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Research Article

Analyses with newly developed microsatellite markers elucidate the spread dynamics of *Tricellaria inopinata* d'Hondt and Occhipinti-Ambrogi, 1985 - a recently established bryozoan along the New England seashore

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Abstract

The majority of bryozoans possess short-lived larvae with limited dispersal potential, yet many species of bryozoans have obtained global distributions. The marine bryozoan *Tricellaria inopinata* d'Hondt and Occhipinti-Ambrogi, 1985 was first described from specimens collected in 1982 in the Lagoon of Venice, but is thought to be native to the northeast Pacific. By 2009, it could be found in various locales in the eastern Atlantic including Spain, France, England, and Ireland. In 2010, *T. inopinata* was found for the first time in the western Atlantic in Eel Pond, Woods Hole, Massachusetts, and shortly thereafter was collected in several other locales in the area. A newly developed suite of polymorphic microsatellite loci was used to investigate this recent range expansion of *T. inopinata* in an effort to understand what makes these animals such successful invaders. We examined various aspects of the population genetics of adult colonies collected from four sites in eastern Massachusetts: Eel Pond, Boston Harbor, Marblehead and Gloucester. There was significant genotypic differentiation between all sites. Higher homogeneity between the Eel Pond and Boston Harbor populations, as well as the potential for Eel Pond acting as a source of migrants for Boston Harbor, suggest that Eel Pond was a source population for the Boston Harbor population. In contrast, levels of genotypic differentiation and a lack of migrants suggested that the Marblehead and Gloucester populations likely did not originate from animals in Eel Pond. Thus, the recent range expansion by *T. inopinata* in the western Atlantic appears to be a result of multiple introduction events.

Key words: invasive species, population structure, dispersal dynamics

Introduction

Due to increases in global connectivity, the number of species being introduced into areas outside of their native range is increasing. Alien species are widely considered the second largest threat to biodiversity globally after habitat loss (Simberloff et al. 2013). Invasive species not only adversely affect biodiversity, but their economic impact can be immense. In response to the growing awareness of the threat of invasive species, the field of invasion ecology has grown substantially (Lockwood et al. 2007), due in part to the increased interest in characterizing the genetics underlying recent invasions. Molecular genetic tools have been proposed as useful for understanding and managing invasive species (Sakai et al. 2001), and there have been recent recommendations for increased investment into developing these tools for analyzing invasions (e.g., Blanchet 2012; Ojaveer et al. 2014). Indeed, these types of genetic tools have been successfully used to re-create past invasion history. For instance, Mackie et al. (2006) used mtCOI haplotype data to infer global invasion patterns in three species of bryozoans. Darling et al. (2012) used microsatellites to implicate shipping traffic in the range expansion of the invasive ascidian *Styela clava* Herdman, 1881 along the northwest coast of North America. Of particular interest is the use of genetic tools to characterize an ongoing range expansion in an invasive species. Such an invasion is ongoing by a marine bryozoan (*Tricellaria inopinata* d'Hondt and Occhipinti-Ambrogi, 1985) recently introduced to North America (e.g., Johnson et al. 2012).

Bryozoans are colonial organisms with indirect development. The phylum is dominated by sessile

species with short-lived, non-feeding, larvae that spend little time in the water column. For example, larvae from *Bugula stolonifera* Ryland, 1960 will usually initiate irreversible settlement and metamorphosis within four hours of release (Woollacott et al. 1989; Wendt and Woollacott 1999). Despite the limited dispersal potential, numerous species of bryozoans have obtained global distributions, most likely in association with shipping traffic, aquaculture, and possibly the aquarium trade (Mackie et al. 2006; Rodgers and Woollacott 2006; Ryland et al. 2011). Watts et al. (1998) examined selected biological characteristics of 197 globally distributed species of bryozoans and found that their ability to adhere to ships best explained the geographic ranges of these animals. Although the ability of bryozoans to adhere to man-made structures and experience anthropogenic dispersal is well documented, how these animals are able to establish and spread following long distance dispersal events remains unclear. Analyses of the recent introduction and spread of *Tricellaria inopinata* to North America, however**,** gives insight into why these animals are so adept at establishing and spreading in introduced areas.

Tricellaria inopinata was first described from specimens collected in the Lagoon of Venice in 1982 (d'Hondt and Occhipinti-Ambrogi 1985), although its native range is thought to be in the eastern Pacific (Dyrynda et al. 2000). The likely vectors of transport into the Mediterranean are shipping traffic and the aquaculture industry (Occhipinti-Ambrogi 1991; 2000), although it is unclear which mode was ultimately responsible. Shortly after its discovery, *T. inopinata* spread throughout the Lagoon and within 20 years was found in the northeastern Atlantic; it was first reported in southern England (Dyrynda et al. 2000) and then from France, the Netherlands, and Belgium (De Blauwe and Faasse 2001). *Tricellaria inopinata* has become a species of interest as it has steadily increased its range in the north Atlantic. Recent surveys have detected *T. inopinata* in Scotland (Beveridge et al. 2011) and Ireland (Kelso and Wyse Jackson 2012). In 2010, *T. inopinata* was found for the first time in the western Atlantic (Johnson et al. 2012). On discovery, the species had already established itself as a thriving member of the fouling community in Eel Pond, Woods Hole, MA, and could be found in dense aggregations throughout the pond. Even within a short period of time following its arrival in Eel Pond, *T. inopinata* was found to negatively affect the pre-existing fouling community. Bryozoans with growth forms similar to *T. inopinata* have decreased in abundance, and *T. inopinata* has been found epibiotic on several different organisms including algae, ascidians, and bryozoans (Johnson et al. 2012). As with the population in the Lagoon of Venice, *T. inopinata* has spread rapidly to surrounding areas. Following its discovery in Eel Pond, *T. inopinata* was found in Gloucester, MA, in 2011 (CD Wells, University of Washington, Seattle, WA, pers. comm.), and a recent survey shows that *T. inopinata* can now be found in several other New England locales (Wells et al. 2014).

The recent introduction of *T. inopinata* to the western Atlantic provides an opportunity to study the dynamics of an invasion as it is occurring, which could provide insight into how such species can colonize and spread after a transport event. The use of population genetic analyses has proven useful in examining the genetics behind invasions in other species (Schwaninger 1999; Mackie et al. 2006; 2012; Darling et al. 2012; Ghabooli et al. 2013); however, suitable genetic markers for conducting fine-scale population genetic analyses have not been developed for *T. inopinata*. The goals of this study were: 1) to develop a microsatellite library for *T. inopinata*, markers that are suitable for fine-scale population genetic analyses, and 2) to use this library to investigate the introduction of *T. inopinata* to North America.

Methods

Animal collection

About 30 adult *Tricellaria inopinata* colonies were collected from each of four sites in Massachusetts, USA, from May to September 2013 (Figure 1). To ensure adequate sampling, attempts were made to collect from multiple areas within each site. Animals were immediately fixed in 95% EtOH and stored frozen (-20°C) until used. Because bryozoan larvae will settle and metamorphose on adults (e.g., Johnson and Woollacott 2010), collected colonies were dissected and inspected for these attached individuals prior to DNA extraction. Further, *T. inopinata* broods embryos within the colony; therefore, only portions of colonies lacking attached individuals and brood chambers were used for genetic analyses. DNA was extracted using the Qiagen DNEasy Blood and Tissue kit. Extracted DNA was quantified using a Nanodrop-1000 Spectrophotometer, and the resulting yield for each extraction used was ≥25 ng/μL.

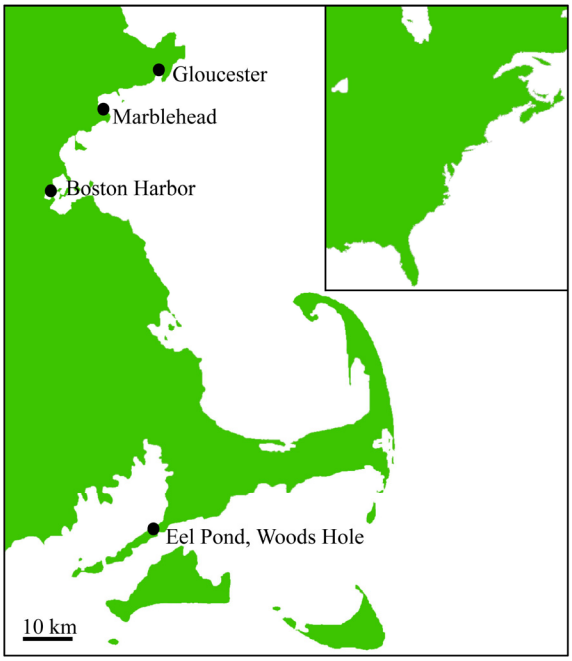

Figure 1. Map of eastern North America (inset) and close-up of eastern Massachusetts showing locations of sampling sites. *Tricellaria inopinata* was first reported in 2010 in Eel Pond, Woods Hole, MA, and was subsequently found in Boston Harbor, Marblehead, and Gloucester. Map created with the WorldMap Platform developed by the Center for Geographic Analysis, Harvard University (worldmap.harvard.edu).

Microsatellite library development

The microsatellite library for *T. inopinata* was developed using next generation sequencing techniques. Initially, total genomic DNA was extracted from one individual collected from Eel Pond, Woods Hole, MA, in June 2013. The colony was fixed in 95% EtOH, and then inspected for the presence of brood chambers and epibiotic organisms. DNA was extracted and quantified as previously described, resulting in a final DNA concentration of 350 ng/μL. The genomic DNA was sheared to an average of 500 bases, and then sequenced using the 2×250 base pair read length configuration on an Illumina MiSeq sequencer, resulting in a total of 15 million reads. Sequencing data were then subjected to the program Trimmomatic v. 0.32 (Bolger et al. 2014), which removes Illumina FastQ data and any adaptors added during the MiSeq sequencing. Trimmed MiSeq data were assembled using ABySS v. 1.3.5 (Simpson et al. 2009), and di-, tri-, and tetra-nucleotide microsatellites were identified using MISA (Thiel et al. 2003). These microsatellites were then manually inspected to verify the repeat motif identified by MISA, as well as to identify sequences with sufficient flanking region allowing for primer design. Primers for appropriate sequences were designed with Primer3 v. 4.0.0 (Untergasser et al. 2012) and tested for amplification. Twelve primer pairs were found to amplify consistently using gel electrophoresis, and were then divided into four groups based on expected size of the amplified product. The 5' ends of the forward primer of these pairs were fluorescently labelled with either 6-FAM, VIC, NED or PET dyes and utilized in a Qiagen Multiplex PCR reaction. Reactions were performed in 10 μL volumes using the protocol published in Johnson and Woollacott (2012). PCR products were diluted with 40μL dH_2O and run on an ABI 3730 xl DNA sequencer using the size standard GeneScan-500 LIZ. The dilution step prevented over-saturation by the fluorescent dyes, which could prevent fluorescent peaks from being scored correctly. Results were analyzed manually using PeakScanner v. 1.0 (Applied Biosystems). Any loci that failed to amplify during the Multiplex reaction were re-run using the same protocol with only the primer of interest included in the reaction. Because bryozoan colonies can asexually reproduce *via* rhizoids (root-like projections that aid in colony support), only individuals with unique genotypes were included in analyses. For the purposes of characterizing these newly developed microsatellite loci, all genotypes were pooled. Descriptive statistics including the number of alleles per locus, expected and observed heterozygosity, and the polymorphic information content were calculated with CERVUS 3.0.7 (Kalinowski et al. 2007). The potential presence of null alleles at each locus was investigated using MICRO-CHECKER v. 2.2.3 (van Oosterhout et al. 2004). Linkage disequilibrium between loci and deviations from Hardy-Weinberg equilibrium (HWE) at each locus were analyzed using GENEPOP 4.3 (Rousset 2008), with Markov Chain Monte Carlo (MCMC) parameters set to 10,000 de-memorizations, 500 batches, and 10,000 iterations per batch. To compensate for conducting multiple comparisons within the same test, significance levels were adjusted using the sequential Bonferroni correction (Rice 1989).

Population comparisons

The descriptive statistics for each of the populations sampled, including the number of individuals genotyped, the percentage of polymorphic loci, the average alleles per locus,

and expected and observed heterozygosity, were calculated with GDA v. 1.1 (Lewis and Zaykin 2002). Estimations of Wright's inbreeding coefficient (F_{IS}) were calculated in Genetix v. 4.05 (Belkhir et al. 1996–2004), with significance determined based on 10,000 permutations. To determine the proportion of the genetic variation at various hierarchical levels, an analysis of molecular variance (AMOVA) was performed in Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010). Genotypic data for each individual were compiled by sampling site, but no further structure was specified in the structure editor. The locus-by-locus AMOVA partitioned the molecular variance among populations, among individuals within populations, and within individuals. Fixation indices were averaged across all loci, with significance determined using 10,000 permutations.

Genotypic differentiation between sampling sites was investigated using several different analyses. First, pairwise F_{ST} values, and their significance, were calculated in Arlequin, with permutations set to 10,000. Additionally, the data were subjected to the exact test for genotypic differentiation conducted in GENEPOP, which analyzes the distribution of diploid genotypes in all pairs and assumes that they are randomly distributed (Raymond and Rousset 1995). Settings for the MCMC run were as previously described. Finally, recent evidence has suggested that estimating population differentiation using F_{ST} values, particularly with microsatellite data, could potentially be problematic (e.g., Jost 2008). To that end, D values, as described by Jost (2008), were estimated using GenAlEx v. 6.5 (Peakall and Smouse 2006) with significance determined based on 9,999 permutations. Isolation by distance among the data was also analyzed in GenAlEx using a Mantel test, which examines the correlation between geographic and genetic distance within the data set. Approximate UTM coordinates were determined for each sampling site, and significance was determined based on 9,999 permutations.

Examining spread dynamics

Although *T. inopinata* was first reported in Eel Pond in 2010 (Johnson et al. 2012), and has subsequently been found at other locales, it remains unclear how these animals were able to spread following their introduction. Further, it is unclear if Eel Pond was the site of the initial introduction event, or if this appearance in the western Atlantic is a result of repeated introduction events. Analyses were conducted to attempt to examine the spread dynamics of these animals. Initially, all populations were investigated for evidence of a genetic bottleneck, an occurrence that is implicit in many long-distance dispersal events. Microsatellite data were analyzed with Bottleneck v. 1.2.02 (Cornuet and Luikart 1996). The program Bottleneck examines allele frequency data and compares the observed genetic heterozygosity to the expected equilibrium heterozygosity. Because during a bottleneck allelic diversity decreases faster than genetic heterozygosity, populations that have experienced a recent bottleneck should have higher than expected heterozygosity. While the program Bottleneck does not require the investigated loci to be in HWE, Cornuet and Luikart (1996) suggested caution when analyzing data that deviates from Hardy-Weinberg proportions. Luikart and Cornuet (1998), however, found that inclusion of loci outside of HWE did not alter results from analyses that only incorporated loci within HWE. Data were analyzed with the two-phase model of mutation (TPM), which is recommended for microsatellite data (Luikart et al. 1998), with the proportion of stepwise mutations set to 95%, variance set to 30, and 10,000 replications. Following this, data were subjected to the program STRUCTURE v. 2.3.4 (Pritchard et al. 2000), which uses a Bayesian algorithm to infer population structure. Depending on the mode of introduction, sampled populations might cluster if individuals from one site established subsequent populations elsewhere. Alternatively, if the introduction is a result of multiple independent events, it might be expected that the sampling sites would fail to cluster. The program was set to use the admixture model, but not incorporate the linkage model or prior population information to pre-assign individuals. The number of clusters (K) was estimated using values of K ranging from 1 to 4, and 10 independent runs for each value, with number of replications set to 1,000,000 and a burn-in set to 100,000. The online program STRUCTURE HARVESTER v. 0.3 (Earl and vanHoldt 2012) was used to determine the ideal number of clusters based on the ΔK method described by Evanno et al. (2005), as well as to process the data for CLUMPP (Jakobsson and Rosenberg 2007). CLUMPP was used to align the cluster assignments from the multiple, independent runs conducted in STRUCTURE. Results from CLUMPP were then used as input for the program DISTRUCT (Rosenberg 2004), which allows for the visualization of the clustering assignments.

Table 1. Accession numbers and characteristics for microsatellite loci developed for *Tricellaria inopinata*. The 5′ end of each forward primer was fluorescently labeled and amplifications were conducted as Multiplex reactions. Primer annealing temperature was 60°C for each primer pair. One failed amplification occurred in Tri.inopinata3 and Tri.inopinata5, resulting in reduced numbers of individuals (*N*) for those loci. Number of alleles and size range in base pairs (bp) are indicated for each locus. Expected heterozygosity (H_E), observed heterozygosity (H_O), and polymorphic information content (PIC) were calculated using CERVUS 3.0.7. Significant deviations from expected Hardy-Weinberg equilibrium indicated with *(GENEPOP 4.2, $P < 0.0001$).

GenBank no./Locus	Repeat motif	Primer sequence (5′- 3′)	*N*	No. of alleles	Size (bp)	H_E	H_O	PIC
KM396464/Tri.inopinata1	(TA)12	F: TGAAGAAGAGTTATGCATGTGTTATAC R: ACAAGCACAGTTACCCTAGATG	105	9	204–222	0.812	0.419*	0.786
KM396465/Tri.inopinata2	(ACT)10	F: TCTGCTCTCACCAACTCAAAG R: ACACCAGGATAGAAACCCGG	105	5	290–305	0.578	0.295*	0.484
KM396466/Tri.inopinata3	(ATG)7	F: GTGAAAGCGAAGGAGATGGC R: ACCTCTCTCCTACGTTTCTGC	104	12	171–264	0.763	0.337*	0.730
KM396467/Tri.inopinata4	(CA)8 (TA)7	F: CGCCACCCAATACACATGAG R: AGCGCACTCAATAGATTTCCAG	105	14	284–326	0.736	0.438*	0.701
KM396468/Tri.inopinata5	(GCT)8	F: AACAGCCATTTCCACCAACC R: GTGACTGATACTTACACACTCTCAC	104	18	296–359	0.893	0.365*	0.879
KM396469/Tri.inopinata6	(GCA)6	F: TCCACCACCTTCAGCCTTAG R: TGCTGCAGACACTAACTTGC	105	5	204–216	0.727	0.724	0.678
KM396470/Tri.inopinata7	(TC)8 CC (TC)6	F: GTTGTGGTTGTCTCTCACCTG R: CCTATGGCAGCAGTTAGAGC	105	8	242–256	0.798	0.686	0.763
KM396471/Tri.inopinata8	(ATA)6	F: ACTTTTCAGCGTGTTCAGCG R: GCTCTCCACAGTTCACGATC	105	8	214–350	0.759	0.257*	0.717

Finally, the potential number and source of migrants within each population was inferred using the program BayesAss v. 3.0 (Wilson and Rannala 2003). BayesAss estimates the posterior probability distribution of the number of migrants between populations (with 95% confidence intervals) using MCMC, without assuming HWE. The MCMC parameters were set to 10,000,000 iterations, a burn-in of 1,000,000, a sampling interval of 1,000, and a randomly generated seed for each run. Five independent runs were conducted and the results compared to ensure consistency. The mixing parameters for allele frequencies, inbreeding coefficients, and migration rates were adjusted until the acceptance rates were within the specified optimal range (~20-60%).

Results

Microsatellite library development

Overall, 105 individuals were genotyped from four collection sites in eastern Massachusetts (Figure 1; Table 1). Of the 12 microsatellite loci tested, only eight were polymorphic. The number of alleles in these loci ranged from 5 to 18, and the polymorphic information content ranged from 0.484 to 0.879. Expected and observed levels of heterozygosity ranged from 0.578 to 0.893 and from 0.257 to 0.724, respectively. Significant deviations from HWE were found for six of the eight loci, resulting from heterozygote deficiencies ($P < 0.0001$). Results from analyses investigating the presence of null alleles (MICRO-CHECKER) suggested that null alleles were present at all loci outside of HWE. The low occurrence of failed amplifications (i.e., individuals being homozygous for null alleles) in these six loci (~0.32%), however, suggested that deviations from HWE stemmed from other causes (e.g., inbreeding, Wahlund effect, etc.). After sequential Bonferroni correction, there was no evidence for linkage disequilibrium for any pairs of loci with the exception of *Tri.inopinata7* and *Tri.inopinata8* in the Marblehead population.

Population comparisons

The number of individuals genotyped within each population ranged from 25 to 28 (Table 2). The average number of alleles per microsatellite locus ranged from 4.1 to 7.4, and all populations were found to be significantly outside of HWE due to a deficiency in heterozygotes. Inbreeding coefficients (F_{IS}) ranged from 0.248 to 0.429, and all were found to be significantly different than zero ($P < 0.0001$).

The AMOVA investigating the overall partition of the genetic variation documented a highly significant spatial component associated with the data, with the Among Populations hierarchical level accounting for ~15% of the variation ($P < 0.0001$, Table 3). Likewise, all of the pairwise comparisons were significantly different (Tables

Table 2. Summary of genetic diversity for populations of *Tricellaria inopinata* sampled in 2013. Number of individuals genotyped (N), percentage of polymorphic loci (P_L), average number of alleles per locus (A), expected (H_E) and observed (H_O) heterozygosity calculated in GDA. Wright's inbreeding coefficient (F_{IS}) calculated in GENETIX v. 4.05. Significant deviations of F_{IS} from zero denoted by * (P<0.0001).

Site	N	P_L	A	H_E	H_O	F_{IS}
Eel Pond	28	1.0	4.1	0.564	0.388	0.315*
Boston Harbor	27	1.0	4.5	0.601	0.454	0.248*
Marblehead	25	1.0	6.5	0.757	0.480	0.371*
Gloucester	25	1.0	7.4	0.769	0.443	0.429*

Table 3. Results from AMOVA investigating the proportion of genetic variation partitioned among various hierarchical levels. Variance components, percentage of variation, and fixation indices were estimated for each locus, and then averaged across all loci. Significance of fixation indices is based on 10,000 permutations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	P
Among populations	3	83.40	0.463	14.68	F_{ST}= 0.1468	<0.0001
Among individuals within populations	101	364.27	0.928	29.46	F_{IS}= 0.3453	<0.0001
Within individuals	105	184.50	1.761	55.86	F_{IT}= 0.4414	<0.0001

Table 4. Matrix of pairwise F_{ST} values (population genetic differentiation, Arlequin ver. 3.5.1.2) below diagonal and results from exact tests for genotypic differentiation (GENEPOP 4.3) above diagonal for collection sites. Chi-square values higher than what could be calculated by GENEPOP denoted by ∞. Bolded values indicate significance ($\alpha = 0.05$).

Site	Eel Pond	Boston Harbor	Marblehead	Gloucester
Eel Pond	-	**39.44**	**∞**	**∞**
Boston Harbor	**0.028**	-	**∞**	**∞**
Marblehead	**0.224**	**0.193**	-	**68.01**
Gloucester	**0.206**	**0.179**	**0.046**	-

Table 5. Matrix of pairwise D values calculated in GenAlEx v. 6.5 (based on Jost 2008). Bolded values indicate significance. For the comparison of Eel Pond and Boston Harbor, P=0.015, for all other comparisons P<0.001).

Site	Eel Pond	Boston Harbor	Marblehead	Gloucester
Eel Pond	-			
Boston Harbor	**0.038**	-		
Marblehead	**0.480**	**0.429**	-	
Gloucester	**0.504**	**0.460**	**0.119**	-

Table 6. Results from BayesAss (v. 3.0) assessing the fraction of migrants within the colonies collected at each sampling site (EP-Eel Pond, BH-Boston Harbor, MH-Marblehead, GL-Gloucester). Numbers in parentheses represent 95% C.I.

		Source			
		EP	BH	MH	GL
Collection site	EP	0.969 (0.017)	0.011 (0.010)	0.011 (0.010)	0.010 (0.010)
	BH	0.301 (0.018)	0.678 (0.011)	0.011 (0.010)	0.010 (0.010)
	MH	0.012 (0.011)	0.012 (0.012)	0.957 (0.022)	0.019 (0.017)
	GL	0.021 (0.015)	0.012 (0.012)	0.041 (0.029)	0.926 (0.032)

4 and 5). Interestingly, pairwise F_{ST} values were relatively low for the Eel Pond and Boston Harbor comparison (F_{ST} = 0.028) and for the Marblehead and Gloucester comparison (F_{ST} = 0.046), although these values were significantly different than zero (P = 0.021 and P < 0.001, respectively). Results from the exact test for genotypic differentiation (GENEPOP) and estimation of D values (GenAlEx) also documented similar results for those comparisons, suggesting higher genetic similarity among those individuals. The Mantel test conducted in GenAlEx failed to detect a significant correlation between genetic and geographic distance (R^2 = 0.015, P = 0.483), suggesting that genetic similarity existed despite an increased geographic distance between some sites.

Examining spread dynamics

The data were initially analyzed to determine if repeated long-distance dispersal events resulted in a potential founder effect in some of these populations. Based on the program Bottleneck,

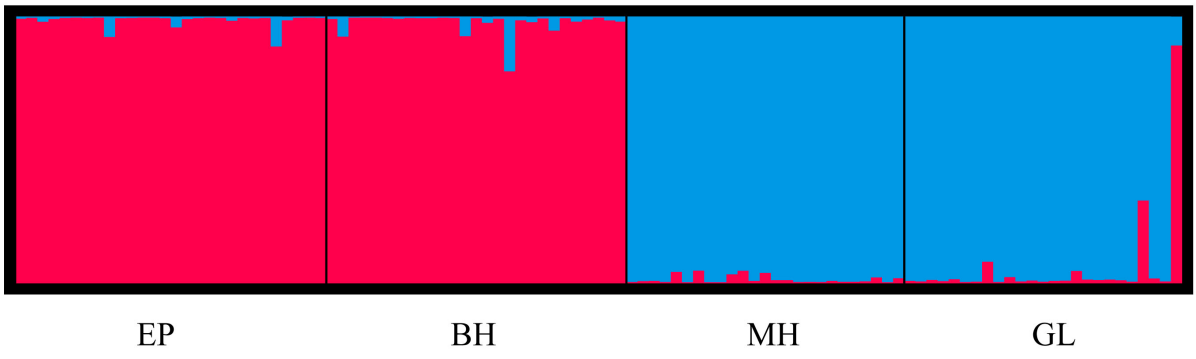

Figure 2. Results from clustering analyses conducted in STRUCTURE (K=2). Individuals within each sampling site are represented by a colored bar, with the height of the bar representing the probability that an individual is assigned to a particular group. Sites sampled were Eel Pond (EP), Boston Harbor (BH), Marblehead (MH), and Gloucester (GL).

on average all loci in each of the populations sampled fit the TPM model, suggesting that none of the sampled sites experienced a recent bottleneck ($P \geq 0.156$ for all sites). The program STRUCTURE was then used to infer population structure in the data set, and to determine if sites clustered in spite of the genotypic differentiation described earlier. The ΔK method described by Evanno et al. (2005) found that the best fit to the data was $K = 2$, with individuals from Eel Pond and Boston Harbor clustered in one group and individuals from Marblehead and Gloucester clustered in the other (Figure 2). Based on the program BayesAss, there was a low number of migrants in most comparisons (Table 6). Indeed, many of the comparisons resulted in confidence intervals that encompassed zero. Conversely, about 30% of the individuals sampled from Boston Harbor were inferred to have originated in Eel Pond. Interestingly, results indicate that this migration was unidirectional as only 1.1% (± 1.0) of individuals sampled at Eel Pond were inferred to have originated from Boston Harbor.

Discussion

Characterization of the genetics underlying recent introduction events and spread dynamics of alien species can provide important information not only as to how these events occur, but also as to the potential application of conservation techniques to limit future occurrences. Results from our analyses of the recent introduction and spread of the marine bryozoan *Tricellaria inopinata* indicated that the populations in the sites sampled have not experienced a recent bottleneck event, in contrast to what might be expected following a long-distance dispersal event. Additionally, although significant differentiation was found between all sampling sites, there was evidence of similarity between some pairs of sites. Overall, these results suggest that: 1) the newly established populations of *T. inopinata* do not appear to be limited by a founder effect due to a reduction in genetic diversity, 2) the Boston Harbor population was likely founded by members of the Eel Pond population, and 3) the introduction of *T. inopinata* to the western Atlantic is a result of multiple introduction events; the Marblehead and Gloucester populations appear to have been founded from a distinct introduction event independent from the event that introduced animals to Eel Pond.

Effects of long-distance dispersal

Due to only a small fraction of the source population being transported to a new area, many dispersal events can result in a reduction in genetic diversity in the founding population (Nei et al. 1975). This reduction in genetic diversity could inhibit the establishment and spread of newly introduced species due to deleterious effects associated with inbreeding. For species to become established in new areas, introduced organisms must overcome this founder effect, which can be accomplished through several mechanisms (see Roman and Darling 2007). For example, a species might have an inherent resilience to inbreeding (e.g., Golani et al. 2007; González-Wangüemert et al. 2014). For instance,

Dupont et al. (2007) documented that introduced populations of the ascidian *Corella eumyota* Traustedt, 1882 were thriving in the English Channel despite extremely low genetic diversity. The authors concluded that because these animals possessed a mixed-mating system (i.e., ability to self-fertilize and outcross), the potential mate-limitation associated with introducing only a few individuals into a new area would not inhibit these animals from colonizing the area. Bryozoans are simultaneous hermaphrodites and could possess similar reproductive capability. Previous work has shown that while self-fertilization can result in significant deleterious effects in offspring survival and reproductive fitness in some species (e.g., Johnson 2010), the ability to self could be population-specific. Hoare and Hughes (2001) found that *Celleporella hyalina* (Linnaeus, 1767) colonies collected from the British Isles were incapable of selfing, whereas colonies collected from different locales were self-compatible (Hughes et al. 2002; 2009). The potential for self-fertilization in *T. inopinata* has not been investigated, but a mixed-mating system in these animals would certainly facilitate their establishment and spread following a long-distance dispersal event.

Alternatively, organisms could overcome a founder effect through an increase in propagule size or number, mechanisms that are increasingly considered important in determining the success of an invasion (Lockwood et al. 2009; Simberloff 2009; Wittmann et al. 2014). An increase in the number of individuals introduced at any one time (propagule size) is thought to allow the resulting population to possess sufficient genetic variability to avoid deleterious effects associated with inbreeding. Likewise, having multiple introduction events (propagule number) can increase the genetic diversity in the introduced population over time, as well as allow the introduced population to withstand potential deleterious environmental fluctuations. The negative effects of an unusual environmental event in a recently introduced population could be offset by the arrival of a new cohort of individuals at a later time. For example, Ghabooli et al. (2014) investigated the genetics underlying the invasion of the ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 into the Mediterranean Sea and found that introduced populations possessed similar genetic diversity relative to their source populations. The authors concluded that the observed pattern of genetic diversity could have resulted from repeated introductions from the ctenophore's native range, as well as input from nearby introduced, established populations (Black Sea). Lejeusne et al. (2014) documented that introduced populations of the oriental shrimp *Palaemon macrodactylus* Rathbun, 1902 often possessed higher genetic diversity than native populations due to introductions from multiple sources within their natural range. Our analyses suggest that multiple introductions played a role in the establishment of *T. inopinata* to North America; Eel Pond potentially accounted for very low numbers of individuals in both Marblehead (1.2% ± 1.1) and Gloucester (2.1% ± 1.5) and had high levels of divergence with both sites. If Eel Pond were the source population for Marblehead and Gloucester, there would likely be lower levels of divergence and higher proportions of migrants, as was found in the Eel Pond and Boston Harbor comparisons.

Although our results suggest that multiple introductions occurred, the extent to which they allowed these introduced populations to thrive is unclear. Eel Pond is a relatively small, enclosed body of water, making multiple introductions from Mediterranean or European waters unlikely. Results from the program BOTTLENECK suggest that none of the sampled sites experienced a recent bottleneck event. Rather than multiple introductions, it is possible that propagule size at the time of introduction allowed these animals to avoid the genetic bottleneck commonly associated with long-distance dispersal events. For many aquatic organisms, the introduction of an elevated propagule size into a new area can occur through the discharge of ballast water (e.g. Simberloff 2009; Lawrence and Cordell 2010). *Tricellaria inopinata* has short-lived larvae (<4h; Johnson et al. 2012) that develop into sessile colonies. While the transport of adult colonies in ballast tanks is possible, it is more likely that colonies were encrusted on ships' hulls or in enclosed areas such as the sea chest. Arborescent bryozoans attach to the substrate via root-like projections, termed rhizoids. Even if the colony dies back, the rhizoids can bud new individuals (Numakunai 1967) that can develop into reproductively mature colonies. Provided sufficient rhizoids remained attached to a ship's hull, an elevated propagule size could be achieved even during a trans-Atlantic voyage, which would enable these animals to avoid a genetic bottleneck and establish and thrive in a new area after just a single introduction event. When they were first discovered in Eel Pond, the animals had already established dense aggregations on

piers and docks throughout the pond (Johnson et al. 2012). No sign of *T. inopinata* was found previously, although Eel Pond was extensively sampled from 2007–2009 (Johnson 2010; Johnson and Woollacott 2010). In Gloucester, *T. inopinata* first appeared in 2011 in low concentrations, but in 2012 could be found in dense aggregations (CD Wells, pers. comm.). These observations, coupled with the results from our analyses, suggest that *T. inopinata* is able to establish and thrive in a new area given only a single introduction event. The role of multiple introductions facilitating colonization success, however, requires further investigation.

Ecological implications and future directions

The establishment and spread of *T. inopinata* in the Mediterranean coincided with a decrease in previously-established bryozoan communities. *Bugula neritina* (Linnaeus, 1758), *B. stolonifera*, and *Scrupocellaria* sp., bryozoans that form erect colonies, were all found to decrease in abundance to the point of disappearing from the Lagoon (Occhipinti-Ambrogi 2000). The two most common bryozoans in Eel Pond, *B. stolonifera* and *B. turrita* (Desor, 1848), have also decreased in abundance following colonization by *T. inopinata* (Johnson et al. 2012). The mechanism is unclear, but *T. inopinata* colonies appear earlier in the growing season (pers. obs.) and achieve reproductive maturity by late-May or early-June, several weeks before *B. stolonifera* and *B. turrita* begin to appear in Eel Pond. This difference in reproductive timing could provide *T. inopinata* colonies with a competitive advantage in occupying available substrate, which could explain the decrease of the other two arborescent bryozoans. The site will continue to be monitored to determine the potential long-term impact of *T. inopinata* in Eel Pond. Similar monitoring by other researchers is also occurring in sites outside of Eel Pond. An assessment of marine species in bays and harbors in New England found *T. inopinata* in several additional sites in the area, including harbors as far north as Hampton Beach, New Hampshire, and as far south as Newport, Rhode Island (Wells et al. 2014). Monitoring the interaction of this bryozoan with other sessile organisms at these additional sites, and incorporating these sites into the types of genetic analyses conducted in our study, will help to further elucidate the spread dynamics of this animal, as well as aid in understanding what makes *T. inopinata* such a successful invader.

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