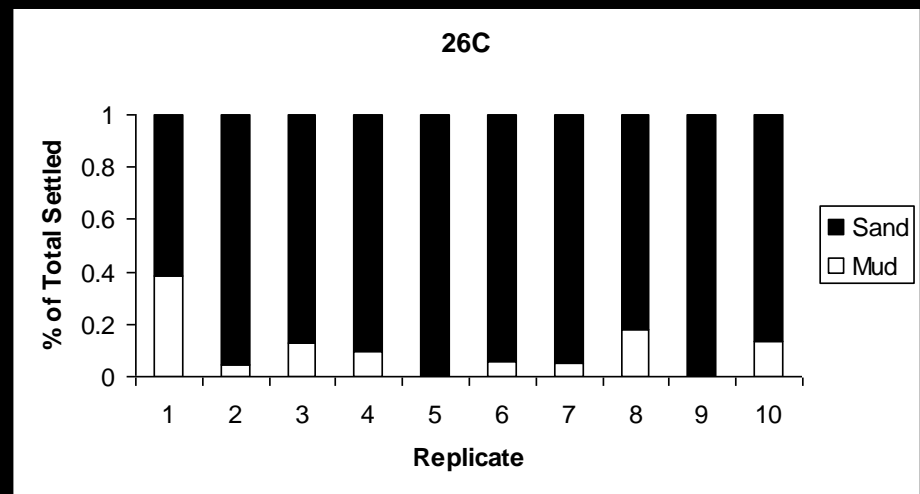


- In lab experiments, *Nematostella* larvae largely swim along the bottom of dishes (5, 15, 60 minutes)

Settlement Decisions



- In 2 choice experiment (mud/sand), settled in sand more than 4X as often



Factors for Limited Dispersal

Potential Explanations

- Benthic egg mass
- Largely benthic larvae
- Potentially poor settlers



Potential Effect

Reduced dispersal period

Reduced dispersal potential

Reduced recruitment success



III. Adaptive Genetic Variation?

Observations

1. Population genetics suggests *Nematostella* has a high degree of genetic structure
2. *Nematostella*'s environment is highly variable, potentially facilitating diversifying selection
3. *Nematostella* genome sequencing project revealed a relatively high polymorphism rate (1 in 125 bps, 10X that of humans)

Hypothesis: *Nematostella* will contain polymorphisms in evolutionarily conserved genes and these alleles will have spatial segregation among natural populations

Pipeline for Identifying Candidate Adaptive Polymorphisms



All polymorphisms



Polymorphisms in coding regions



Polymorphisms that result in amino acid change (= nonsynonymous)



Nonsynonymous polymorphisms in conserved domains



Nonsynonymous polymorphisms that



- 1) Result in amino acid with different chemistry (more likely to effect protein function)
- 2) Result in an amino acid that is evolutionarily uncommon

Polymorphic Gold!

Polymorphisms in Conserved Domains

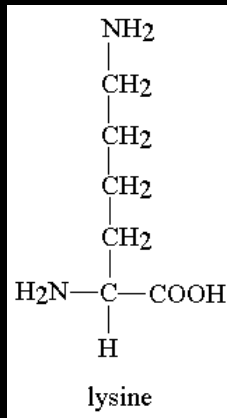
- Using StellaSNP's gene classification, we identified nonsynonymous polymorphisms in conserved domains
- The resulting amino acids must either:
 - 1) Differ in biochemical properties indicating potential effect on protein function
 - 2) One of the amino acids is phylogenetically uncommon among other organisms

Rx SNPs

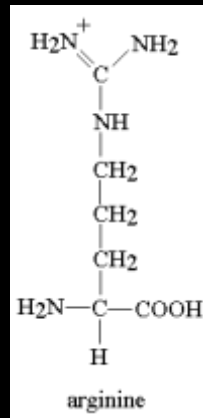
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      *           20           *           40           *           60
NV_RX1 : RRNRTIFTTIFQLHELERAF EKSHYPDVYDREELAKKISLPEV RVQVWFQNRRAKWR RQENMEM : 63
NV_RX2 : RRNRTIFTTIFQLHELERAF EKSHYPDVYDREELAKKISLPEV RVQVWFQNRRAKWR RREKMEM : 63
Hom_RX  : RRNRTIFTTIYQLHELERAF EKSHYPDVYSREELAKKVNLP EVRVQVWFQNRRAKWR RQEKLEV : 63
Xeno_RX : RRNRTIFTTIYQLHELERAF EKSHYPDVYSREELAKKVNLP EVRVQVWFQNRRAKWR RQEKLEV : 63
Drom_RX : RRNRTIFTTIYQLHELERAF EKSHYPDVYSREELAKKVNLP EVRVQVWFQNRRAKWR RQEKSES : 63
          RRNRTIFTTIFQLHELERAF EKSHYPDVYDREELAKKISLPEV RVQVWFQNRRAKWR RQEK E
  
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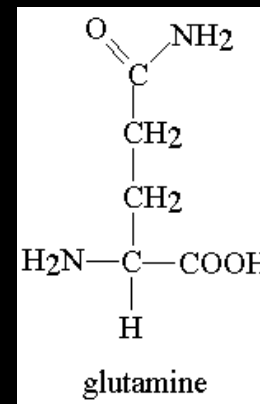
K



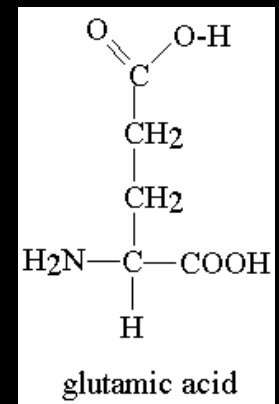
R



Q



E



Presence of lysine rare, no other reported instances in rx genes

Presence of glutamic acid rare, no other reported instances in rx genes

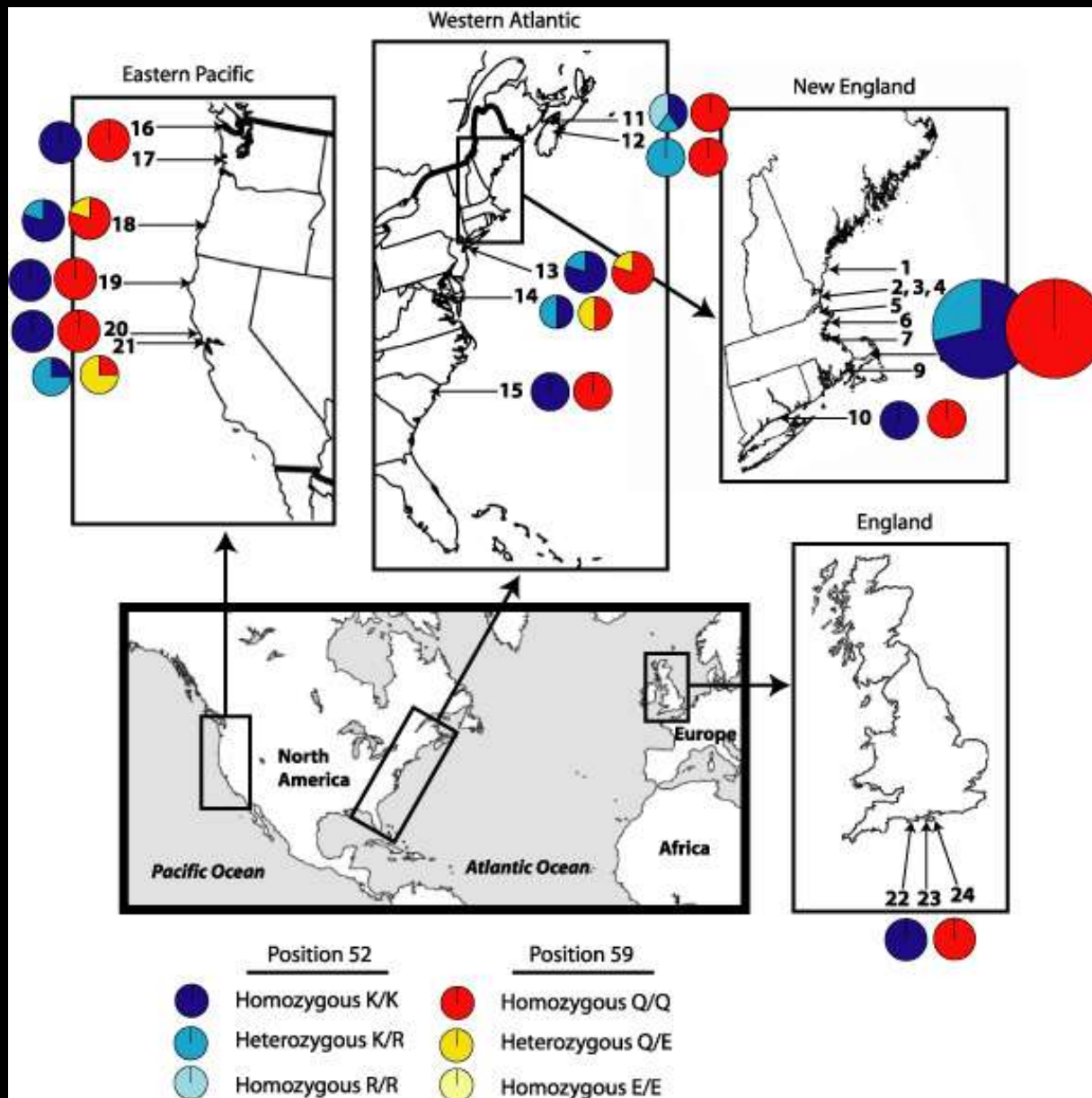
Rx SNPs

Position 52

Despite K being rare in *rx* genes, it is predominant in natural populations (K = 85%) and represented in most genotypes (KK = 72%, KR = 26%)

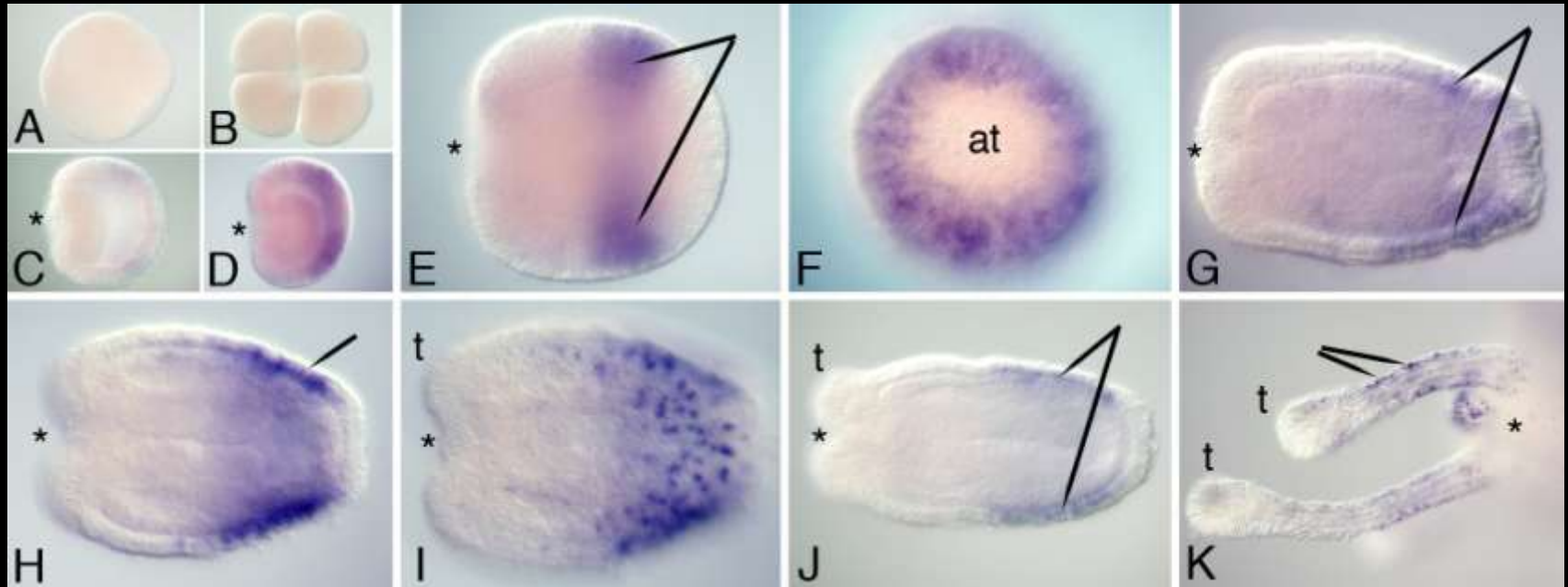
Position 59

E is rare in other *rx* genes and rare in *Nematostella* populations (E = 4%, QE = 7.5%, EE = 0%)



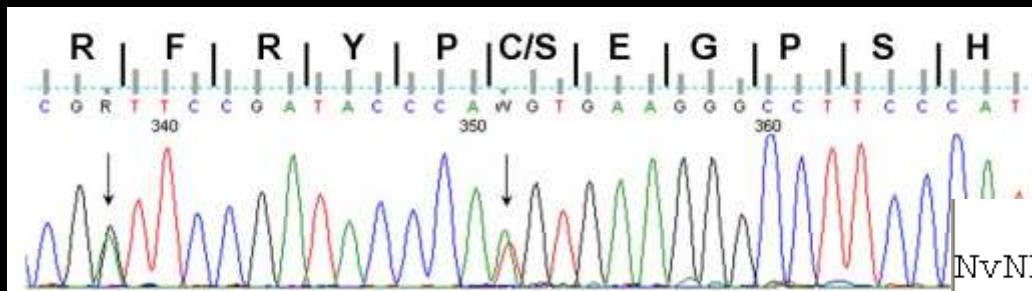
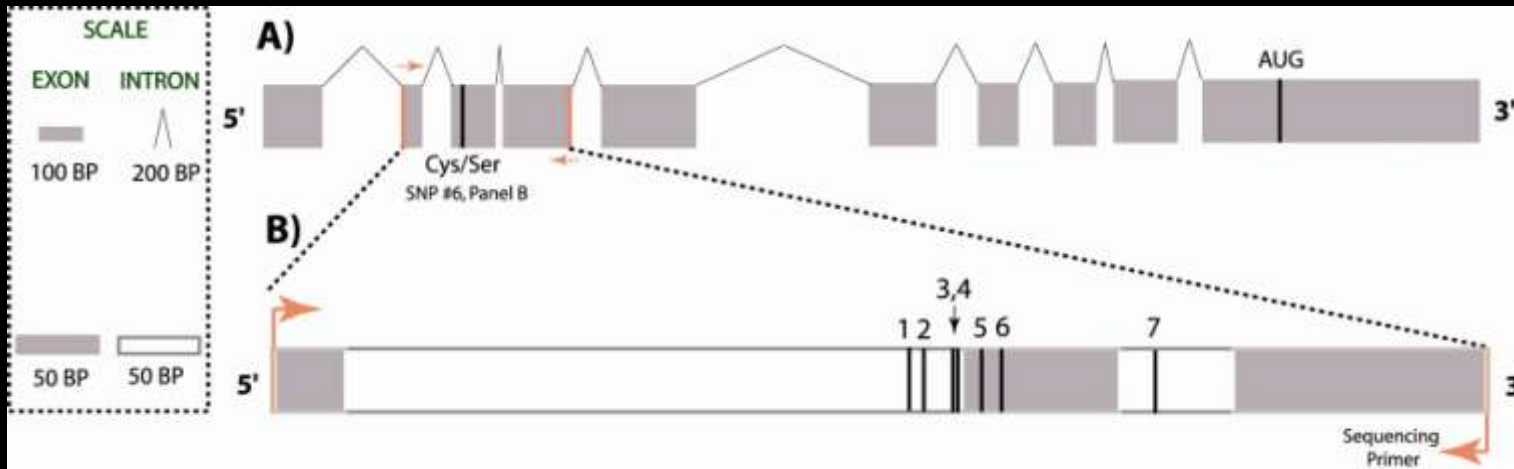
3 of 4 haplotypes present in natural populations showing recombination, R/E not observed

Do these variants matter?



- Would need to compare alleles to assay for functional differences (e.g. DNA binding)

Nematostella NF-κB

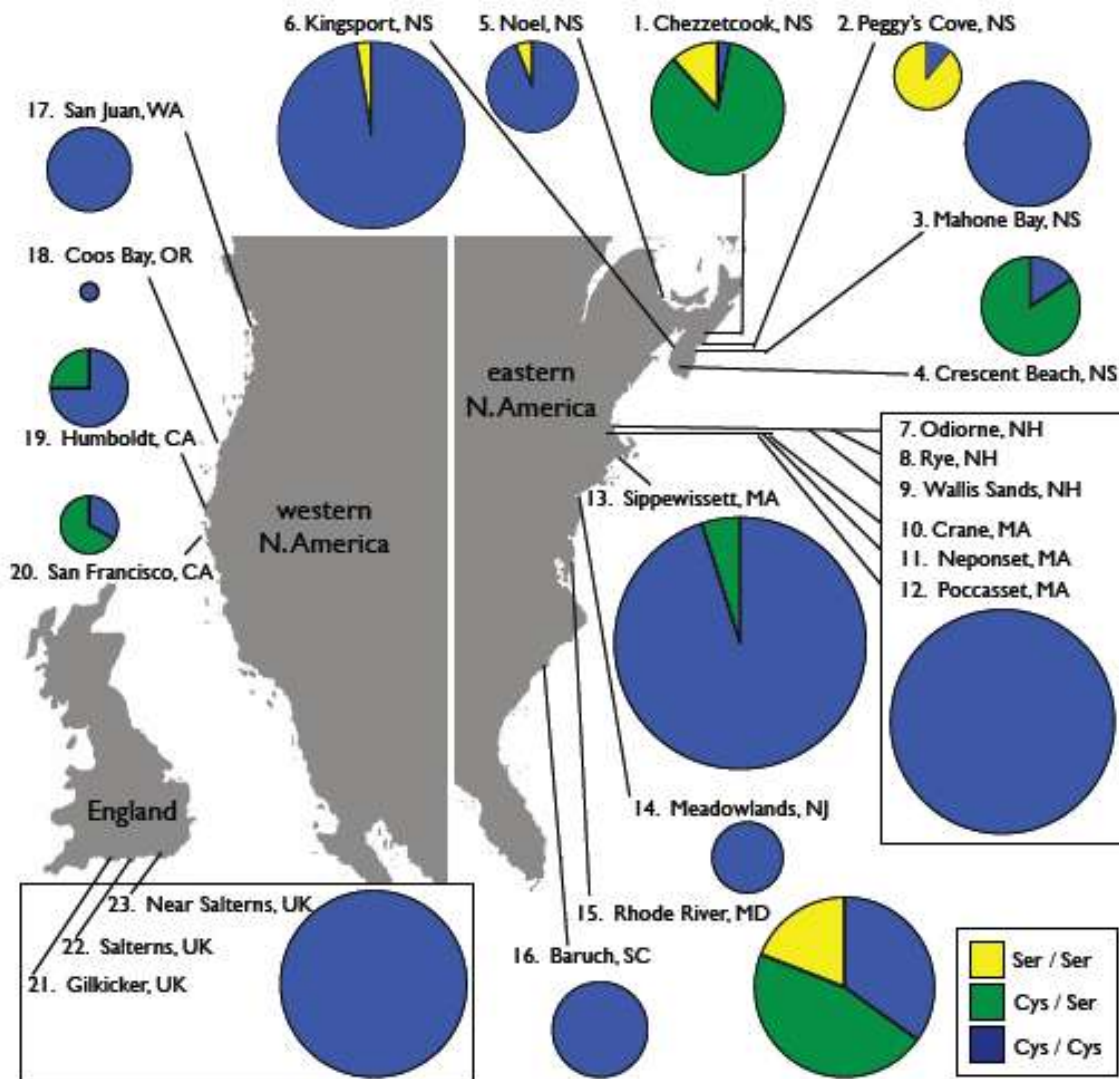


- Cysteine in every other organism

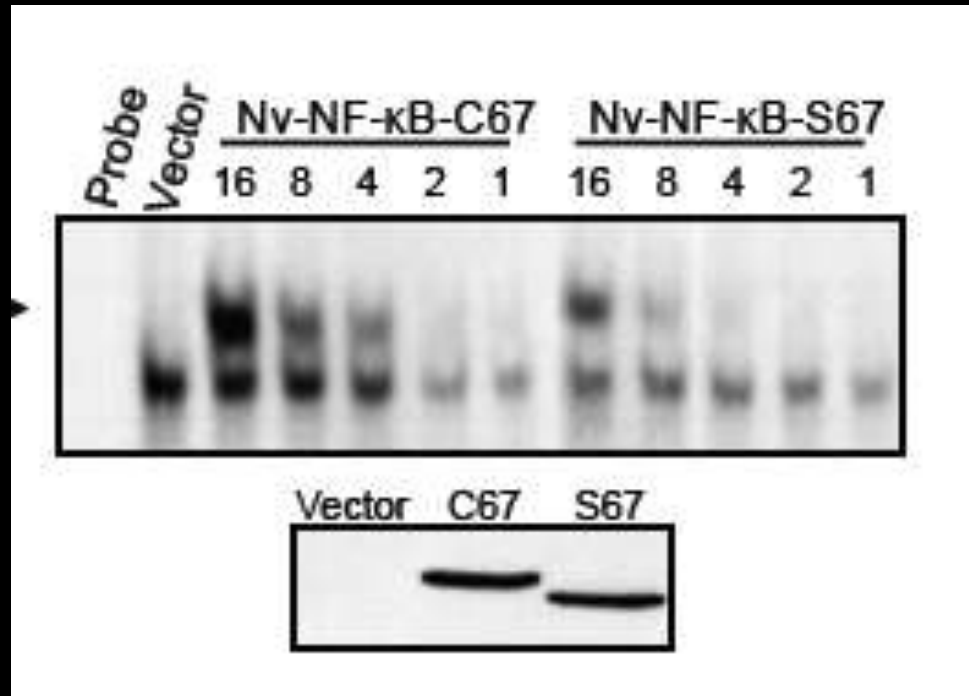
*

NvNFκB	: RFRYPSE [*] EGPSHGGLPG	: 16
HsNFκB1	: RFRYVCEGPSHGGLPG	: 16
MmNFκB1	: RFRYVCEGPSHGGLPG	: 16
GgNFκB1	: RFRYVCEGPSHGGLPG	: 16
XlNFκB1	: RFRYVCEGPSHGGLPG	: 16
SpNFκB	: RFRYGCEGPSHGGLPG	: 16
MmNFκB2	: RFRYGCEGPSHGGLPG	: 16
HsNFκB2	: RFRYGCEGPSHGGLPG	: 16
GgNFκB2	: RFRYVCEGPSHGGLPG	: 16
XlNFκB2	: RFRYVCEGPSHRGLPG	: 16
DmDorsal	: RFRYECEGRSAGSIPG	: 16

Distribution of Polymorphism



Functionality of the NF- κ B SNP



The SNP has two main functional consequences:

- 1) Altered DNA binding affinity
- 2) Decreased sensitivity to oxidative stress

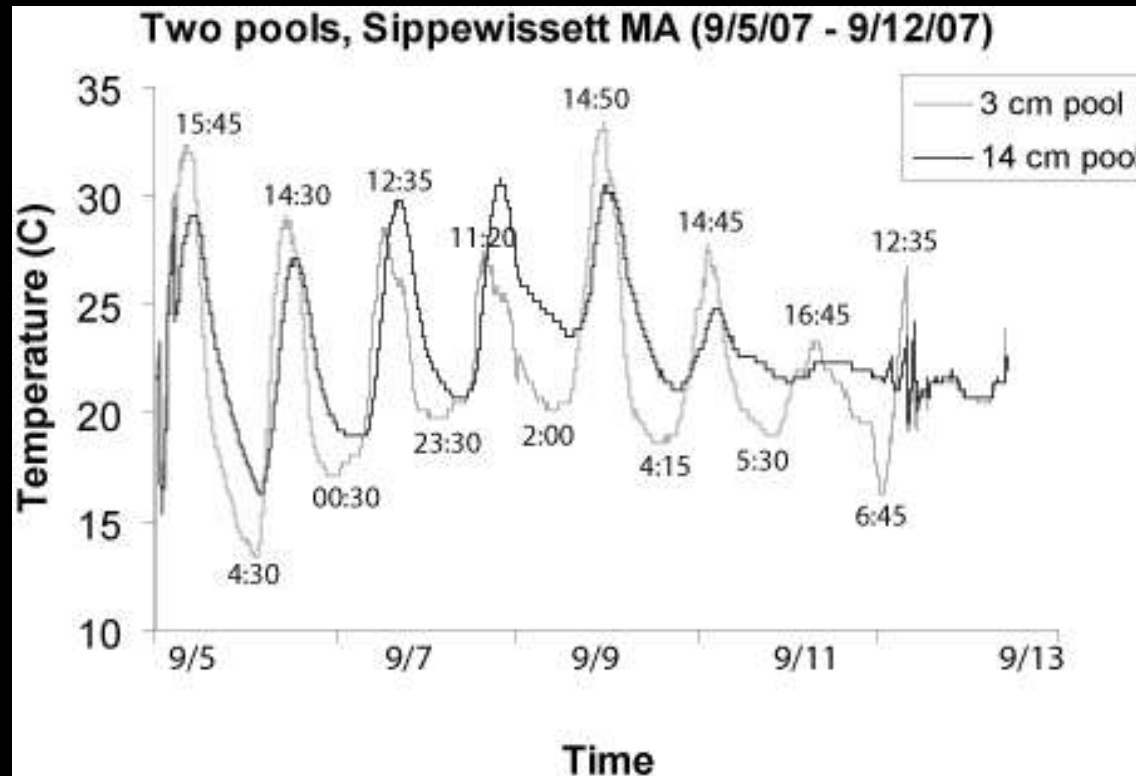
IV. Local Adaptation to Temperature

Observations:

- Lots of genetic diversity with restricted gene flow provides fodder for local adaptation
- *Nematostella* populations along the Atlantic coast occupy a steep latitudinal thermocline

Hypothesis: *Nematostella*, similar to other estuarine species, will show evidence of local adaptation to temperature in growth and development.

..In addition, large temporal / spatial variation



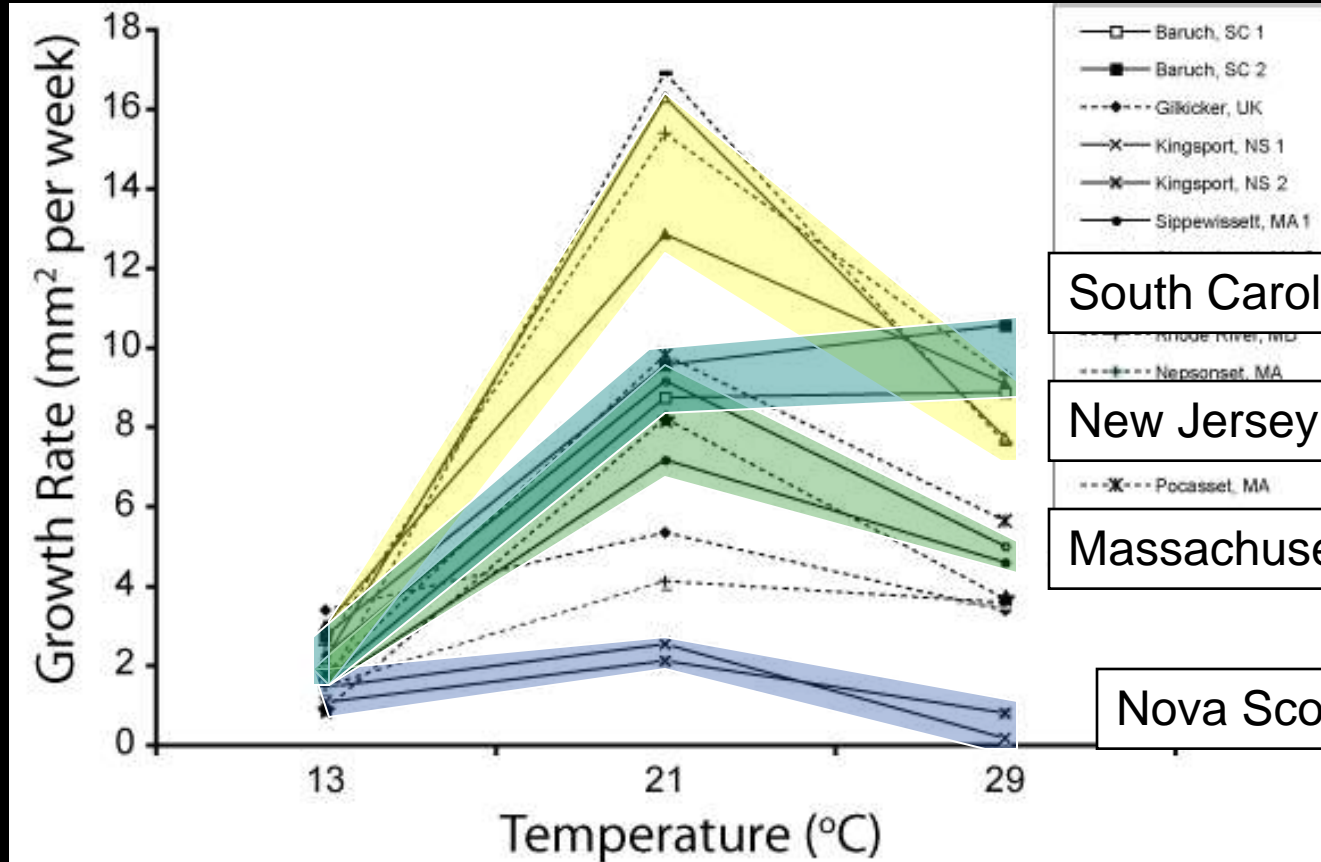
- Daily temperature changes of up to 20° and larger variance in shallower pools

Common-garden Experiments. I.

Growth Rate

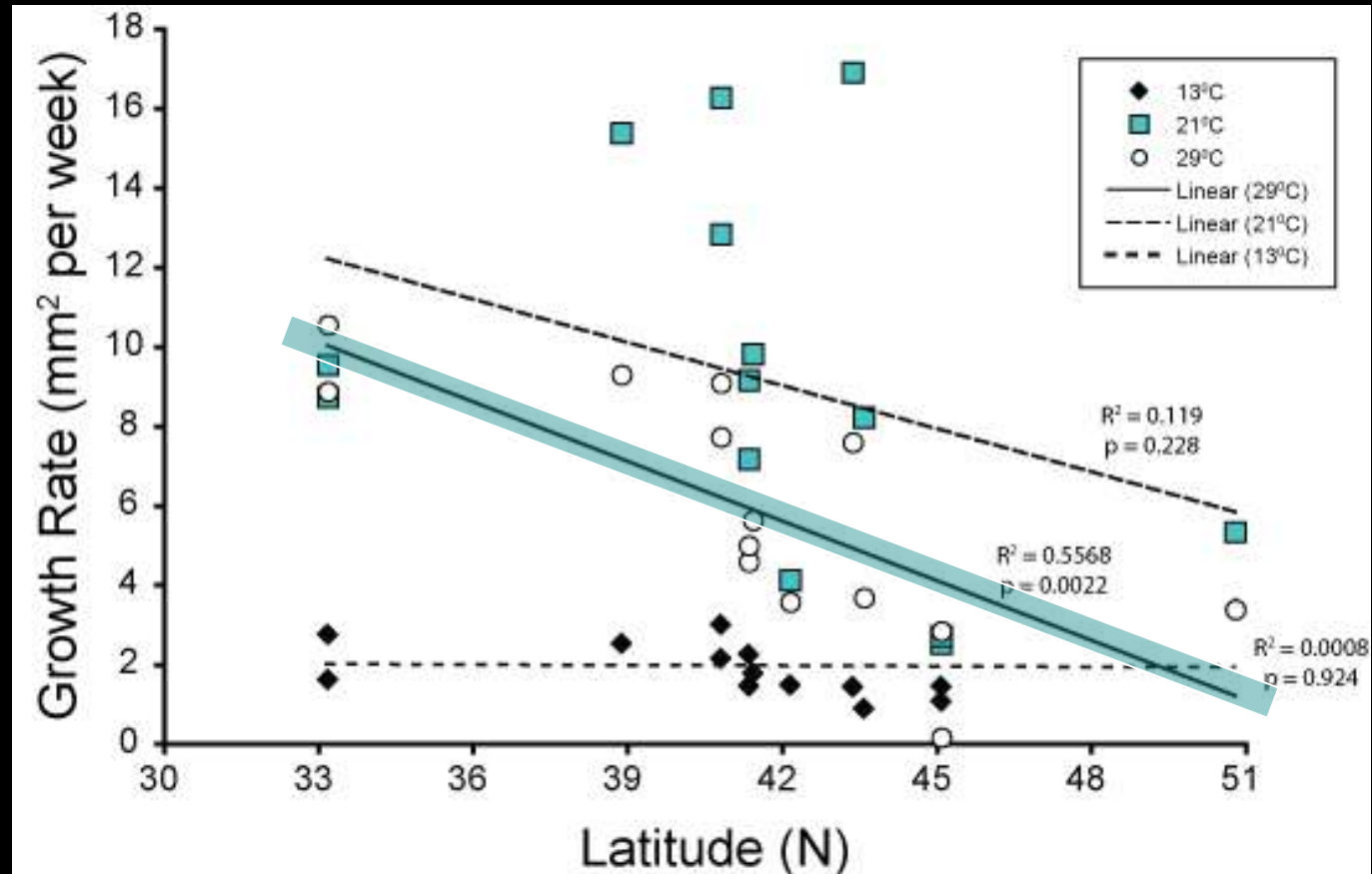
- Growth Rate
 - Generated 14 clonal lines from individuals collected from 10 locations, mostly along Atlantic coast of North America
 - Reared at 3 temperatures (13°C, 21°C, 29°C) indicative of mean summer temperatures from New England to southern US
 - Measured growth via 2-D projections (i.e. area)

Growth Rate of Clones in 3 Temps



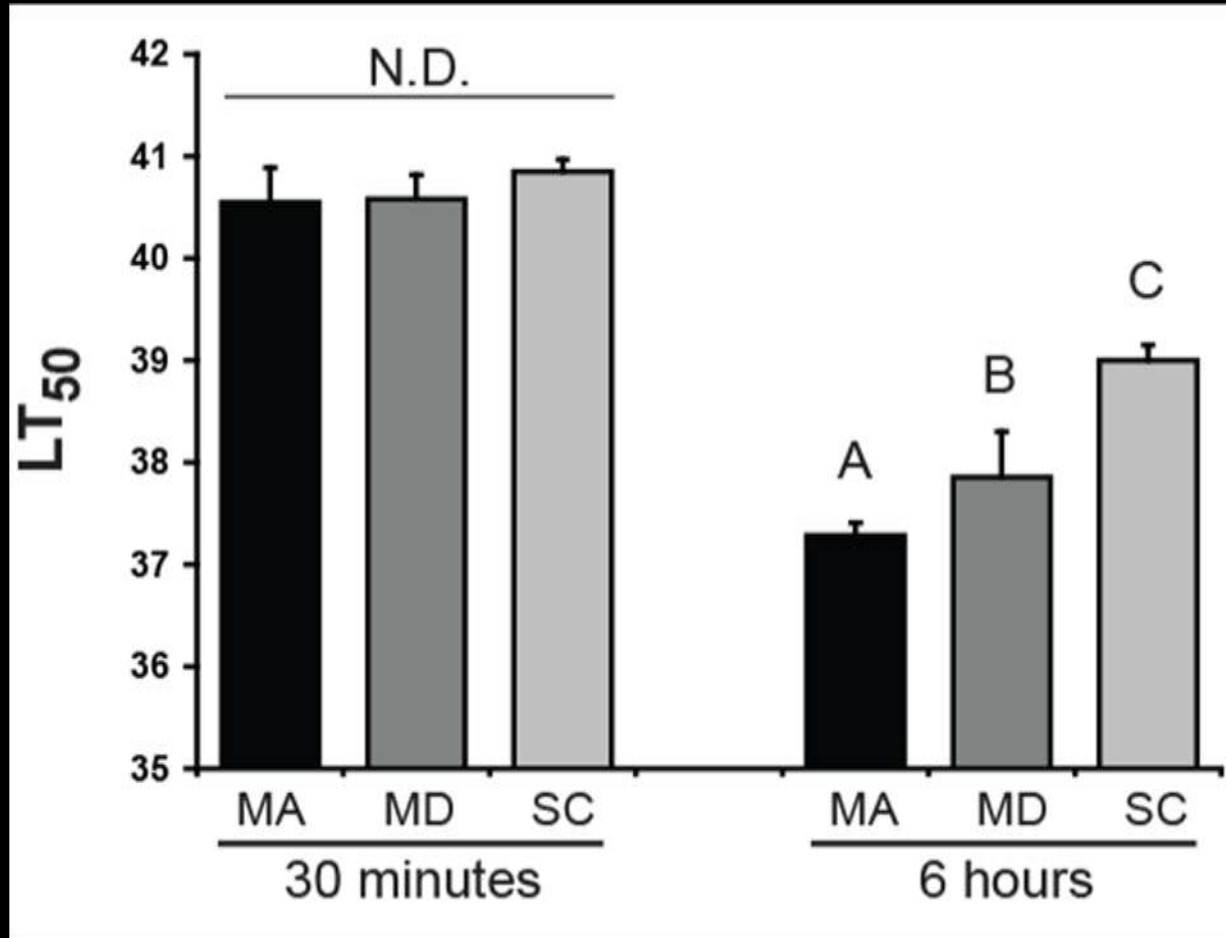
- Significant effects of clone ($p < 0.0001$), temperature ($p < 0.0001$), and interaction of clone X temperature ($p < 0.00001$)

Latitude and Warm Temperatures



- Significant, negative relationship between latitude and growth rate at highest temperature (29°C)

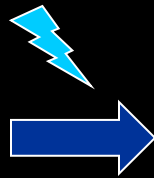
Lethal temperatures for juveniles from 3 geographic locations



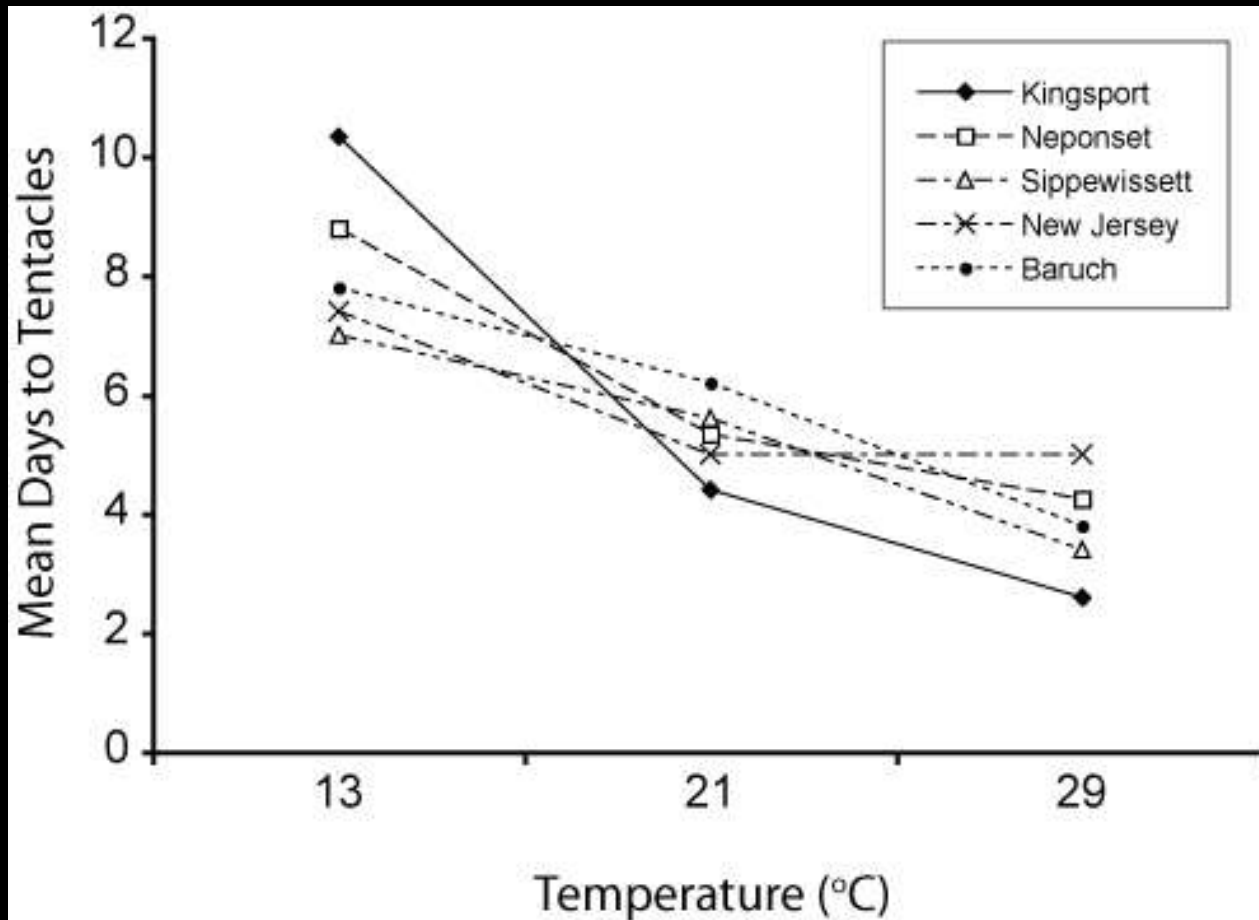
Common-garden Experiments. II.

Regeneration Rate

- Regeneration Rate
 - Used 5 clonal lines from 5 locations to measure oral regeneration rate in same 3 temperatures
 - Cut in half and follow development of aboral half



Regeneration and Temperature



- Regeneration rate only significantly affected by temperature ($p < 0.0001$), not clone ($p = 0.811$) or interaction of clone X temperature ($p = 0.717$)

Summary of Temperature Experiments

- Temperature has a significant effect on growth and regeneration rates in *Nematostella*
- Geographic origin was only significantly related to growth rate and duration of exposure to stressful temperatures
- Growth rate at the warmest temperature was negatively correlated with latitude and there was no local adaptation to cooler temperatures in more northern populations

Nematostella and Its Environment

- *Nematostella* is a year-round, infaunal resident of estuaries (a dynamic environment)
- Variety of stressors (natural, anthropogenic)

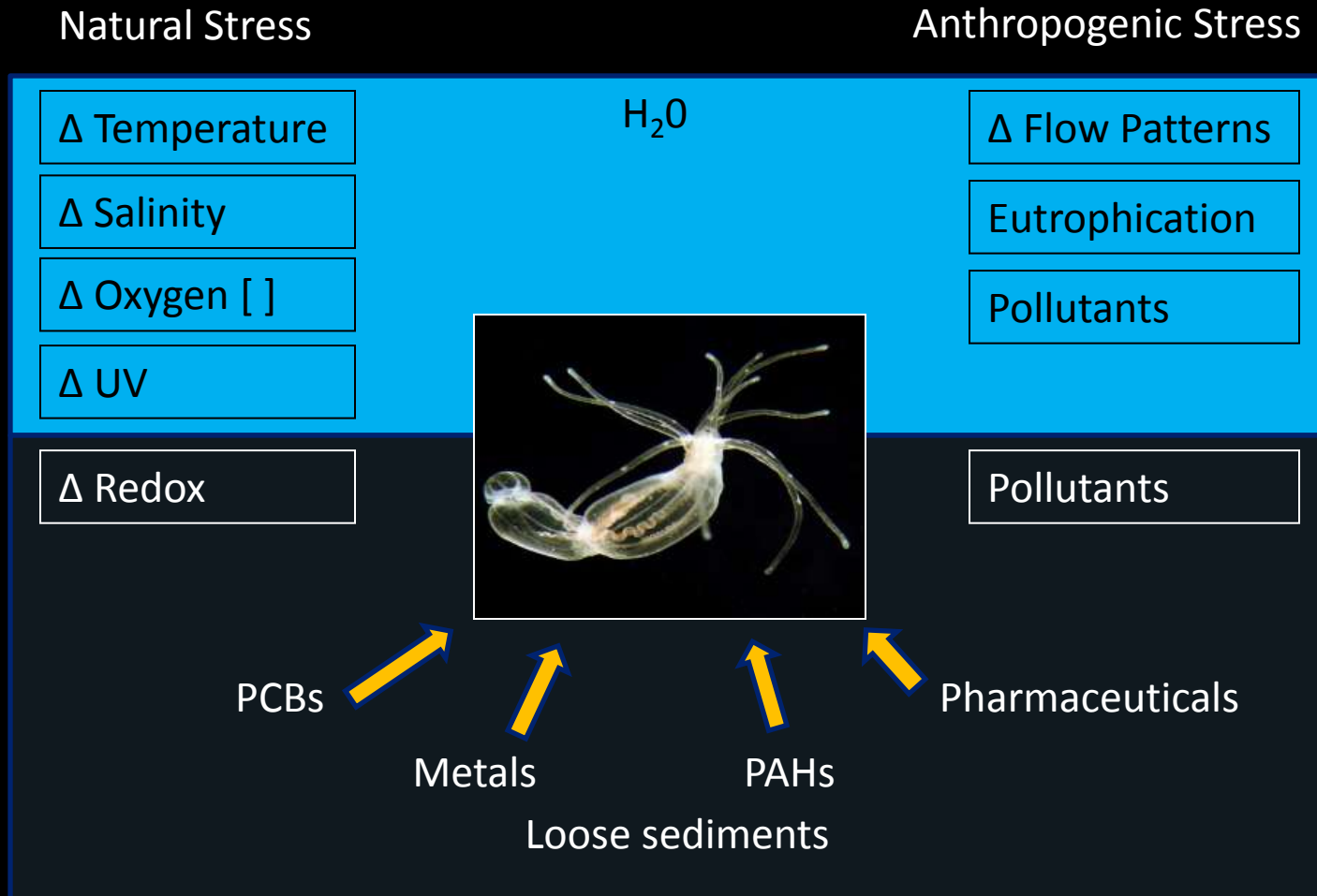


Most molecular work on *Nematostella* thus far has been conducted on developmental pathways



- *Nematostella* is poised for studies to understand the evolution and expression molecular mechanisms involved in ecological stress

Living in a Dynamic World



What molecular mechanisms are involved in responding to these environmental signals?

Transcriptional Regulators of Stress Responses

Antioxidant Response

Metal Response

Xenobiotic Response

Temperature Response

Hypoxia Response



Heat
Oxidative Stress
Metals



Oxidative Stress



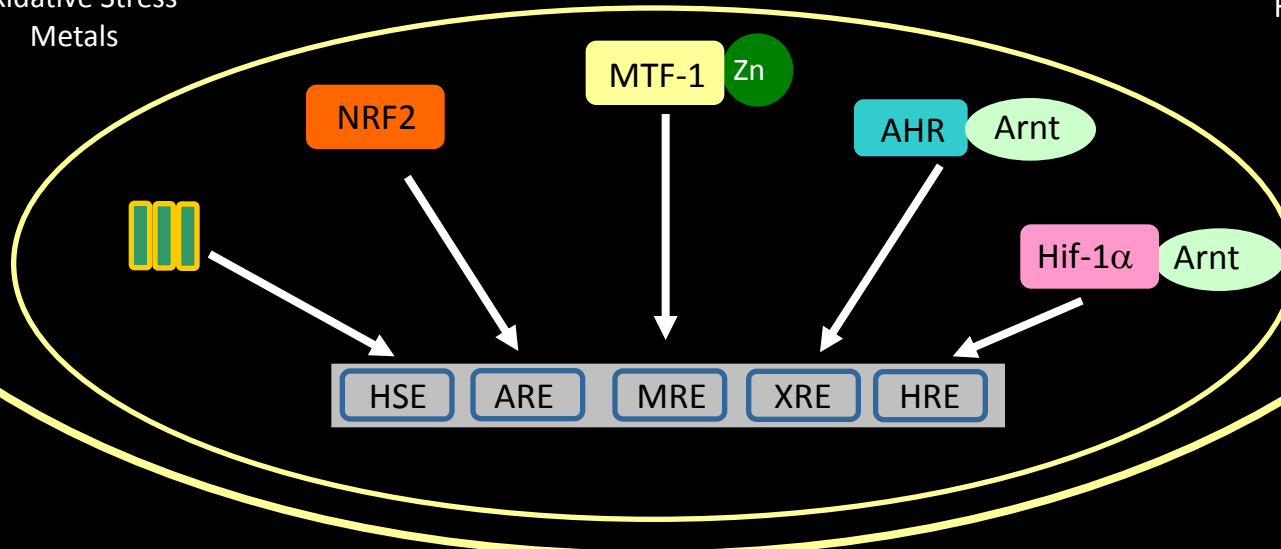
Metals



Aromatic
Hydrocarbons



Hypoxia



General Approach

- I. Utilize genome to identify homologs potentially involved in response to particular stressors
- II. Study expression of genes in these responses
 - Candidate genes (quantitative PCR)
 - Microarray (Ann Tarrant and Jed Goldstone)
 - Deep sequencing with Illumina Hi-Seq
- III. Identify target genes and their expression potentially regulated by transcription factors

Phases of Biotransformation

Toxic
Compound

Phase 1: facilitated by cytochrome p450s (n = 75)

Oxidized
metabolite

Phase 2: facilitated by glutathione pathway, specifically GST; sulphotransferases. (n = 25, GSTs, GCLC, etc.). Also, typically involves response to ROS generated during Phase 1 (SODs, peroxidases, catalase: n = 11)

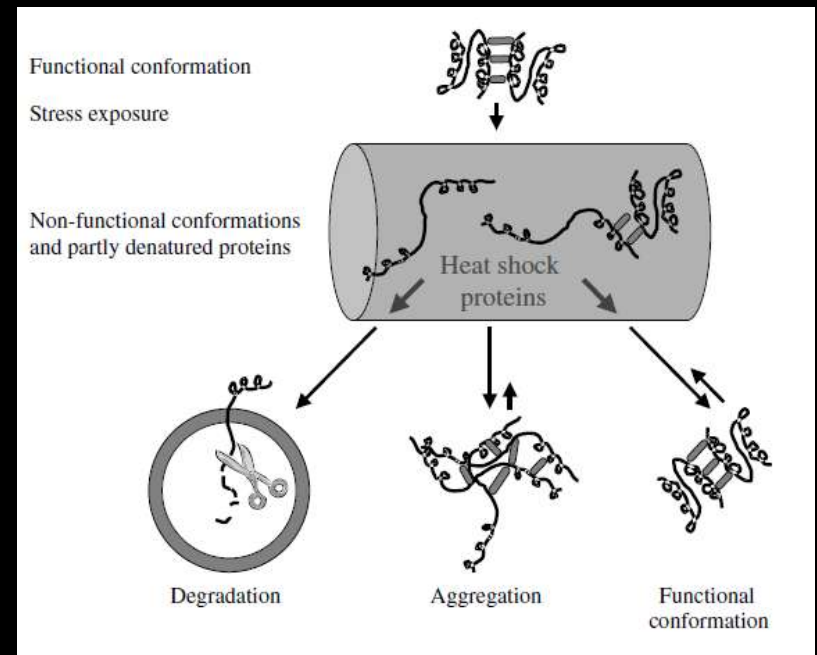
Conjugated
compound

Phase 3: Excretion of the modified compound, e.g., ABC transporters (n = 11).

Two Examples: Heat and Metal Stress

- 1) *Nematostella* experiences extreme changes in temperature on a daily basis
- 2) Estuaries are common sites of concentrated pollutants, including metals and PAHs.

A general stress responsive set of genes are the heat shock proteins (HSPs).



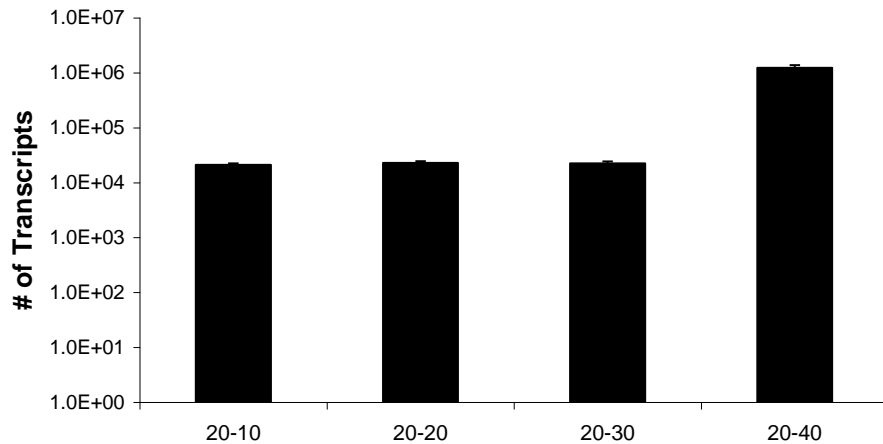
What are HSPs?

- Heat shock proteins (Hsps) are multiple functional proteins that largely serve as protein chaperones
 - Required for developmental processes
 - Upregulated in response to cellular stress
 - Commonly complex with transcription factors to sequester in cytoplasm
- Hsps are categorized in size classes

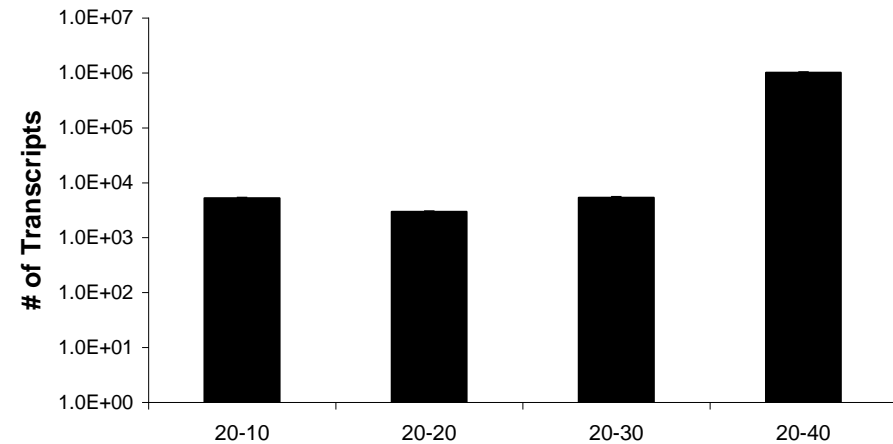
Example 1. Temperature Stress

Exposed *Nematostella* adults to temperature shifts from 20°C to 10, 30, and 40°C for 6 hours

HSP70A



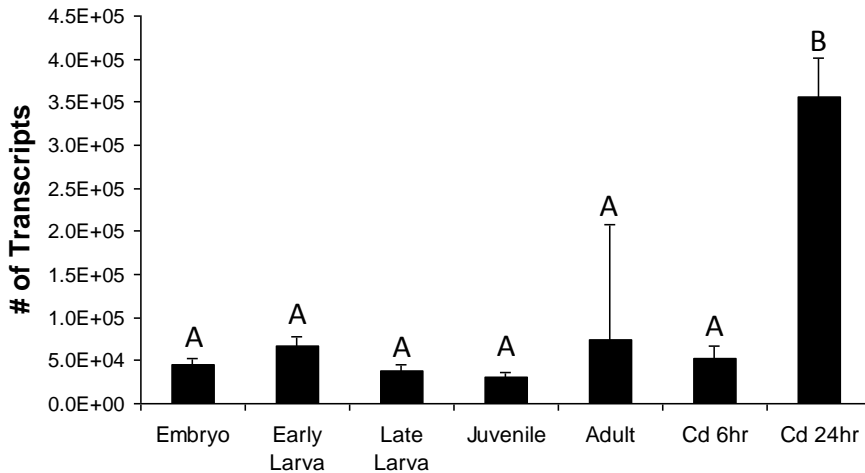
HSP90



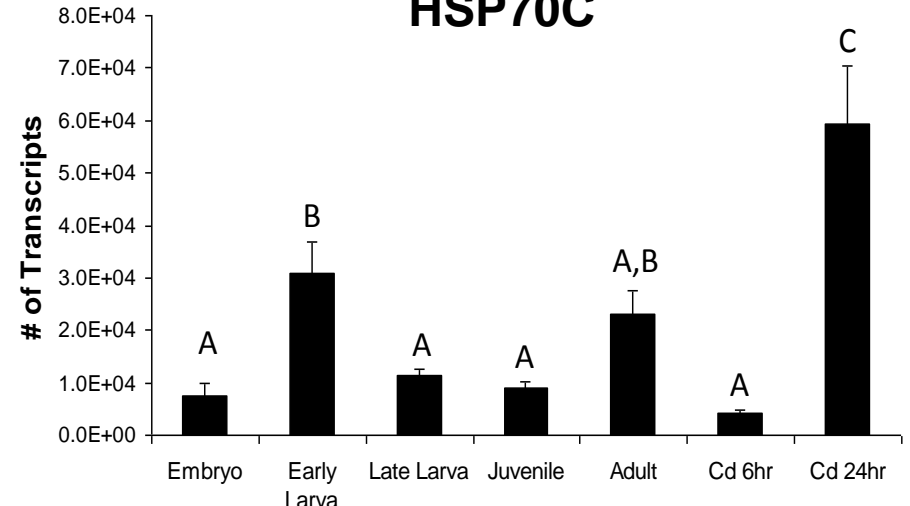
Example 2. Cadmium Stress

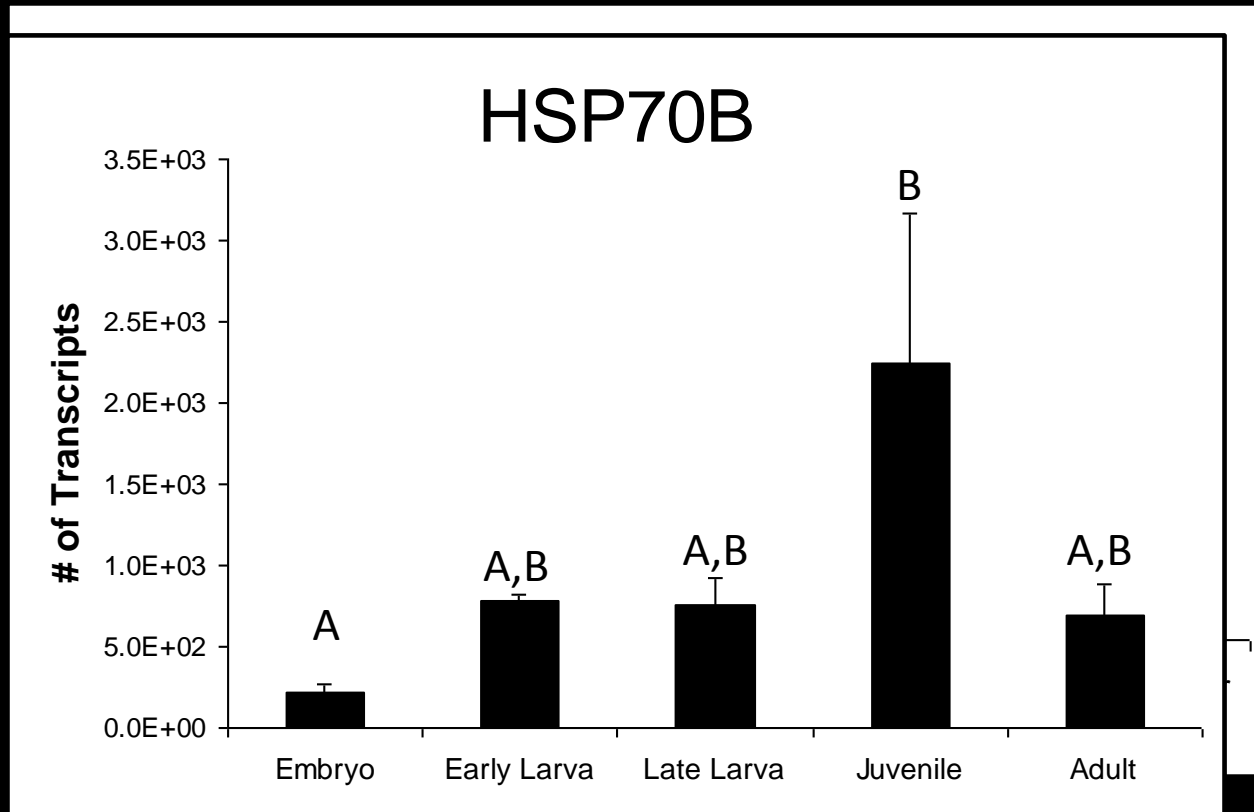
Exposed *Nematostella* adults to 100 ppm cadmium for 6 and 24 hours

HSP70A



HSP70C



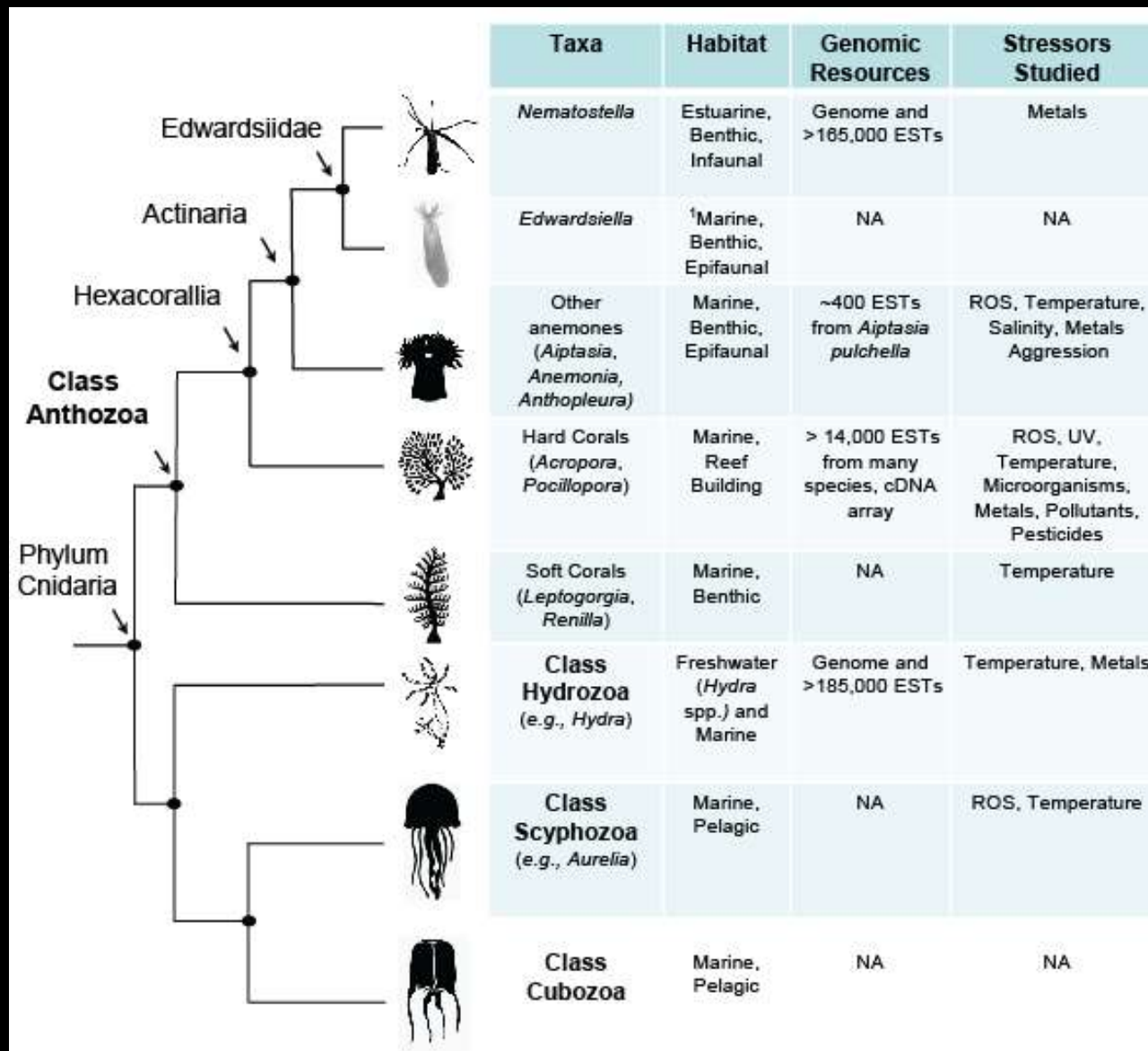


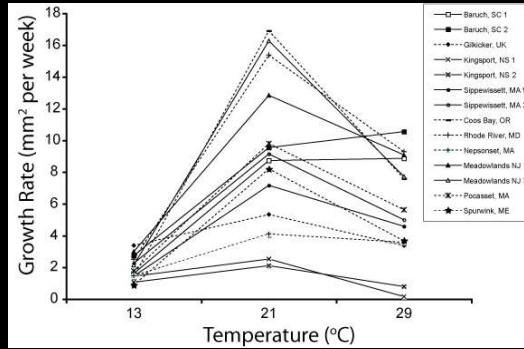
- Greater than 100X increase in transcription in response to cadmium

Summary

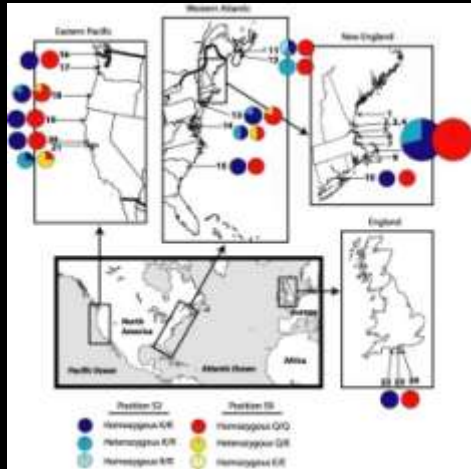
- *Nematostella* contains orthologs (and many paralogs) to genes involved in toxic metal and general stress
- Heat stress at environmentally relevant temperatures results in large induction for specific HSPs
- Cadmium dosing caused upregulation of most HSPs
- Together, these, and other genes, will be useful biomarkers for field populations

Complementing Study of Stress in Cnidarians

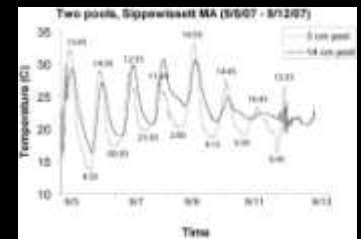
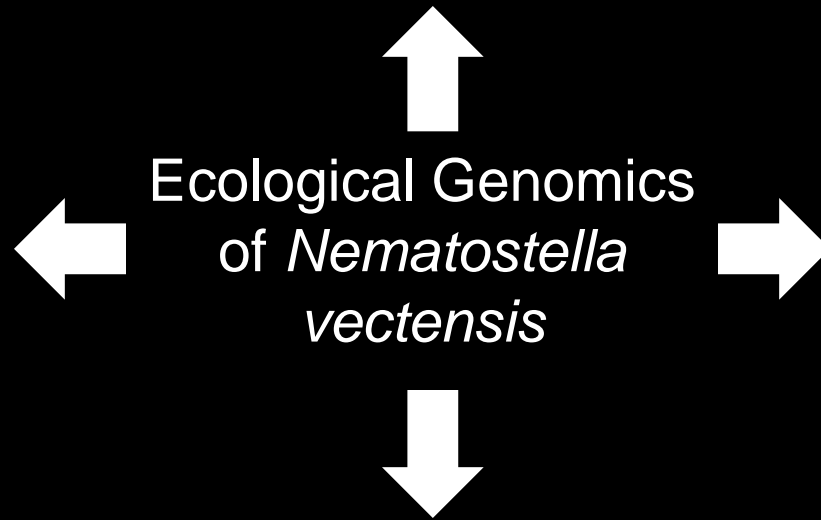




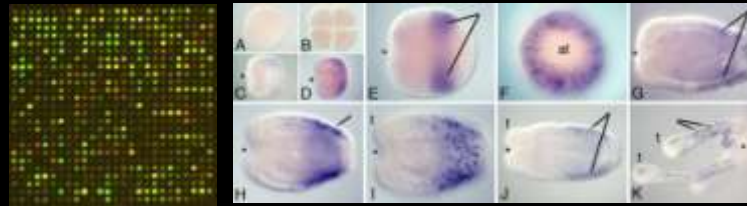
Phenotypic variation



Population genetics



Ecology



Gene expression

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