Regional population structure of a widely introduced estuarine invertebrate: *Nematostella vectensis* Stephenson in New England

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Abstract

Nematostella vectensis is an infaunal anemone occurring in salt marshes, lagoons and other estuarine habitats in North America and the United Kingdom. Although it is considered rare and receives protection in England, it is widely distributed and abundant in the United States, particularly along the Atlantic coast. Recent studies suggest that both anthropogenic dispersal and reproductive plasticity may significantly influence the genetic structure of N. vectensis populations. Amplified fragment length polymorphism (AFLP) fingerprinting of individuals from nine populations in the northeastern United States indicates that stable populations are maintained by both asexual and sexual reproduction; in some cases asexually reproducing lineages exist within sexually reproducing populations. F statistics reveal extraordinarily high degrees of genetic differentiation between populations, even those separated by very short distances (less than 100 m). Genetic distances show little to no correlation with geographical distances, consistent with a role for sporadic, geographically discontinuous dispersal coupled with limited gene flow. No single genotype was found at more than one site, despite apparent homogeneity of habitat. In contrast with reported genotypic distributions for Nematostella in the United Kingdom, where a single clonal genotype dominates at multiple sites through southern England, our data thus fail to support the hypothesis of a general-purpose genotype in the northeastern United States. However, they are consistent with important roles for reproductive plasticity, sporadic introductions and complex local population dynamics in determining the global and regional distribution of this species.

Keywords: AFLP, anthropogenic dispersal, clonality, estuary, introduced species, *Nematostella vectensis*, reproductive plasticity

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Introduction

The starlet sea anemone *Nematostella vectensis* is a euryhaline, eurythermal anemone inhabiting salt marshes, saline lagoons and other sheltered estuarine environments. Despite the extraordinarily high productivity of such habitats, dramatic variability in both salinity and temperature results typically in extremely low diversity, dominated primarily by transient species (Long & Mason 1983; Little 2000). *N. vectensis* is one of few estuarine specialists capable of stably occupying these marginal environments.

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The current global distribution of *N. vectensis* is highly discontinuous, with populations in geographically isolated regions in the eastern Pacific, western Atlantic, northern English Channel and western North Sea (Hand & Uhlinger 1994). Although the species appears to be widespread and abundant in North America, particularly along the United States Atlantic coast, it is currently listed as vulnerable in the IUCN *Red Data Book* (last assessed in 1996), and remains protected in Britain under the Wildlife and Countryside Act. The past several decades have seen active conservation of English populations, ranging from preservation and restoration of habitat to artificial colonizations of relatively well-protected sites (Williams 1975; Williams 1983; Williams 1987; Williams 1988; Sheader *et al.*

1997). Under the most recent (2001) review of the UK Biodiversity Action Plan, conservation targets for *N. vectensis* include maintenance of viable populations at all current sites and reintroduction into approximately five novel sites by 2005.

Several lines of evidence suggest that the global distribution of N. vectensis cannot be explained by natural dispersal mechanisms alone. Although the species has been reported from both sides of the North Atlantic, it has never been reported from intervening arctic habitats (most notably Iceland), despite detailed biotic surveys of estuarine habitats in those regions (Carlgren 1939; Ingolfsson 1994, 1999). This led Hand & Uhlinger to suggest as early as 1994 that N. vectensis populations in England were a recent introduction from North America, and this hypothesis has been recognized and expanded by others (Hand et al. 1994; Sheader et al. 1997; Pearson et al. 2002). More recently, genetic studies of N. vectensis in England revealing the dominance of a single genotype throughout the region (Pearson et al. 2002) indicate the possibility of extreme founder events leading to population increases driven primarily (if not entirely) by asexual reproduction. Chapman and colleagues (unpublished), building on previous data (Kozloff 1983; Carlton unpublished), assessed populations of N. vectensis in the Northeast Pacific (where the species is distributed discontinuously and associated with estuaries of large navigable harbours) and found that the species satisfies seven major criteria for introduced status: (1) discontinuous global distribution; (2) insufficient natural dispersal to account for current distribution; (3) local distributions that are restricted or discontinuous relative to known native fauna; (4) adaptation to broader range of environmental conditions than experienced throughout its occupied geographical range in the northeast Pacific and England; (5) association with other known introduced species; (6) probable association with human-mediated dispersal mechanisms such as shipping; and (7) physiological characteristics incongruous with native fauna.

N. vectensis is thus probably one of a collection of widely introduced estuarine species that have been dispersed by human activities to geographically isolated regions of the globe. In North America alone, 298 introduced marine species have been reported from various coastal environments (Ruiz et al. 2000). Estuaries are particularly susceptible to foreign introductions, due to their frequent association with navigable harbours and their low native biodiversity (Cohen & Carlton 1998). N. vectensis possesses a number of characteristics that make it an ideal colonizer of these habitats: it is an excellent generalist, capable of tolerating considerable variability in salinity and temperature, even on very small temporal scales; it ships well, withstanding dessication and prolonged starvation; and it can reproduce asexually, allowing establishment of clonal populations from single individuals.

One motivation for characterizing the genetic structure of N. vectensis in New England is to understand more clearly the probable relative importance of anthropogenic and natural dispersal mechanisms at local, regional and global scales. Introduced species are among the major threats to global biodiversity. To understand the causes and consequences of introductions, it is critical to reconstruct their history and the routes by which they are occurring. It is similarly important to understand the evolutionary, ecological and developmental factors that affect the outcome of these anthropogenic introductions. A number of recent studies have used molecular markers to identify and track the sources of introduced populations (Downie 2002; Facon et al. 2003; Scheffer & Grissell 2003) and to compare the genetic structure of natives vs. non-natives (Amsellem et al. 2000; Tsutsui et al. 2000; Meunier et al. 2001; Tsutsui et al. 2001; Walker et al. 2003). Introduced populations generally exhibit markedly lower genetic diversity than populations from a species' native range (Holland 2000). A comparison of the genetic structure of N. vectensis in New England vs. England may bolster the hypothesis that N. vectensis is native to eastern North America and introduced in England (Pearson et al. 2002).

A second motivation for this study was to deduce the relative contributions of sexual and asexual reproduction to the genetic structure of natural populations of N. vectensis. Sexual reproduction involves the spawning of egg masses through the mouth, followed by external fertilization, and the development of a ciliated planula larva (Hand & Uhlinger 1992). Asexual reproduction occurs by transverse fission (Hand et al. 1992). While Pearson et al. (2002) report that asexual reproduction predominates in wild populations in England, the situation appears to be reversed in laboratory cultures (Hand & Uhlinger 1995). N. vectensis is so inclined towards sexual reproduction in laboratory cultures that even in the prolonged absence of males, females will spawn regularly while undergoing fission rarely (Hand et al. 1994). The unfertilized eggs undergo rapid degradation. However, some individuals are noteworthy for their proclivity to undergo fission, suggesting the possibility of significant genotypic variation in this trait (Hand et al. 1994). Because egg production is related to body size, and because wild-caught animals tend to be small relative to well-fed laboratory animals (< 1.0 cm vs. upwards of 5.0-10.0 cm in length), sexual reproduction may be more rare in the field than in the laboratory. Stable single-sex populations have been reported in the wild (Hand et al. 1994), and some of these populations have been observed to exhibit exceptionally high rates of population increase via asexual reproduction (Sheader et al. 1997). No male anemones have been reported from the United Kingdom, and recent genetic studies confirm the presence of asexually reproducing female clones, one of which comprises 61% of the total population and is represented at significant frequency at all English sites (Sheader *et al.* 1997; Pearson *et al.* 2002).

Molecular markers have been used recently to assess the contribution of sexual and asexual reproduction to population structure in a number of systems, including terrestrial plants (e.g. Kjolner et al. 2004) insects (e.g. Vorburger et al. 2003a) and marine invertebrates, including cnidarians such as anemones (Shaw 1991) and corals (Nishikawa & Sakai 2003; Miller & Ayre 2004). Each of these systems shares a fundamental similarity with N. vectensis - reproductive plasticity - but each of these systems differs from N. vectensis taxonomically, environmentally or developmentally in ways that could affect selection on reproductive traits. For example, parthenogenetic reproduction from unfertilized eggs can produce a larva with the same ontogeny and the same dispersal ability as a larva produced by a fertilized egg (e.g. aphids). However, asexual reproduction in N. vectensis yields a sessile propagule that is entirely distinct in terms of its ontogeny and dispersal ability from the motile planula larva produced via sexual reproduction. Although N. vectensis is an estuarine specialist, and the realized dispersal distance of its planula is likely to be substantially lower than the planulae of oceanic cnidarians such as corals.

Here we describe the population structure of N. vectensis from nine locations in the northeastern United States based on amplified fragment length polymorphism (AFLP) fingerprinting. AFLPs have been used with great success in other systems to determine population structure at the interspecific level (e.g. Dodd et al. 2002; Douek et al. 2002; Kai et al. 2002; Rivera-Ocasio et al. 2002; see Mueller & Wolfenbarger 1999 for review). Furthermore, the ease with which numerous markers can be generated by the AFLP protocol contributes to the identification of clonal populations and the investigation of asexual vs. sexual reproduction, as the large number of markers facilitates analysis of multilocus disequilibrium (Van Der Hulst et al. 2000; Garcia et al. 2002; Brem & Leuchtmann 2003; Eckert et al. 2003). Although AFLP markers suffer from the limitation of complete dominance, a number of statistical methods have been devised to provide unbiased estimators of various population genetic parameters from such markers (Lynch & Milligan 1994; Zhivotovsky 1999; Krauss 2000; Holsinger et al. 2002).

Materials and methods

Sample collection

In New England, *N. vectensis* is found most commonly in tidally restricted pools within high salt marsh habitats. These pools typically contain flocculent top sediments from which anemones are easily extracted by sifting. At each collection site, all suitable pools were examined for the presence of N. vectensis; whenever possible, animals were collected from multiple pools. Within each pool, individual anemones were collected from randomly selected points throughout the extent of suitable habitat. The possibility of asexual reproduction coupled with limited adult dispersal necessitated the sampling of multiple points at each site (even for small sites) to limit the possibility of bias toward spatially restricted clonal populations. Animals were isolated by sifting sediment over standard window screens (~1 mm mesh size). Animals were maintained alive in 50 mL conical tubes in marsh water for transport to the laboratory, where they were transferred to culture in freshly prepared one-third strength (~12 ppt) artificial seawater (Instant Ocean; Aquarium Systems). Cultured anemones were maintained as described (Hand et al. 1992). Live samples from Spurwink River, Maine, were kindly provided by Dr Michael Mazurkiewicz.

AFLP fingerprinting

N. vectensis DNA was extracted using the DNeasy kit (Qiagen) on small tissue fragments removed from the pedal end of each individual. Individuals used for DNA extraction were starved for a minimum of 5 days prior to extraction; this significantly minimized the risk of spurious amplification of contaminating DNA. Anemones were allowed to recover and returned to culture for possible future study. AFLP fingerprints were generated utilizing commercially available restriction site adaptors and fluorescently labelled AFLP primers (PE Applied Biosystems). All other DNA modifying enzymes were obtained through New England Biolabs. Reactions were performed following modifications of the PE Applied Biosystems Plant Mapping protocol. Restriction/ligation reactions were performed on approximately 50 ng DNA with 1 U MseI, 5 U EcoRI, 1 Weiss unit T4 DNA ligase and 1:10 dilutions of MseI and EcoRI adaptors. The total volume of reactions was 11 µL, and reaction buffer included 50 µg/mL bovine serum albumin, 50 mм Tris-HCl (pH 7.8), 10 mм MgCl₂, 10 mм dithiothreitol and 1 mM ATP. Restriction/ligation was run at 37 °C for 2 h, after which products were diluted 1:10 in 10 mм Tris-HCl (pH 8.0).

Primary amplification was carried out using 4 μ L of diluted restriction/ligation product and 1 μ L of preselective primers in a standard 20 μ L polymerase chain reaction (PCR) reaction containing 1.5 mM MgCl₂, 1× Mg-free *Taq* buffer (Promega), 100 μ M dNTPs and 1 U *Taq* polymerase. Reactions were run on an MJResearch PT200 thermocycler with the following cycling parameters: 120 s at 72 °C; 20 cyles of 20 s at 94 °C, 30 s at 56 °C, 120 s at 72 °C and 30 min at 60 °C. Products were diluted 1:20 in 10 mM Tris-HCl (pH 8.0).

Selective amplifications were conducted using 3 µL diluted primary amplification, 250 pM unlabelled (*MseI*)

primer and 50 pm fluorescently labelled (*Eco*RI) primer in a standard 20 µL PCR reaction as described above. After denaturation at 94 °C for 2 min reactions were run through 10 cycles, each cycle beginning with 20 s at 94 °C, followed by a 30 s annealing step at temperatures decreasing from 66 °C to 57 °C with each cycle, followed by a 120 s extension at 72 °C. Reactions were subjected to an additional 20 cycles of 20 s at 94 °C, 30 s at 56 °C and 120 s at 72 °C, followed by a final 30 min extension at 60 °C. Primer pairs *Eco*RI-ACT/*Mse*I-CAC and *Eco*RI-ACT/*Mse*I-CAT were used to generate all AFLP profiles.

Fingerprints were visualized on 5% Long Ranger XL polyacrylamide gels (BioWhittaker Molecular Applications), 36 cm well-to-read distance, using an ABI-377 automated sequencer with GENESCAN analysis software. Polymorphic bands were sized using internal GENESCAN-500 ROX size standards and scored semi-automatically using GENOTYPER software. Automated sequencer, size standard and analysis software were all obtained through PE Applied Biosystems.

Data analysis

The genetic relatedness of N. vectensis populations was assessed using relatedness trees generated by the UPGMA method. We used Nei's standard genetic distance (Nei 1972) as the coefficient of relatedness, and adopted Lynch & Milligan's (1994) Taylor expansion method for estimation of null allele frequency. We found other distance measures (Jaccard and Dice coefficients) and an alternate clustering algorithm (neighbour-joining) to predict identical tree topologies. Neighbour-joining analysis of individual samples was performed in PAUP, version 4.01 (Swofford 2003), using mean character difference as the distance metric. Mantel tests of correlation between genetic and geographical distance were implemented using TFPGA software version 1.3 (Miller 1997); for these tests, geographical distance was measured as total length of intervening coastline between sites.

Population genetic structure was determined both by analysis of molecular variance (Excoffier et al. 1992) using the ARLEQUIN software package version 2.0 (Schneider et al. 1997), and by the determination of F statistics. Dominant markers such as AFLPs present some statistical difficulties for estimation of the latter, as the classical approach of using the square root of the null homozygote frequency to determine null allele frequency gives biased estimates (Lynch et al. 1994; Krauss 2000). Lynch & Milligan's modified approach has been shown to reduce bias, but requires the assumption of Hardy-Weinberg equilibrium (Lynch et al. 1994). More recently, Bayesian approaches have been developed that allow estimation of F statistics without prior knowledge of population substructure (Zhivotovsky 1999; Holsinger *et al.* 2002). Here we estimate F_{ST} using both Nei's G_{ST} [calculated using POPGENE version 1.31 and

assuming full inbreeding (f = 1)] and the Bayesian parameter θ_b (determined with a uniform prior distribution of inbreeding coefficient f using HICKORY version 0.8; Holsinger *et al.* 2002).

We employed two indices to assess the degree of clonality in N. vectensis populations. The first (P) estimates the likelihood of obtaining the observed (or higher) number of individuals with identical multilocus genotypes, given the estimated frequency of alleles in the population (Tibayrenc et al. 1990). In some cases (such as that of Crane Reserve, MA, where 28 individual anemones show identical genotypes at all marker loci) such analysis proves unnecessary given the large number of loci analysed and the relatively large sample size. However, for ambiguous cases (e.g. Rye Harbor, where two of 12 individuals were found with the same genotype) this index provides some indication of the confidence with which these individuals can be said to be clonal. We also determined, using MULTILOCUS software version 1.3 (Agapow & Burt 2001), the modified index of association, $\bar{r}_{\rm D}$, to provide an indication of the degree of multilocus disequilibrium. The classical index of multilocus disequilibrium (I_A ; see Brown *et al.* 1980) suffers from dependence on the number of loci included in the analysis, limiting the ability to compare results between studies; the modified index normalizes for the number of loci and thus avoids this difficulty (Agapow, Burt 2001). Assessment of disequilibrium provides independent evidence of clonality, but is complicated by the fact that disequilibrium can result not only from asexual reproduction but also from inbreeding; it has thus been interpreted with some caution, primarily as independent confirmation of likelihoods estimated by P.

Results

Summary of AFLP results

Anemones were collected from nine sites throughout New England, ranging from Southern Maine to Connecticut (Fig. 1, Table 1). Of these populations, six were previously unreported (Hand *et al.* 1994). Although habitat at all sites was typical of New England salt marsh, sites varied widely in the degree of human-mediated modification, ranging from relatively pristine (Sippewissett), to heavily impacted by nearby development (Neponset, Odiorne Point, Rye Harbor), to recently restored (Crane). Sample sizes from each site depended primarily on site accessibility and population density. In total, 212 individuals from the region were genotyped. The two primer pairs utilized to generate AFLP fingerprints yielded a total of 169 markers used in subsequent analysis; of these, 154 (91.1%) were polymorphic when considered across the entire data set.

The occurrence of clonal populations provided a test for repeatability of AFLP fingerprints. For 28 clonal individuals

Name of site	State	Ν	No. of distinct genotypes	Frequency of most common genotype (%)	% Polymorphic markers	$H_{\rm E}$
1 Spurwink	ME	31	31	NA	46.15	0.1531
2 Odiorne Point	NH	15	15	NA	38.46	0.1459
3 Wallis Sands*	NH	22	22	NA	13.46	0.0321
4 Rye Harbor*	NH	12	11	17	15.38	0.0555
5 Old Town Hill*	MA	6	3	67	3.55	0.0135
6 Crane*	MA	30	3	93	1.78	0.0058
7 Neponset River*	MA	36	16	44	28.99	0.0713
8 Sippewissett Marsh	MA	36	36	NA	37.87	0.1084
9 Clinton*	CT	24	20	17	17.75	0.0628
All populations		212	157	13.2	91.12	0.2665

Table 1 Summary of AFLP results for nine *N. vectensis* populations. Population numbers correspond to Fig. 1. A total of 169 AFLP markers were used in the analysis

NA, not applicable; $H_{\rm E'}$ average expected heterozygosity, determined using Lynch & Milligan's Taylor expansion estimate for dominant markers; *previously unreported populations.



Fig. 1 Geographic distribution of New England sampling sites. (A) Atlantic coastline of North America, showing sampling region (grey shaded box). (B) Location of New England sampling sites, map expanded from shaded box in (A); site numbers correspond to Table 1.

from Crane Reservation, MA, identical fingerprints were obtained amounting to 28 independent AFLP reactions performed on the same genotype. All measurable electropherogram peaks in these fingerprints were 100% repeatable across all 28 reactions; this was true for both primer sets. Independent confirmation of repeatability was obtained by subjecting nine individuals (three individuals from Neponset, three from Odiorne Point and three from a cultured laboratory population) to three independent rounds of AFLP fingerprinting for both primer sets.

Reproductive plasticity

Of the nine populations, five exhibited genotype distributions indicative of asexual reproduction. In three cases (Neponset, Old Town Hill and Crane), single genotypes comprised a significant fraction of the entire genetic diversity (Table 1). Diversity, as indicated by average expected heterozygosity $(H_{\rm F})$, varied widely among populations, but was generally low. The population at Crane exhibited the most dramatic clonality, with all but two individuals (93%) possessing identical genotypes. This clonal genotype was found to persist over the course of 2 years of sampling at this site; a preliminary survey in the year preceding the collections analysed here found 100% of individuals (n = 5) exhibiting the same dominant genotype (data not shown). In two cases (Neponset and Clinton) multiple asexual lineages were identified. The large number of markers used in the study makes it highly unlikely that identical genotypes might have arisen randomly by recombination; *P*-values for all asexual lineages, even those consisting of just two individuals, reject this null hypothesis (Table 2). A modified multilocus index of association further confirms significant association of alleles in these populations (Table 2). It is notable that even in the case of sexual populations (e.g. Spurwink) there is significant linkage disequilibrium (P < 0.01; values are significant but an order of magnitude lower than for asexual populations), suggesting high levels of inbreeding (see below). Only in the case of the population at Wallis Sands was there no evidence of significant linkage disequilibrium (P = 0.26). No single genotype was observed in more than one population.

Genetic differentiation between populations

For hierarchical analysis of molecular variance (AMOVA), populations were divided into geographical groups north and south of Cape Cod, which represents the most significant regional geographical barrier to potential gene flow. The vast majority of variation (59.23%) was

	Index of asso	ociation	Likelihood of most common genotype		
Population		r _D	Genotype frequency (% total)	iency P	
Clinton	Observed	0.32074	16.7	4.856×10^{-21}	
	P-value	< 0.01	8.3	1.152×10^{-21}	
Neponset	Observed	0.293256	44.4	3.809×10^{-53}	
1	P-value	< 0.01	13.9	1.438×10^{-14}	
			5.6	1.324×10^{-7}	
Rye Harbor	Observed	0.150604	16.7	1.775×10^{-10}	
	P-value	< 0.01			
Crane	Observed	ND*	93.3	ND	
	P-value	ND*			
Old Town Hill	Observed	0.6	66.7	ND	
	<i>P</i> -value	< 0.01			
Odiorne	Observed	0.323227	NA	NA	
	<i>P</i> -value	< 0.01			
Sippewissett	Observed	0.018629	NA	NA	
	P-value	< 0.01			
Spurwink	Observed	0.018095	NA	NA	
	P-value	< 0.01			
Wallis Sands	Observed	0.005882	NA	NA	
	<i>P</i> -value	0.26			

Table 2 Contribution of asexual reproduction to genetic structure of *N. vectensis* populations, \bar{r}_D modified index of association; *P*, likelihood of obtaining equal or higher number of observed identical genotypes, given population allele frequency. Values of *P* are given for all genotypes occurring in more than one individual

NA, not applicable; ND, not done; *index of association values for the Crane population give unreliable results due to the high frequency of a single clonal genotype.

Source of variation	Degrees of freedom	Sum of squares	Variance components	% Total	<i>P</i> -value
Among populations	8	3644.296	14.75694	59.23	< 0.0001
Among subpopulation within populations	5	300.714	6.17885	24.80	< 0.0001
Within subpopulations	198	787.557	3.97701	15.96	< 0.0001

Table 3 Hierarchical AMOVA for New England *N. vectensis* populations. Populations consist of all individuals collected at a single site; subpopulations consist of individuals collected from single isolated pools; subpopulations were present only within Sippewissett, Clinton, and Odiorne populations

attributed to differences between populations, regardless of group (P < 0.0001, Table 3). Variation between individuals within populations (24.80% of total) was also highly significant (P < 0.0001), while variation attributed to differences between groups (15.96%) was relatively low but significant (P < 0.0001).

Determination of $F_{\rm ST}$ values further supports an extremely high degree of genetic divergence between populations (Table 4). Bayesian $F_{\rm ST}$ analogue θ_b , determined with an undefined prior distribution of inbreeding coefficient f (no prior assumption of population structure) gave extremely high estimates of genetic differentiation (Table 4) between populations throughout the region ($\theta_b = 0.7234$). In two cases, genetic variation was also found to be high between individual pools within a single sampling site. θ_b calculated for subpopulations at Sippewissett and Clinton indicate significant genetic structure within these sites ($\theta_b = 0.3075$ for Sippewissett, $\theta_b = 0.5899$ for Clinton). In one case (Odiorne Point), sampling of a single site on multiple occasions (once in the summer of 2002, a second time in the summer of 2003) allowed preliminary assessment of temporal change in genotype distribution; $\theta_b = 0.4177$ for these temporal subpopulations. Estimates of F_{ST} using Nei's G_{ST} and assuming full inbreeding (f = 1) gave similar results (Table 4). Estimators of F_{ST} requiring assumption of Hardy–Weinberg equilibrium were in marked disagreement with those used here, and are unlikely to be biologically relevant.

Neighbour joining analysis of individual genotypes provides a visual representation of the divergence between populations (Fig. 2). In all cases, genotypes are distributed into well-differentiated groups corresponding to individual populations. In the case of Clinton and Odiorne Point populations, subgroups corresponding to spatial (Clinton) or temporal (Odiorne) subpopulations are also well differentiated. Sippewissett subpopulations show significant,

Table 4 F_{ST} analogues calculated for all populations and for subpopulations at Sippewissett, Clinton and Odiorne Point. Subpopulations at Sippewissett and Clinton are geographical subpopulations (isolated individual pools within the sampling site); at Odiorne Point, subpopulations correspond to two collections taken roughly 1 year apart during the summers of 2002 and 2003. θ_b , Bayesian estimator of F_{ST} (Holsinger *et al.* 2002), with undefined prior distribution of inbreeding coefficient *f*, given with 95% credible interval (Bayesian analogue of 95% confidence interval); Nei's G_{ST} determined assuming full inbreeding (*f* = 1); ND, not done

	_	
Population	$ heta_b$	$G_{\rm ST}$
All populations		
Mean	0.7234	0.7170
95% CI	0.7006-0.7439	ND
Sippewissett		
Mean	0.3075	0.3345
95% CI	0.2383-0.3836	ND
Clinton		
Mean	0.5899	0.5627
95% CI	0.4848-0.6892	ND
Odiorne Point		
Mean	0.4177	0.6523
95% CI	0.3072-0.5332	ND

but less dramatic, correspondence of subpopulations to individual groups (not indicated in figure).

Relationships between populations

UPGMA clustering (Fig. 3) reveals no correlation between genetic relatedness and geographical proximity, except in the case of subpopulations within single sites (highly supported groups comprising Sippewissett and Clinton subpopulations). The Mantel test of correlation between genetic and geographical distance (conducted with Sippewissett and Clinton subpopulations collapsed into two single populations) quantitatively supports this conclusion (r = -0.118; P = 0.604).

Discussion

Contributions of sexual and asexual reproduction to population genetic structure

AFLP analysis of New England populations of *N. vectensis* indicates that both asexual and sexual reproductive strategies contribute significantly to overall genetic structure. Several populations exhibit high degrees of clonality, with one population (Crane) approaching complete monoclonality, while three populations (Spurwink, Sippewissett and Wallis Sands) show no evidence of asexual reproduction. It is probable that the Crane population is, in fact, monoclonal, as the frequency of polymorphic markers is extremely low (Table 1) and may be explained by the accumulation of somatic mutations. In intermediate cases it seems likely that asexual lineages exist within predominantly sexually reproducing populations. Without longitudinal study of these populations it is impossible to determine whether or not these lineages are stable and capable of persisting despite the possibility of recombination with sexually reproducing conspecifics.

Although our data do suggest a substantial contribution of asexual reproduction to overall genetic structure, the level of clonality observed in New England is far less pronounced than that reported for English populations of N. vectensis. Previous studies demonstrated that over 60% of the entire English population possesses the same single genotype; that genotype was found at significant frequencies (3-100%) in all English populations surveyed (Pearson et al. 2002). In contrast, the overall observed degree of clonality in New England was only 29.7% (frequency of all genotypes observed in more than one individual; compare to 82.8% in England), the most frequent single genotype (from Crane) accounted for 13.2% of all individuals assayed and no single genotype was found in more than one population. In part, the recognition of greater diversity in the present study may be the result of the greater resolution afforded by the use of AFLP markers; genetic analyses of English anemones were based on only 31 polymorphic RAPD makers generated from six primer pairs, in contrast to the 169 AFLP markers used here.

This study identified a handful of abundant asexual lineages in New England, amid a background of predominantly sexual reproduction. Three hypotheses may explain the apparent success of particular asexual lineages. First, asexual reproduction may be a particularly adaptive strategy in marginal habitats characterized by infrequent colonizations and low diversity, such as the estuarine habitats populated by N. vectensis. In these marginal and highly variable habitats, selection may favour either a highly fitting 'general-purpose genotype' (Baker 1965; Lynch 1984) or a niche specialist (Vrijenhoek 1984; Vrijenhoek 1998a,b). Regardless of whether selection has favoured a generalist genotype or a niche specialist, asexual reproduction will preserve favourable combinations of alleles in the offspring of well-adapted individuals. If the adaptive benefits of generalism or niche specialization are sufficiently great, asexual lineages may be able to persist despite the presence of sexually reproducing conspecifics, or may even displace them.

Another possible explanation for the local abundance of a specific asexual lineage may be its proclivity to reproduce asexually. In *Nematostella*, asexual propagules are incapable of dispersal, while sexually produced planula larvae are capable of dispersal. A lineage that engages preferentially in asexual fission even where sexual reproduction is feasible may come to achieve local numerical dominance,



Fig. 2 Neighbour-joining tree for all 212 individuals. Scale bar shows genetic distance based on mean character difference. Branchpoints associated with clonal lineages are marked by a number indicating the number of individuals with that genotype.

particularly if the recruitment of new larvae is low. This can occur even if the adult phenotype is not better adapted to the local conditions than the average phenotype of sexually produced offspring. Data from laboratory populations of *N. vectensis* suggest that there may be pronounced variation among genotypes in rates of asexual reproduction (Hand *et al.* 1994).

Finally, the establishment of a stable asexual lineage may be a stochastic event initiated sporadically in reproductively plastic populations. In this case, the persistence of asexual lineages would be primarily a function of demography, and not selective advantage. In populations of extremely low density, sexual reproduction may be prohibited if the mechanics of fertilization require a sufficiently dense population (Yund 2000). Colonizations of novel sites will typically involve the transfer of a small number of animals into a previously uninhabited site, resulting initially in a low-density, low-diversity population. In such cases, local clonal lineages may become dominant (Baker 1965). Reproductive plasticity of *N. vectensis* could thus dramatically affect genetic structure of colonizing populations. The potential exists for a single individual to found an entire stable population, and sporadic dispersal of one or few individuals may thus result in the establishment of clonal populations.

It is important to note, however, that adaptive and demographic hypotheses for the persistence of clonal populations need not be mutually exclusive. The dramatic



Fig. 3 UPGMA dendrogram for New England *N. vectensis* populations. Bootstrap values over 50% (based on 1023 randomizations) are shown next to corresponding nodes. Geographic subpopulations at Sippewissett and Clinton sites and temporal populations at Odiorne were included as separate populations in the analysis. Scale bar is Nei's genetic distance. For Mantel test of correlation between genetic and geographical distances, r = -0.118 (P = 0.604).

reductions in genotypic diversity and decreased frequency of sexual recombination that accompany extreme founder events may result in population structures conducive to either adaptive sweeps by particularly fit genotypes (Lynch 1984), or competitive exclusion leading to local niche specialization (Vrijenhoek 1998b). The likelihood of disrupting relatively fit genotypes through recombination may be elevated in cases of reduced local diversity, in which welladapted allelic combinations may be rare. Such conditions are likely to be met frequently in populations introduced to novel sites through range expansion or episodic dispersal.

Previous genetic studies of N. vectensis have raised the possibility that the dominant English genotype may be a general-purpose genotype with broad environmental tolerances (Pearson et al. 2002). This hypothesis has been invoked in other systems to explain the common observation of asexual taxa occupying broader geographical ranges and more disturbed and/or marginal habitats than closely related sexual taxa ('geographical parthenogenesis'; Lynch 1984; Bierzychudek 1989; Myers et al. 2000; VanDoninck et al. 2002). However, the hypothesis has received only mixed empirical support. Although generalism does appear to characterize certain widely distributed asexual taxa (Michaels & Bazzaz 1989; VanDoninck et al. 2002), the majority of empirical tests have failed to detect significantly broader tolerances in such taxa when compared to sexually reproducing relatives (Weider 1993; Kenny 1996; Parker et al. 2003; Vorburger et al. 2003a). Most recently, the potential importance of adaptive generalism in the establishment of marginal populations has renewed interest in the general-purpose genotype hypothesis as it relates to the evolutionary genetics of introduced species (Lee 2002; Parker *et al.* 2003). Work has also been conducted on species with mixed life-history strategies (i.e. species with true reproductive plasticity). Tests for generalism in obligately parthenogenetic populations (relative to cyclically parthenogenetic conspecifics) of both *Daphnia pulex* and *Myzuz persicae* failed to uncover any evidence of general-purpose genotypes in these species (Weider 1993; Vorburger *et al.* 2003b).

At present, there is no direct evidence that any particular asexual lineages of N. vectensis are competitively superior to their sexually reproducing counterparts, either as generalists or niche specialists. The latter possibility seems unlikely for New England populations, given the apparent local homogeneity of habitat from which anemones have been collected. Individual pools, which in some cases harbour asexual lineages within sexually reproducing populations, are lacking in the ecological complexity requisite for niche specialization. There is, however, significant circumstantial evidence to suggest that asexual lineages are more prevalent in recently colonized locales. Pacific populations of *N. vectensis* are thought to be recent introductions (Chapman et al., unpublished; Kozloff 1983; Carlton 2003), and the large majority of populations in this region appear to be entirely female, suggesting the dominance of clonal propagation (Hand et al. 1994). The regional genetic structure in the northwestern United States may thus parallel that reported for England, and both may contrast rather dramatically with that along the Atlantic coast of the United States, where mixed-sex populations and sexual reproduction are far more common (Hand et al. 1994). It is notable that the one probable monoclonal population found in the current study (Crane) inhabits a site that underwent tidal restoration in 1999 (Hutchins et al. 2001); the species was not found within suitable habitat throughout an expansive area of marsh outside of the restoration area. The clonal population at this site may thus be the result of a recent introduction to a disturbed habitat. Unfortunately, biotic surveys from the prerestoration marsh did not include infaunal macroinvertebrates, and it is not known whether N. vectensis presence at the site predated the restoration. The greater genetic diversity and the proportionately smaller role for asexual reproduction seen in New England vs. England are consistent with the hypothesis that New England is part of N. vectensis' native range. Additional AFLP data from both outlying populations (England and the Pacific coast of the United States) and contiguous populations (mid-Atlantic and southeastern United States) could confirm this hypothesis if such data reveal western Atlantic source populations for the possibly introduced populations in England and the Pacific.

Geographic distribution of N. vectensis

Our analysis fails to identify any signature of isolation by distance within the New England region. The Mantel test

of correlation between genetic and geographical distance shows no evidence of geographical structure (r = -0.118; P = 0.604), and UPGMA clustering reveals a pattern of genetic relatedness that is strikingly incongruous with geographical distributions (Fig. 3). For example, although geographical subpopulations within Sippewissett and Clinton sites group closely together, individual populations isolated by only several kilometres fail to cluster together in genetic distance analyses. Most conspicuous is the failure of the Rye Harbor population to group with its nearest geographical neighbours. Odiorne Point, Wallis Sands and Rye Harbor populations lie together within a 7km stretch of New Hampshire coastline; however, while Odiorne Point and Wallis Sands populations cluster in a single group, the Rye Harbor population lies at significant genetic distance, clustering together in a relatively wellsupported group with Crane and Clinton populations. The results of our analyses are thus consistent with the hypothesis of sporadic dispersal of N. vectensis, perhaps by anthropogenic means. Similar results were reported previously by Pearson et al. (2002), who found no evidence of correlation between genetic and geographical distances, although those results are influenced clearly by the presence of a single genotype in all sampled populations. In both studies, the combination of reproductive plasticity and episodic, discontinuous dispersal of small numbers of anemones offers one reasonable explanation for the observed regional distribution. However, it is also possible that any signature of isolation by distance would be swamped by the dramatic genetic structuring of populations caused by complex local population dynamics (see below). The significant shift in genetic relatedness between Odiorne and other populations over the course of 1 year (Fig. 2) may reflect such dynamics. This combination of episodic dispersal and complex local dynamics may mask genetic continuity of populations over broader spatial scales, potentially complicating future attempts to determine the native and introduced ranges of this species. Such complications may be common for introduced coastal marine organisms, with multiple introductions between and within coastlines requiring a 'metainvasion' framework for the reconstruction of historical biogeography (Davies et al. 1999).

Potential causes of high levels of genetic differentiation between populations

Genetic differentiation between populations of *N. vectensis* is unusually high, suggesting either very limited gene flow between sites, extreme population dynamics resulting in frequent bottlenecks, or some combination of the two. While adult *N. vectensis* are effectively sessile, planula larvae are active swimmers, and in laboratory cultures can persist for 7–14 days before undergoing successful

metamorphosis (Hand *et al.* 1992). This pelagic period could permit dispersal over tens to hundreds of kilometres in oceanic currents (Strathmann *et al.* 2002). However, empirical studies have indicated that the difference in potential vs. realized dispersal can be large, as larval behaviour or restricted water flow can significantly reduce dispersal distances, resulting in larval retention (e.g. Bingham & Young 1991; reviewed in Jackson 1986). Furthermore, work with cnidarians has shown a degree of parental habitat homing in settlement behaviour that may significantly reduce realized dispersal distances (Siefker *et al.* 2000). Recruitment of larvae from geographically distant populations thus may be biologically limited by larval behaviour. Gene flow may be limited further by physical barriers restricting access of larvae to open ocean.

Although the genetic divergence between populations is striking, even more remarkable is the high degree of genetic differentiation between subpopulations within single sites. At Sippewissett Marsh, subpopulations inhabiting four isolated pools separated by no more than 100 m revealed significant differentiation, as indicated by F_{ST} analogues. This variation exists despite regular tidal inundation of the marsh (several times a month, on average), which ought to provide ample opportunity for both migration and larval recruitment from neighbouring pools (Dr Ivan Valiela, personal communication). Genetic isolation is even more dramatic for two subpopulations at Clinton, CT. These two populations, although within approximately 25 m of each other, are separated by a large tidal creek, which may provide additional restrictions to gene flow between the two pools.

Nevertheless, it seems unlikely that restrictions to migration and recruitment alone could account for such extreme levels of genetic divergence within sites. Observed differentiation may thus be a result, at least in part, of local population dynamics. Temporal habitat variability is likely to be far more significant than spatial variability, particularly for temperate New England marshes. Seasonal population fluctuations may lead to frequent bottlenecks, and unless gene flow between subpopulations is unrestricted such fluctuations could result in conspicuous genetic structuring of the metapopulation. Demographic studies of English N. vectensis populations have revealed such seasonal fluctuations in population density, with densities varying over three orders of magnitude. At one English site, N. vectensis abundance was observed to fluctuate from under 100 m⁻² to over 2500 m⁻² and back again in the course of a single calendar year (Sheader et al. 1997). The hypothesis is supported further in part by the observation of a high degree of genetic differentiation between temporal subpopulations at Odiorne Point. Determination of F_{ST} analogues for temporally distinct samples has been used in other studies to detect population dynamics (Takami et al. 2004); although our data are limited, they do support the hypothesis of significant temporal variability in genotype distribution, even within a single site.

Conclusions

Limited data are available on the population structures of species stably inhabiting salt marsh and lagoonal habitats. The current study, in concert with previous genetic and demographic studies of N. vectensis (Sheader et al. 1997; Pearson et al. 2002), suggests that many of the characteristics allowing this species to specialize in these marginal and highly variable habitats may also make it particularly well suited to colonization of novel sites, and thus to sporadic introduction by human or other means. Our data suggest that the regional population genetic structure of N. vectensis in New England is strongly influenced by (1) reproductive plasticity, (2) episodic, geographically discontinuous dispersal and (3) extreme population dynamics on small spatio-temporal scales. All these observations are also consistent with observed global distribution patterns. In particular, the high frequency of asexually propagated populations in the Northwestern United States and England is consistent with founder events followed by clonal expansion and subsequent local reintroductions. This mechanism probably operates within coastlines as well, particularly those coastlines modified and fragmented by heavy development and subjected to frequent shipping.

The observed distribution pattern of *N. vectensis* genotypes reinforces the importance of life-history strategies in shaping evolutionary responses to range expansion and introduction (Parker *et al.* 2003). The data presented here, coupled with previous findings, suggest that *N. vectensis* will be a very useful model for exploring the ecology, developmental mechanisms and evolution of reproductive plasticity. The genetic data reveal the existence of small, tractable wild populations in which both sexual and asexual reproduction are occurring. Both sexual and asexual reproduction are tractable in the laboratory, and there is evidence for genotypic variability in rates of asexual propagation (Hand *et al.* 1994). Finally, proven molecular protocols exist for studying developmental mechanisms at the molecular level (e.g. Finnerty *et al.* 2004).

Further genetic studies of *N. vectensis* throughout its global range will be required to determine the native range of the species. Although diversity in New England is far greater than that observed in England, diversities may be even higher in regions such as the southeastern United States, where more continuous habitat may contribute to increased gene flow and decreased frequency of clonal populations. In addition, longitudinal studies of local populations, both genetic and demographic, will probably provide greater insight into population dynamics and the potential role of local extinctions, re-colonizations and bottlenecks in maintaining local genetic structure.

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This research is part of an ongoing investigation of reproductive plasticity in *N. vectensis vectensis* across various levels of the biological heirarchy. John Darling is a postdoctoral researcher interested primarily in evolutionary responses of introduced populations and the genetic consequences of introductions. Adam Reitzel is a graduate student studying the physiological bases of reproductive plasticity in *N. vectensis* and the evolutionary relevance of alternative life-history strategies. Work in John Finnerty's laboratory at Boston University takes advantage of *N. vectensis* as a model system to explore questions in comparative genomics, developmental evolution and evolutionary ecology.