

# Human-mediated transport determines the non-native distribution of the anemone *Nematostella vectensis*, a dispersal-limited estuarine invertebrate

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**ABSTRACT:** Sessile invertebrates are common invaders of estuarine ecosystems. To expand their non-native ranges, these invasive taxa must contend with the geographically and ecologically discontinuous nature of estuarine habitats, in many cases without the benefit of highly dispersive larval phases. In addition, their population dynamics may reflect contributions from both sexual and asexual reproduction. Here we use genetic methods to explore the population structure of *Nematostella vectensis*, a dispersal-limited salt marsh anemone, along the Pacific coast of North America. Analysis of 9 highly polymorphic microsatellite loci reveals that asexual reproduction is critical to both maintenance of local population density and regional population expansion. While high levels of genetic differentiation among populations ( $F_{ST} > 0.3$ ) reflect general restrictions to natural dispersal, the observation of 2 clones distributed across multiple, widely separated sampling sites indicates that long distance dispersal of adult anemones is a major contributor to the spread of *N. vectensis*. We argue that anthropogenic transport represents the most likely mechanism driving this spread, although rafting may offer an alternative explanation for the observed distribution.

**KEY WORDS:** *Nematostella vectensis* · Invasive species · Clonality · Asexual reproduction · Dispersal · Microsatellites · Estuarine

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## INTRODUCTION

The past half century has seen a dramatic rise in the incidence and effect of aquatic biological invasions, as globalization of trade and the efficiency of international shipping and travel vastly increase opportunities for populations to disperse beyond their native range limits (Cohen & Carlton 1998, Ruiz et al. 2000). A substantial proportion of these invasions have occurred in estuarine ecosystems, largely due to their association with common vectors of introduction such as commercial shipping and recreational boating (Wonham et al. 2000, Carlton 2001, Floerl & Inglis 2005). The ecological consequences of estuarine invasions can be substantial (Grosholz 2002), and the associated costs in terms of lost ecosystem services may require significant investment in the management of threats posed

by introduced populations (Williams & Grosholz 2008). Understanding the population dynamics of introduced taxa is a critical prerequisite to effective management, as knowledge of life history and demographic characteristics provides important insights into the potential for dispersal to and colonization of new habitats, the likely geographic patterns of range expansion and the possible responses to novel environmental challenges (Sakai et al. 2001).

Sessile benthic invertebrates are frequent invaders of estuarine ecosystems, as they often contribute to fouling assemblages and, as a result, are prone to human-mediated transport (Carlton & Geller 1993). However, full appreciation of the population dynamics of these taxa is complicated by 2 important aspects of their biology. First, many sessile invertebrates are capable of asexual reproduction (Jackson et al. 1985),

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and patterns of population growth and spatial expansion may reflect contributions of both sexual and asexual increase. Asexual reproduction is known to have significant effects on the population structure of reef-building corals (Stoddart 1984, Miller & Ayre 2004, Whitaker 2006, Foster et al. 2007) and other anthozoans (Ayre 1984, Shaw 1991, Sherman & Ayre 2008), as well as some other invertebrate taxa (Zilberberg et al. 2006). However, the potential effect of reproductive plasticity on invasion dynamics in marine invertebrate taxa remains understudied. Second, estuaries are discrete habitats, typically separated by both geographic and ecophysiological boundaries, and dispersal between these habitats depends on complex hydrological, physiological and behavioral mechanisms (Bilton et al. 2002). Differences in larval strategies (i.e. larval retention versus larval dispersal) can result in widely differing patterns of genetic connectivity between estuaries, with corresponding implications for evolutionary diversification and range expansion (Bilton et al. 2002, Levin 2006).

The salt marsh anemone *Nematostella vectensis* Stephenson, 1935 provides a promising system within which to examine the interactions of reproductive plasticity and dispersal on the population dynamics of an introduced estuarine species. Recent genetic evidence indicates that *N. vectensis* has been introduced to both England and the west coast of the USA from its presumed native range in the northwestern Atlantic Ocean (Reitzel et al. 2008). The species is capable of asexual reproduction through either physal pinching, in which an aboral fragment of the adult anemone is cleaved off and subsequently develops new head structures, or polarity reversal, in which the adult develops a new oral crown at its aboral pole before undergoing cleavage through the body column (Reitzel et al. 2007). Both of these mechanisms involve transverse fission of the adult anemone, and it is likely that both are important to the maintenance of population density in natural populations (Darling et al. 2004, Reitzel et al. 2008). Although a high frequency of anemones undergoing polarity reversal (i.e. 2-headed anemones) have been observed in some wild populations, physal pinching is clearly the predominant form of asexual reproduction in laboratory populations (Reitzel et al. 2008). In addition, genetic and experimental studies suggest that *N. vectensis* possesses extremely low dispersal capacity, even at spatial scales of tens of meters (Stocks & Grassle 2001, Darling et al. 2004). This is despite the fact that development proceeds through a ciliated planula stage capable of active swimming for up to 14 d in culture (Hand & Uhlinger 1992). The observed limitations on dispersal may be related to either behavioral or physical restrictions on the access of planulae to the open ocean (Bil-

ton et al. 2002); regardless, they suggest that natural colonization of geographically distant sites should be rare.

Here we use genetic data to investigate the contributions of both clonal reproduction and long distance dispersal to the regional expansion of *Nematostella vectensis* on the Pacific coast of North America. We examine 7 populations throughout the known range of *N. vectensis* in this region, and use 9 highly polymorphic microsatellite loci to assess (1) the prevalence of asexual reproduction based on the observation of repeated multilocus genotypes (MLGs), (2) genetic connectivity between populations, and (3) the spatial extent of clonal genotypes. Our findings suggest that the expansion of *N. vectensis* in the northeastern Pacific Ocean has probably been driven primarily, if not entirely, by human dispersal vectors. The contribution of these vectors to contemporary patterns of genetic diversity observed in this species may have implications for the expected genetic patterns in other invasive estuarine invertebrates with facultative asexual life histories.

## MATERIALS AND METHODS

**Sample collection.** Adult *Nematostella vectensis* were collected from throughout the species' known range on the Pacific coast of North America, using methods described previously by Darling et al. (2004). Additional attempts to sample anemones south of San Francisco Bay were unsuccessful, which is consistent with a lack of reports on *N. vectensis* presence in southern California. The distribution of the species observed in the present study is consistent with previous observations (Hand & Uhlinger 1994); in addition to being restricted to estuarine habitats with soft substrates, the species does not appear to be continuously distributed throughout suitable habitat in the northeast Pacific Ocean.

**Molecular methods.** Samples were either preserved in 95% ethanol or returned alive to the laboratory, where they were maintained in one-third strength (salinity 13) artificial seawater. In most cases, a fragment of tissue approximately 2 mm long was removed from the pedal end of each individual, and whole genomic DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions. In the case of very small individuals, the entire specimen was processed for DNA.

Microsatellite loci *Nv4*, *Nv8*, *Nv13*, *Nv16*, *Nv17*, *Nv24*, and *Nv29* were amplified according to protocols described in Darling et al. (2006). Each 15  $\mu$ l PCR contained 0.5 U *Taq* DNA polymerase (Invitrogen), 1 $\times$  Mg-free PCR buffer, 1  $\mu$ M each forward and reverse primer

(for *Nv17* only 0.1  $\mu$ M of each primer was used), 0.67 mM dNTPs, 1.7 mM  $MgCl_2$ , and approximately 5 ng of DNA template. Cycling parameters consisted of 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at the locus-specific annealing temperature, and 1 min at 72°C, with a final 15 min extension step at 72°C. Amplified products were diluted with Hi-Di formamide and sized on an ABI 3730 DNA analyzer (Applied Biosystems). Microsatellite data was scored using Genemarker v1.70 software (SoftGenetics).

**Data analysis.** Distinct multilocus genotypes (MLGs) were identified and assigned to clones using GENCLONE (Arnaud-Haond & Belkhir 2007). In addition, this software was used to calculate  $p_{gen}$ , the probability of each MLG arising through sexual recombination given observed allele frequencies. When multiple ( $n$ ) instances of the same MLG were observed, we also calculated  $p_{sex}$ , the probability of  $n$  occurrences of that MLG arising through independent instances of sexual reproduction. Departures from Hardy-Weinberg equilibrium were allowed, and each value was calculated both across the entire region and within individual populations. To assess the spatial extent of clonal genotypes, we determined the probability of clonal identity within 4 distance classes. The maximum spatial extent of these 4 classes was, respectively, 0 km (within individual collection sites), 477 km (inclusive of single collection sites and their nearest neighbors), 837 km (inclusive of the 2 nearest neighbors of each site), and 1627 km (all collection sites). Distances were measured in kilometers along the coast in ArcView GIS v9.1 (ESRI). Genotypic richness ( $R$ ), Simpson's diversity index ( $D$ ), and Simpson's evenness index ( $E_D$ ) were calculated for each population in GENCLONE, and allelic richness ( $A$ ) and expected heterozygosity ( $H_E$ ) were calculated in FSTAT v2.9.3 (Goudet 2001).

To determine the degree of genetic differentiation between populations, we calculated pairwise  $F_{ST}$  values using GENEPOP v4.0 (Rousset 2008), with 1000 bootstrap replicates to assess statistical support. To visualize genetic relationships among *Nematostella*

*vectensis* populations, we employed 3-dimensional factorial correspondence analysis in GENETIX v4.0.5 (Belkhir et al. 2004). Since the presence of repeated MLGs can significantly affect the results of these analyses, we conducted them on both an unedited dataset and a truncated dataset in which every MLG within each population was counted only once; in other words, repeated instances of single MLGs were removed from individual populations but retained in separate populations. While the most meaningful analysis of population structure is probably the one based on allele frequencies calculated from unique MLGs, we include the unedited analysis for comparison.

## RESULTS

### Genetic diversity within populations

Genetic diversity measures varied widely between populations (Table 1). Repeated MLGs were observed in all samples, but the proportion of the most common genotype and the genotypic richness differed dramatically. Coos Bay (CB) had the most genotypically diverse population observed ( $R = 0.2889$ ,  $D = 0.8415$ ), but even this population was dominated by a single MLG representing 28% of all individuals. In the most extreme case, the population from San Juan Island (SJI) in Puget Sound consisted of only 3 MLGs ( $R = 0.1250$ ,  $D = 0.4706$ ), with the most common one comprising over 70% of the sample. Although most MLGs observed occurred as singletons (Fig. 1), these comprised only a small proportion of the total dataset (approximately 8%; Table 2) due to the high frequency of occurrence of a number of clones.

With only 2 exceptions, all repeated MLGs could be attributed confidently to asexual reproduction (Table 2). In the case of the most common MLG from Humboldt Bay (HB) (Clone 1), we could not reject the possibility that the number of observed individuals with

Table 1. *Nematostella vectensis*. Summary of collection sites on the US Pacific coast and genetic diversity measures.  $N$  = sample size;  $G$  = number of genotypes;  $P$  = proportion of most common genotype;  $R$  = genotypic richness;  $D$  = Simpson's diversity;  $E_D$  = Simpson's evenness;  $A$  = Allelic richness;  $H_E$  = expected heterozygosity. Latitude and longitude are given in decimal degrees; see Fig. 2 for map of locations

Sample	Location	Latitude	Longitude	$N$	$G$	$P$	$R$	$D$	$E_D$	$A$	$H_E$
SJI	San Juan Island, Washington	48.504494	-122.898981	17	3	0.71	0.1250	0.4706	0.5051	1.8701	0.4016
GH	Gray's Harbor, Washington	46.953151	-124.058762	18	5	0.33	0.2353	0.7974	0.8876	2.5770	0.4044
WB	Willapa Bay, Washington	46.559184	-123.938433	41	7	0.32	0.1500	0.8073	0.8821	2.7294	0.4864
YB	Yaquina Bay, Oregon	44.621459	-124.033935	33	7	0.61	0.1875	0.6136	0.5074	2.6423	0.4486
CB	Coos Bay, Oregon	46.953151	-124.058762	46	14	0.28	0.2889	0.8415	0.7656	3.1486	0.5178
HB	Humboldt Bay, California	40.769651	-124.206431	26	3	0.50	0.0800	0.5569	0.7486	1.8746	0.4200
TB	Tomales Bay, California	38.166395	-122.907986	9	4	0.33	0.3750	0.8056	0.8533	2.6250	0.5470

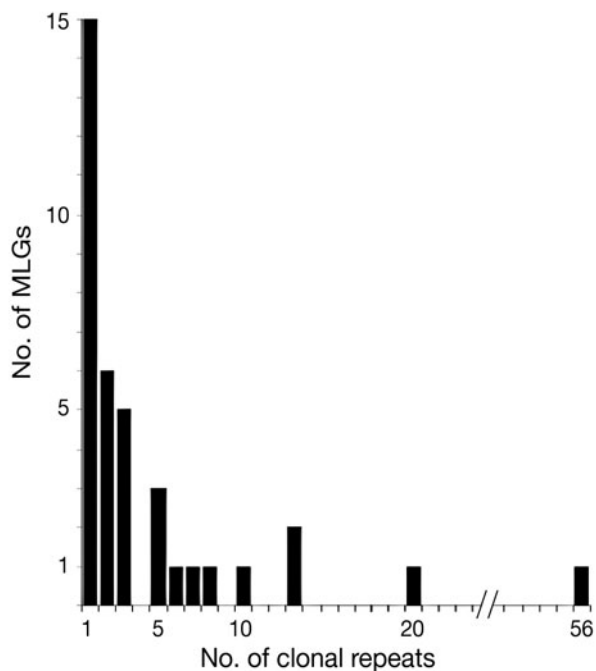


Fig. 1. *Nematostella vectensis*. Frequency distribution of distinct multilocus genotypes (MLGs)

the same genotype may have arisen through sexual recombination ( $p_{sex} = 0.4435$ ). The same held true for one MLG observed 4 times in the San Juan Islands ( $p_{sex} = 0.1833$ ). These somewhat counterintuitive results can be attributed to the extremely limited allelic diversity in these populations ( $A = 1.8746$  for HB,  $A = 1.8701$  for SJI), which renders the occurrence of the observed MLGs likely even when alleles are recombined randomly. In all other cases,  $p_{sex}$  for repeated MLGs within populations was less than  $\alpha = 0.05$ , and the hypothesis of sexual reproduction was rejected.

### Spatial extent of clones

Two MLGs were shared between multiple populations (Table 2 & Fig. 2). The most common of these (Clone 1) was observed in 4 populations (SJI, Yaquina Bay [YB], CB, HB), and comprised nearly 30% of the entire dataset; the second (Clone 2) was found at SJI, CB, HB, and Tomales Bay (TB), and represented approximately 11% of all individuals. The geographic extent of these single clones was broad, with Clone 2

Table 2. *Nematostella vectensis*. Clonal diversity. Individual clones extending across multiple collection sites are highlighted in gray.  $p_{gen}$  = likelihood of individual genotype arising through sexual reproduction, given allelic frequencies in the regional population;  $p_{sex}$  = likelihood of obtaining the total number of observations of the same genotype in the regional population, given allelic frequencies; ns = repeated genotypes with non-significant values of  $p_{sex}$  ( $>0.05$ ) within individual samples; na = not applicable. See Table 1 for definition (location) of sample abbreviations

Clone	Population							Count	Frequency	$P_{gen}$	$P_{sex}$
	SJI	GH	WB	YB	CB	HB	TB				
1	12			20	11	13 ns		56	0.294737	0.000214	3.71E-154
2	4 ns				1	12	3	20	0.105263	5.79E-05	1.94E-55
3			13					13	0.068421	6.77E-10	3.03E-92
4					13			13	0.068421	2.08E-05	2.15E-38
5			10					10	0.052632	1.47E-08	2.33E-56
6					8			8	0.042105	1.14E-06	3.87E-30
7			7					7	0.036842	3.55E-07	1.21E-28
8		6						6	0.031579	3.51E-08	1.04E-28
9			5					5	0.026316	1.64E-08	3.79E-24
10				5				5	0.026316	3.51E-05	7.96E-11
11		5						5	0.026316	3.67E-06	9.49E-15
12							3	3	0.015789	5.01E-07	4.51E-09
13			3					3	0.015789	8.20E-11	1.21E-16
14		3						3	0.015789	2.85E-07	1.46E-09
15				3				3	0.015789	1.97E-07	6.93E-10
16		3						3	0.015789	1.30E-07	3.02E-10
17							2	2	0.010526	4.36E-09	8.28E-07
18					2			2	0.010526	1.70E-08	3.22E-06
19			2					2	0.010526	1.78E-09	3.38E-07
20					2			2	0.010526	1.88E-08	3.57E-06
21					2			2	0.010526	2.92E-06	0.000555
22				2				2	0.010526	4.08E-08	7.75E-06
Singletons	1	1	1	3	7	1	1	15	0.078947	na	na
Total	17	18	41	33	46	26	9	190			

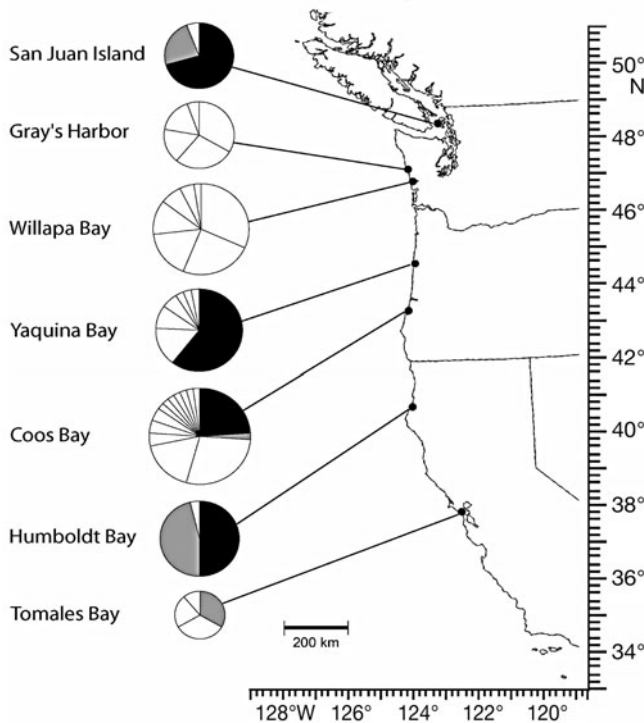


Fig. 2. *Nematostella vectensis*. Geographic distribution of clones. Pie graphs are scaled to sample size; pie graph segments indicate relative frequency of MLGs observed at each collection site. Shared Clones 1 and 2 are shown in black and gray, respectively; all private clones are shown in white. Approximate west longitude and north latitude are shown on x- and y-axes, respectively

Table 3. *Nematostella vectensis*. Clonal subrange analysis. Distance classes are defined as described in 'Materials and methods'. p = probability of 2 individuals drawn from within the distance class having the same MLG

Distance class	Minimum distance	Average distance	Maximum distance	p
1	0	0	0	0.2571
2	34	306	477	0.0441
3	523	717	837	0.1538
4	1000	1214	1627	0.1519

Table 4. *Nematostella vectensis*. Genetic differentiation between populations. Pairwise  $F_{ST}$  values are given for the complete dataset (below diagonal) and for the dataset with clones reduced within individual samples (see 'Materials and methods' for details). Values that are not significant ( $p > 0.05$ ) are indicated in **bold** text. See Table 1 for definition (location) of sample abbreviations

	SJI	GH	WB	YB	CB	HB	TB
SJI	–	<b>-0.16</b>	0.31587	<b>-0.02672</b>	<b>0.00453</b>	0.20293	<b>-0.03115</b>
GH	0.30995	–	0.31587	<b>-0.00916</b>	<b>0.0196</b>	0.20293	<b>-0.04121</b>
WB	0.37376	0.19665	–	0.19675	0.16539	0.12928	0.2441
YB	0.00709	0.24249	0.31137	–	<b>0.01823</b>	0.11159	0.0304
CB	0.06637	0.26128	0.26058	0.05274	–	0.15971	<b>0.01471</b>
HB	<b>-0.02023</b>	0.31288	0.3765	0.02258	0.0713	–	0.16704
TB	0.07151	0.26791	0.30328	0.08206	0.05892	0.06075	–

spread over 1627 km and Clone 1 over 1150 km. Analysis of clonal subrange (Table 3) indicated that the likelihood of 2 randomly chosen MLGs being identical was highest within populations (distance class 1, maximum distance = 0 km;  $p = 0.2571$ ). This likelihood declined precipitously when the subrange was increased to include each population's nearest neighbor (Class 2;  $p = 0.0441$ ), but rose again when the subrange was expanded to include the 2 nearest neighbors (Class 3;  $p = 0.1583$ ) or the entire range (Class 4;  $p = 0.1519$ ).

### Genetic relatedness between populations

Genetic differentiation between *Nematostella vectensis* populations was dramatic, as revealed by pairwise estimates of  $F_{ST}$ . When the full dataset was considered, all pairwise comparisons were statistically significant with the exception of SJI and HB (Table 4, below diagonal). Levels of differentiation were extremely high in some cases, with  $F_{ST}$  values over 0.3. Willapa Bay (WB) and Gray's Harbor (GH), in particular, were highly diverged from all other populations; interestingly, despite their geographic proximity, they also exhibited significant genetic differentiation from each other ( $F_{ST} = 0.1967$ ). When we considered a dataset from which repeated MLGs had been removed from individual populations,  $F_{ST}$  values generally decreased, and a number of pairwise comparisons were no longer statistically significant (Table 4, above diagonal). The most dramatic changes were the overall reduction in differentiation between GH and other populations, and the increase in differentiation between SJI and HB, now statistically significant at  $F_{ST} = 0.20293$ . Notably, WB remained highly diverged from all other populations.

Factorial correspondence analysis of individual MLGs was consistent with genetic distance analysis (Fig. 3). In particular, the populations from SJI and HB cluster very tightly together, and WB and GH both fall far outside the diffuse cluster comprising the remaining populations. Spatial relationships between populations did not change substantially when the truncated dataset was considered, although the overall percentage of genetic variance explained by the 3 factors was reduced from 91.19 to 85.25% (not shown).



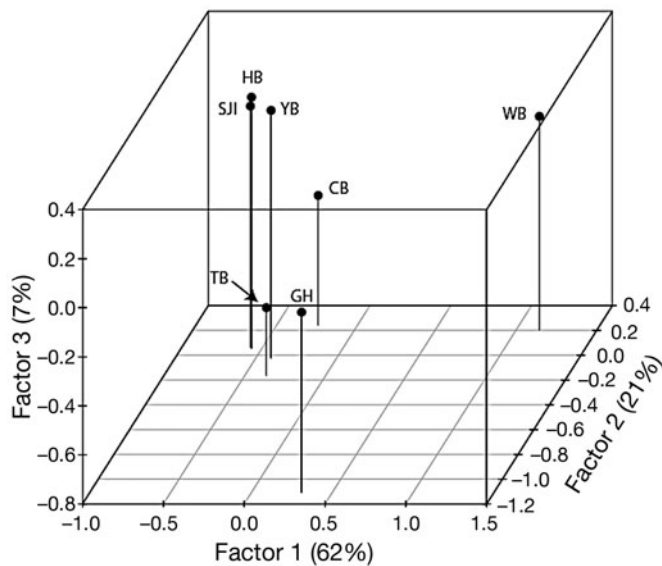


Fig. 3. *Nematostella vectensis*. Factorial correspondence analysis of microsatellite data. Only results from analysis of the edited dataset (unique MLGs only) are shown

## DISCUSSION

### Asexual reproduction and colonization success of *Nematostella vectensis*

Previous studies have revealed an important role for reproductive plasticity in generating the patterns of genetic variation observed in *Nematostella vectensis* populations (Pearson et al. 2002, Darling et al. 2004, Reitzel et al. 2008). While some populations appear to derive exclusively from sexual reproduction, others can be dominated by single MLGs to the point that at least one population has been reported consisting solely of a single clone (Darling et al. 2004). Typically, wild populations of *N. vectensis* appear to be maintained by a combination of both sexual and asexual reproduction, with clonal lineages existing in populations largely consisting of unique, sexually produced MLGs (Darling et al. 2004). However, evidence of asexual reproduction appears to be much more pronounced in those populations that have presumably been introduced by human activity (Pearson et al. 2002, Reitzel et al. 2008).

The distribution of MLGs reported here is consistent with these earlier observations. All populations possessed clonal lineages, with the dominance of single clones varying widely among populations, and all repeated MLGs could be confidently ascribed to asexual reproduction. The only clonal lineages with non-significant values of  $p_{\text{sex}} > 0.05$  within individual samples were Clone 1 at Humboldt Bay and Clone 2 at San Juan Island. However, since these clones were also

found widely distributed at other sites, it is unlikely that they derived from sexual recombination and is probable that the high values of  $p_{\text{sex}}$  are an artifact of low allelic diversity (Table 1). Thus, although most MLGs occurred as singletons (Fig. 1), the vast majority of individuals could be assigned to clonal lineages, and only about 8% of all anemones could not confidently be attributed to asexual reproduction (Table 2).

Still, the elevated clonal diversity observed at many sites (Table 1) suggests that population density is not achieved solely through expansion of dominant clones. The relatively high frequency of unique MLGs in the populations at Coos Bay and Yaquina Bay (0.15 and 0.09, respectively) indicates that sexual reproduction contributes substantially to genetic diversity at these sites, despite the prominence of clonal genotypes. The distribution of genetic diversity at Coos Bay and Willapa Bay may similarly indicate the joint contributions of sexual and asexual reproduction. The large number of clonal lineages observed at these sites (Table 2) suggests that MLGs generated through recombination may later expand asexually, giving rise to populations with significant clonal diversity despite the presence of dominant clones. Frequent adoption of mixed asexual and sexual reproduction within single populations, observed now throughout the native and introduced ranges of *Nematostella vectensis*, suggests that there may be significant individual variation in response to the cues triggering reproductive decisions for this species.

The capacity to reproduce asexually has been often cited as a characteristic conducive to invasion success (Sakai et al. 2001), though the correlation has been reported most thoroughly in plant systems (Ruesink & Collado-Vides 2006, Wright & Davis 2006, Burns 2008, Milbau & Stout 2008). Only a few studies have explored the role of asexual reproduction in the successful invasions of invertebrate taxa (Ting & Geller 2000, Facon et al. 2003, Stadler et al. 2005), and none have directly tested the hypothesis that asexuality contributes to invasiveness. It seems likely that the capacity for asexual reproduction may mitigate a number of the negative demographic and genetic effects associated with colonization (Roman & Darling 2007).

One benefit of clonality is the possibility of replication and temporal persistence of especially fit genotypes. For instance, Sherman & Ayre (2008) have found that fine scale dominance of genotypes in the actinarian *Actinia tenebrosa* reflects the expansion of locally adapted clones. On broader scales, the dominance of particular MLGs over large geographic ranges has suggested to some the possibility of general purpose genotypes with high relative fitness across a wide variety of habitat types (Lynch 1984), though recent empirical evidence for this hypothesis is equivocal (Kenny

1996, Schmid et al. 1999, Van Doninck et al. 2002, Vorburger et al. 2003). Pressure for the maintenance of such genotypes may be particularly strong in marginal habitats such as those populated by *Nematostella vectensis* (Vorburger et al. 2003).

There is no direct evidence for the competitive superiority of any *Nematostella vectensis* genotype, although single widespread MLGs have been observed elsewhere in the species range, most notably in England (Pearson et al. 2002, Reitzel et al. 2008). The dominance of 2 clones over multiple sites — including those at Coos Bay and Yaquina Bay, where diversity is high and sexual reproduction appears to be common — suggests that there may be some selective advantage in maintaining their high frequency across the region. It is possible that these lineages exhibit ecological tolerances that confer higher relative fitness in the estuarine habitats frequented by *N. vectensis* on the US west coast. Alternatively, these 2 clones may display a predilection for asexual reproduction. The tendency toward fission may have a genetic basis, as certain populations have been observed with much higher rates of asexual reproduction than others under similar environmental conditions (Reitzel et al. 2008).

In the absence of selective advantage, it is possible that the broad spatial extent of any single MLG may reflect the outcome of stochastic sampling. The genetic composition of the propagule pool colonizing a novel site will be determined by that of the source population, and common MLGs may persist if founding populations expand principally or exclusively by asexual reproduction. Strong founder effects accompanied by asexual reproduction could have yielded dominant clones in newly established populations of *Nematostella vectensis* on the Pacific coast. If these then served as sources for secondary introductions, clonal dominance would be expected to extend over a broad geographic range. Such dominance of particular genetic types driven by sampling effects has been observed in other invasive taxa (Voisin et al. 2005). However, it is important to note that stochastic and deterministic explanations for clonal dominance may not be mutually exclusive. For instance, the predominance of a single invasive lineage of the brown alga *Undaria pinnatifida* in Europe is probably due to selective advantage artificially imposed on aquaculture stocks in Asia where the species originated, and not necessarily to selective advantage in the non-native range (Voisin et al. 2005). Thus, although widespread MLGs may exhibit adaptive superiority in those populations from which they originate, they may not necessarily do so across their entire range. Detailed knowledge of invasion history may be required for adequate experimental assessment of the potential selective advantage of widespread MLGs.

### Dispersal and range expansion of *Nematostella vectensis*

Connectivity between estuarine populations may be limited by the geographic and ecological boundaries separating estuarine habitats (Bilton et al. 2002). Numerous genetic studies of both benthic invertebrates (Perrin et al. 2004, Virgilio & Abbiati 2004, Schmidt et al. 2006) and coastal fish taxa (Pampoulie et al. 2004, Watts & Johnson 2004, Bradbury et al. 2008) bear out this prediction, revealing significant genetic differentiation between estuarine populations, in many cases despite the presence of potentially dispersive larval forms. Empirical evidence, thus, suggests that estuarine taxa, particularly those lacking long-lived planktonic larvae, are likely to exhibit marked genetic structure as a result of limited gene flow between populations, with corresponding increases in the potential for local adaptation and limitations to the possibility of range expansion. Human-mediated dispersal could significantly alter these dynamics, however. For instance, Pettengill et al. (2007) have observed only very slight genetic differentiation between widely separated populations of the invasive, dispersal-limited polychaete *Hydroides elegans*, and suggest that frequent anthropogenic transport of adults and larvae may mitigate natural limits to population connectivity. Similarly, oyster farming probably contributes substantially to the regional expansion of the invasive gastropod *Ocenebrellus inornatus* in France, despite the lack of a dispersive larval phase (Martel et al. 2004).

The association of *Nematostella vectensis* with human transport vectors results in patterns of range expansion and genetic connectivity that are unexpected given observed limits to natural dispersal (Reitzel et al. 2008). It is highly unlikely that either of the 2 most dominant clones observed in the current study arose more than once ( $p_{\text{gen}} = 0.000214$  for Clone 1,  $p_{\text{gen}} = 5.79 \times 10^{-5}$  for Clone 2), and their wide distribution must then result from dispersal between embayments. Thus, their discontinuous geographic distribution indicates gene flow occurs between populations separated by over 1600 km (San Juan Islands to Tomales Bay, the maximum extent of Clone 2; see Fig. 2).

Genetic studies consistently reveal high levels of differentiation between *Nematostella vectensis* populations, even those separated by only tens of meters (Darling et al. 2004, Reitzel et al. 2008), and experimental studies of invertebrate dispersal within estuaries have revealed that *N. vectensis* rarely colonizes novel sites within embayments where populations are already established (Stocks & Grassle 2001). In the present study, pairwise  $F_{\text{ST}}$  values between some populations are extremely high, even when repeated clonal

genotypes are removed from the analysis (Table 4), and the frequency of shared clones is low between neighboring sites (Table 3). Since planulae are produced sexually, the only possible explanation for the broad geographic range of *N. vectensis* clones is the dispersal of adult polyps, or fragments of adult polyps, despite the sessile nature of these forms.

Although we find anthropogenic transport to be the most likely explanation for the broad extent of clonal genotypes, it should be noted that rafting presents a plausible alternative. *Nematostella vectensis* polyps occasionally cling to epifaunal substrates (J. Darling & A. Reitzel pers. obs.), and so may associate with marsh grass or algal rafts. Connectivity between populations of many coastal invertebrates with limited dispersal capacity may be mediated by rafting (Thiel & Gutow 2005), and observations of some species suggest that this mechanism may result in dispersal distances of many hundreds of kilometers (McCormick et al. 2008). In addition, some studies indicate that both anthropogenic transport and rafting together may contribute to the dispersal of certain taxa (Watts et al. 1998). As both mechanisms provide opportunities for long distance dispersal events and broad increases in geographic range, their effects may be difficult to distinguish. However, in the case of *N. vectensis* we find the rafting hypothesis less compelling than the hypothesis of anthropogenic transport for several reasons. First, frequent rafting results in surprisingly low genetic differentiation within regional populations (Dias et al. 2006), a pattern not observed in *N. vectensis*. In addition, the conspicuous genetic differentiation (and lack of shared MLGs) between Gray's Harbor and Willapa Bay suggests that dispersal vectors for *N. vectensis* are episodic and idiosyncratic, and do not necessarily connect estuaries in close geographic proximity. Finally, the direct dispersal of clonal propagules between San Juan Island and Humboldt Bay, without colonization of intervening habitat, is a pattern that seems difficult to explain based on a current-driven dispersal model.

Ballast water is commonly cited as a favored means of anthropogenic dispersal by many coastal and estuarine invasive species (Ruiz et al. 2000, Watts & Johnson 2004, Wonham & Carlton 2005). However, this vector seems unlikely in the case of *Nematostella vectensis*. Not only are adult anemones generally infaunal, but they are typically found in habitats where their entrainment in ballast water tanks would be improbable. Dispersal propagules are much more likely to travel as components of fouling communities, on recreational watercraft or other equipment (e.g. waders, fishing gear). *N. vectensis* polyps have an impressive adhesive quality (J. Darling & A. Reitzel pers. obs.) and are capable of passively attaching to

most surfaces. High thermal and salinity tolerances, combined with resistance to starvation and desiccation (Darling et al. 2004), could enable propagules to persist for many days outside of their natural environments.

Small scale intra-coastal vectors may play important roles in driving the range expansion of many invasive species (Wonham & Carlton 2005). A recent study of Elkhorn Slough, California, revealed the presence of numerous invasive taxa present elsewhere along the west coast, despite the absence of any commercial ports (Wasson et al. 2001), and fouling of recreational boats is probably a major vector for the movement of species between such habitats (Floerl & Inglis 2005). Fouling has been implicated in the global expansion of numerous invasive taxa, including crustaceans (Zardus & Hadfield 2005, Otani et al. 2007), bryozoans (Marchini et al. 2007), mollusks (Ricciardi 1998) and tunicates (Bullard et al. 2007). We suggest that this vector may be playing a central role in the expansion of the range of *Nematostella vectensis* in the eastern Pacific Ocean.

## Conclusions

The broad geographic distribution of multiple MLGs reveals that transport of adult *Nematostella vectensis* must be frequent enough to confer connectivity between multiple embayments throughout the region, thus overcoming documented natural limits to dispersal and confounding expectations for genetic and demographic patterns within this species. If adult anemones are more likely than planulae to be associated with human transport vectors, which would certainly hold true in the case of fouling, then asexual reproduction may provide the most efficient mechanism for the production of dispersive propagules, and could result in broad distributions. The high frequency with which estuarine taxa are dispersed by anthropogenic vectors, along with the facultative asexuality exhibited by many such species, suggests that the patterns observed in *N. vectensis* may be reflected in other species, both introduced and native.

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