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Consequences of a poecilogonous life history for genetic structure in coastal populations of the polychaete *Streblospio benedicti*

CHRISTINA ZAKAS^{*,†} and JOHN P. WARES^{*}

^{*}Department of Genetics, University of Georgia, Athens, GA 30602, USA

[†]Department of Biology and Center for Genomics & Systems Biology, New York University, NY 10003, USA

Abstract

In many species, alternative developmental pathways lead to the production of two distinct phenotypes, promoting the evolution of morphological novelty and diversification. Offspring type in marine invertebrates influences transport time by ocean currents, which dictate dispersal potential and gene flow, and thus has sweeping evolutionary effects on the potential for local adaptation and on rates of speciation, extinction and molecular evolution. Here, we use the polychaete *Streblospio benedicti* to investigate the effects of dimorphic offspring type on gene flow and genetic structure in coastal populations. We use 84 single nucleotide polymorphism (SNP) markers for this species to assay populations on the East and West Coasts of the United States. Using these markers, we found that in their native East Coast distribution, populations of *S. benedicti* have high-population genetic structure, but this structure is associated primarily with geographic separation rather than developmental differences. Interestingly, very little genetic differentiation is recovered between individuals of different development types when they occur in the same or nearby populations, further supporting that this is a true case of poecilogony. In addition, we were able to demonstrate that the recently introduced (~100 ya) West Coast populations probably originated from a lecithotrophic population near Delaware.

Keywords

developmental dimorphism; gene flow; marine introductions; pelagic dispersal; poecilogony

Correspondence: Christina Zakas, Fax: +1 212-995-4015; christinazakas@gmail.com.

C.Z. is interested in the evolution and genetic basis of larval dimorphisms. J.P.W. uses molecular approaches to understand distribution of diversity at all spatial scales.

Data accessibility

Transcriptome assembly reads are available at the U.S. National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) Accession number SRP009880.

Sequence data for each SNP used, as well as individual genotypes are in DRYAD entry doi: 10.5061/dryad.6rh86.

Introduction

Major transitions between development modes are a common occurrence in the evolutionary history of most animal phyla and result in profound evolutionary consequences. Marine invertebrates exhibit strikingly divergent reproductive strategies, even among closely related species, maximizing either adult fecundity or offspring survival (Strathmann 1985, 1990). Pelagic larval forms generally adhere to two modes: *planktotrophic* larvae are small, obligately feeding offspring that can spend weeks to months developing in the water column, while *lecithotrophic* larvae are larger, nonfeeding and have a greatly reduced larval development time of hours to days. By influencing migration and gene flow, larval type has sweeping micro- and macroevolutionary impacts on a lineage (Wray 1995; Hart 2000; McEdward 2000). Planktotrophic taxa persist longer in the fossil record, with limited potential for local adaptation due to the swamping effects of high gene flow (Vermeij 1982; Caley *et al.* 1996; Pechenik 1999). In contrast, lecithotrophic development increases the potential for local adaptation (Sotka 2005), population structure (Palumbi 1994, 1995; Hellberg 1996; Todd *et al.* 1998; Collin 2001), rates of protein evolution (Foltz *et al.* 2004), and speciation (reviewed in Krug 2011). The evolutionary forces that underlie the maintenance of this variation have been studied extensively in congeneric species across a great range of taxa (Blake 1969; Perron 1986; Byrne 1991; Hart *et al.* 1997; Arndt & Smith 1998); however, very few studies have investigated the phylogeographic and population genetic consequences of larval development mode within a single species, thus eliminating a number of potentially confounding species-specific effects.

Transitions from planktotrophy to lecithotrophy occurred frequently in most Bilaterian phyla (Hart *et al.* 1997; Duda & Palumbi 1999; Jeffery & Emler 2003; Meyer 2003; Collin 2004). However, intraspecific variation in larval type, known as *poecilogony*, is apparently rare (Hoagland & Robertson 1988; Bouchet 1989). Most taxa exhibit only one reproductive mode, precluding direct comparisons between long- and short-lived larval morphs. Cases of poecilogony, where one species (or individual) produces distinct types of embryos that develop into either planktotrophic or lecithotrophic larvae, are limited to a few polychaete worms (Blake & Kundenov 1981; Levin 1984; Qian & Chia 1992) and one group of herbivorous sea slugs (Krug 2001).

The poecilogonous marine annelid *Streblospio benedicti* is an ideal system to study the evolutionary consequences of larval mode. *S. benedicti* inhabits muddy estuarine sediments and adult females either produce many ‘Small’ (60–70 µm in diameter) planktotrophic larvae that spend weeks feeding in the water column, or fewer, ‘Large’ (100–200 µm) larvae that complete development in a few days and retain a gut and can— but do not need to— feed (Pernet & McArthur 2006). Adult females can only produce either Large or Small larvae, and larval mode is highly heritable (Levin *et al.* 1991). Both larval morphs are found in native Western Atlantic populations (Levin & Bridges 1994; Schulze *et al.* 2000), but populations in the eastern Pacific are nonindigenous, and only consist of the Large morph. Adults of both morphs are indistinguishable in morphology and at mitochondrial loci (Schulze *et al.* 2000). Larval development expressed in the offspring produced by a given female does not change with maternal condition. Quantitative genetic studies indicate that poecilogony reflects an allelic polymorphism in *S. benedicti*, maintained by trade-offs

between suites of correlated life history traits (Levin & Creed 1986; Levin & Huggett 1990; Levin *et al.* 1991; Levin & Bridges 1994).

Here, we use *S. benedicti* as a model system to test for population genetic consequences of variation in larval development mode. While the evolutionary expectations for either planktotrophic *or* lecithotrophic development have been explored, the evolutionary implications of a poecilogonous larval life history mode remain unaddressed. We use 84 SNP loci (described in Zakas *et al.* 2012) to estimate the amount of genetic structure between (i) geographic populations and (ii) populations of different larval types. Such a study was last performed with mitochondrial DNA markers by Schulze *et al.* (2000), who concluded there was little geographic structure and no distinction between populations of the distinct larval types. Multilocus codominant allelic data provide a greater opportunity to determine the relationships between these larval types and diversity across a broad geographic survey. Here, we discuss our expectations for dispersal and gene flow in a poecilogonous species and determine the amount of genetic structure among geographical populations on the East and West Coast of North America. Finally, we use these SNPs to test the hypothesis that the recent (~100 ya) US West Coast introduction of *S. benedicti* originates from populations near the Chesapeake Bay (Blake 1965).

Methods

Collections and genotyping

Individuals of *Streblospio benedicti* were collected from 13 populations along the US East and West Coast as described by Pernet & McArthur (2006). Sites and collection dates are shown in Table 1, Fig. 1. The West Coast females collected from these populations are definitively of the Large morph; therefore, we assume all individuals from these populations shared this strategy. The University of South Carolina Baruch Marine Institute (BR) is the only location in our sampling where both larval types can be consistently found together. Brooding females were identified as either 'Small' or 'Large' using an Olympus SZ61 dissecting microscope as described by Pernet & McArthur (2006). Adults of both larval types are morphologically indistinguishable when broods are not present.

We performed DNA isolations for individual adults following a standard phenol–chloroform extraction (Doyle & Doyle 1987). Brooding females were agitated to induce the release of their larvae prior to DNA extraction. For each of the 480 individuals, nucleic acid quality and concentration were evaluated with a Nanodrop ND-1000 spectrophotometer. Golden Gate genotyping (Illumina) was conducted on 50 ng of DNA according to manufacturer's protocol at the Georgia Genomics Facility (www.dna.uga.edu). Each individual was genotyped for 96 SNPs developed by Zakas *et al.* (2012) using the Illumina BeadXPress Golden Gate genotyping assay. These SNPs were chosen from a transcriptome, which was generated for a different study, and consisted of pooled larvae and juveniles of Large individuals from site LB (Table 1) based on criteria described by Zakas *et al.* (2012). Genotypes were assigned and annotated using GenomeStudio (Illumina) as described by Zakas *et al.* (2012). Of the 96 SNPs genotyped, 84 were both polymorphic and returned a high call rate across individuals (average call rate for a locus is 87% (SD = 25%)) and were used in subsequent analyses.

Outlier detection

Because inclusion of outlier loci can greatly bias estimates of population evolutionary history (reviewed in Luikart *et al.* 2003), we tested for loci that may be under selection. We used the program BayesScan v2.0 (Foll & Gaggiotti 2008) to detect outlier loci based on F_{ST} , as it has the lowest type I and II error compared with other neutrality tests (Narum & Hess 2011). Using default settings (5000 iterations, 20 pilot runs, burn-in length of 50 000 iterations and a thinning interval of 10), we identified loci that favoured the model including selection with a posterior probability of 97–100%. This identifies loci that are decisive outliers and may be subject to diversifying selection (high- F_{ST} values), or balancing/purifying selection (low F_{ST} values). In addition, we also identified outlier loci using just the East Coast populations. We excluded the West Coast populations because they probably underwent a recent bottleneck, which would bias the outlier detection (Excoffier *et al.* 2009). We performed all of our analyses both including and excluding these outlier loci.

Geographic population structure

We initially grouped individuals according to their geographic location. To test for equilibrium models of gene flow, we used a Mantel test to determine whether there is isolation by distance (IBD) along the East Coast using the program *Zt* v1.1 (Bonnet & Van de Peer 2002). We calculated pairwise F_{ST} as a measure of genetic distance between populations using Arlequin 3.5 (Excoffier & Lischer 2010). We corrected genetic distance for the one-dimensional coastline by using $F_{ST}/(1-F_{ST})$ as described by Rousset (1997). We also applied a partial Mantel test in *Zt* to control for the effect of larval type on IBD.

Under conditions of IBD, it is still possible for strong regional genetic structure to exist between populations on the East Coast (Moyle 2006). We wanted to determine whether *S. benedicti*, like many other coastal marine populations, exhibits a phylogeographic break centred near Long Island Sound as suggested by the synthesis of Wares (2002). We performed an analysis of molecular variance (AMOVA) using Arlequin 3.5 with the Northern region containing ME and NS and the Southern region including DE with the remaining southern populations (DNS, IRI, MC, BR, CH, SP3, SP6). Significance was assayed from 1023 permutation tests.

Larval type population structure

We are especially interested in determining the amount of population differentiation that is not due to geographic distance, but rather to larval type. We grouped the 191 individuals of known larval type into Small and Large ‘regions’ and performed an AMOVA to test for genetic structure between the two larval types. There is only one site where both larval types consistently cohabitate (BR), and we calculated F_{ST} between the known Small and Large larval morphs within this population. As aforementioned, we used BayesScan to test for outlier loci for this grouping. We wanted to test for an overall difference in gene flow between predominantly Small and Large populations on the East Coast. We did this graphically by plotting the pairwise F_{ST} vs. distance for all population comparisons, and determining if Small vs. Small populations demonstrated a greater amount of gene flow (considering both slope and magnitude of the regression) than Small vs. Large populations. We used an analysis of covariance (ANCOVA) to determine significance. When also used

50 randomly chosen loci with the program Powsim (Ryman & Palm 2006) to determine the probability of detecting a low F_{ST} of 0.02 between larval populations.

Introduction to the West Coast

To determine the most likely source population(s) for the West Coast introduction, we performed an assignment test for each of the West Coast individuals using GeneClass2 (Piry *et al.* 2004). This program determines the most likely population based upon the simulation algorithm described by Paetkau *et al.* (2004), which calculates likelihood by creating a test distribution of simulated individuals through drawing haplotypes and using Monte Carlo resampling algorithms. We wanted to determine the utility of using outlier loci for assignment tests in this species for future studies, so we also performed an assignment test using only the 12 high- F_{ST} outlier loci.

We used the program Bottleneck 1.2.02 (Piry *et al.* 1999) to test for the signatures of recent bottlenecks in the West Coast populations and included DE because it is the most likely source population we assessed based on the previous assignment test and historical records (Cohen *et al.* 1995; Wasson *et al.* 2001). Bottleneck uses a coalescent simulation to test for an excess of heterozygosity relative to mutation-drift equilibrium based on the number of alleles and the sample size. For a recent bottleneck, the allelic diversity is reduced faster than the heterozygosity resulting in an excess of heterozygotes compared with expected. We use the results of two statistical tests under an infinite alleles model: the sign test and the standard difference test (recommended for >20 loci), as well as a mode-shifted allele frequency distribution due to loss of rare alleles (Cornuet & Luikart 1996) to determine whether a recent bottleneck has occurred on the West Coast. We also used AMOVA to determine individual population inbreeding coefficients (F_{IS}).

To test whether the West Coast populations originated from separate introductions from the East Coast, we compared pairwise genetic distance among West Coast populations vs. pairwise distances between West Coast and the DE populations (OR-LB, OR-SF, LB-SF vs. OR-DE, SF-DE, LB-DE) using a one-tailed paired *t*-test. If these two distributions of genetic distances are significantly different, meaning the West Coast populations are more closely related to each other than to DE, it suggests that the West Coast populations are probably the result of a single introduction that has spread (Liebert *et al.* 2006). A one-tailed test is appropriate because of the directionality of the hypothesis: we are only testing for lower pairwise F_{ST} across the West Coast. We also tested for IBD in the West Coast populations using a Mantel test. We used Genalex v6 (Peakall & Smouse 2005) to calculate the effective number of alleles (N_A) as well as the mean observed (H_O) and expected (H_E) heterozygosity for DE and the West Coast populations.

Results

All of the 84 SNPs were variable across populations and there were no private alleles that designate geographic populations, regions or larval type, although this is likely because the SNPs chosen are variable in the transcriptome of the Large morph and may not represent regions that differ between larval morphs (Zakas *et al.* 2012). The number of effective

alleles, observed and expected heterozygosity, and significant F_{IS} values are reported in Table 2. The distribution of F_{ST} values for all loci is shown in Fig. 2.

Outlier analyses

BayeScan identified 20 loci that were decisive outliers based on their F_{ST} values in the total set of populations (Fig. 2). Twelve of the SNPs appeared to be subject to diversifying selection, while eight appeared to be under balancing or purifying selection (as determined by the alpha value). These loci and their summary statistics appear in Table 3. When including only the East Coast populations, a subset of these loci remain outliers and two additional loci were also detected as significant outliers (Table 3). Some of these loci may not truly be under selection, but the false-positive rate is low in BayeScan using the 97% threshold (Narum & Hess 2011).

Geographic population structure

The Mantel test revealed that there is significant and strong IBD along the East Coast: $r^2 = 0.6671$ ($P = 0.01$). This appears to be due to true migration-drift equilibrium as the pattern still persists south of the proposed phylogeographic break at DE ($r^2 = 0.67$, $P = 0.02$). Using a partial Mantel test to control for the effect of larval type, we recovered a very similar value: $r^2 = 0.66$ ($P = 0.01$). When the outlier loci were removed, the IBD became even stronger and more significant $r^2 = 0.88$ ($P = 0.001$).

The AMOVA demonstrates that there is strong and significant population structure with the proposed break above DE (Table 4a, FFig. 1). There is also significant structure for populations within regions (S_C) as would be expected with IBD. A Neighbor-joining tree based on pairwise population F_{ST} is shown in Fig. 3 to illustrate the genetic separation among populations. The population grouping in this tree, along with the strong among-regional structure (F_{CT}) and increase in within-region structure (F_{SC}) when the West Coast is included, led us to believe that there may be another significant regional break within the Southern region. We performed a set of post hoc AMOVAs where the DE and the West Coast were grouped into a third region (First region: NS, ME; Second region: DE, OR, SF, LB; Third region: DNS, IRI, MC, BR, CH, SP3, SP6, Fig. 1). This grouping also has a high and significant F_{CT} value (and has the largest F_{CT} value of any grouping that included the West Coast). With the AMOVA that includes the West Coast, the F_{CT} value is stronger than when the West Coast populations are not included. Interestingly, this regional grouping splits the DE and DNS populations, which are only ~19 km apart on the Delaware shoreline (Table 4b).

Larval type population structure

When individuals are grouped according to larval type, there is apparent genetic structure between predominantly Small and Large populations: $F_{ST} = 0.107$ ($P < 0.001$) but this value is confounded by geography. Within a single location (BR), the F_{ST} for individuals grouped by larval type is low (0.049, $P = 0.040$) compared with the pairwise F_{ST} values across nearby geographic regions (average pairwise F_{ST} for adjacent populations is 0.112 and the average pairwise F_{ST} for BR and its two neighboring populations is 0.11). This supports the conclusion that the majority of population genetic structure is driven by geography rather

than larval type. Unlike when populations were grouped based on geography, BayeScan did not detect any outlier loci using this grouping by larval type. When we compared the distributions of pairwise F_{ST} for Small vs. Small populations and Small vs. Large populations, we recovered an interesting trend where the Small vs. Large populations had consistently higher pairwise F_{ST} values (Fig. 4). These distributions are significantly different ($P < 0.001$). When using a subset of 50 randomly chosen loci, we found a 95% probability of detecting a F_{ST} of 0.02 between populations separated by ~100 years when using simulations in the program Powsim (Ryman & Palm 2006).

Introduction to the West Coast

All West Coast individuals were assigned to the DE population with the highest probability, with the exception of one individual from LB [which has equal probability of over 97% of belonging to multiple populations (IRI, MC, SP3, SP6)]. On average, the assignment probability to DE as the source population is 0.72 (SD = 0.33) for LB individuals, 0.82 (SD = 0.25) for OR and 0.67 (SD = 0.32) for SF. No other single East Coast location had an average assignment probability greater than 21% (IRI, also in Delaware). When only the 12 high- F_{ST} outlier loci were used, DE was still the highest assigned source population, but with lower probability [LB = 0.46 (SD = 0.31), OR = 0.43 (SD = 0.32) and SF = 0.50 (SD = 0.30)]. The second most likely population of origin was DNS with the average probability being 0.08 (SD = 0.22). When the 12 high F_{ST} outlier loci were used, only two LB individuals did not have DE as their most likely source population. This suggests that the 12 high- F_{ST} outlier loci alone, instead of the full SNP data set, can be used for assignment tests in other populations. Individuals that had a lower probability (<0.8) of being assigned to DE had a similarly low probability of being assigned to any other population sampled, and this did not appear to be a result of fewer genotyping calls in these individuals.

In all West Coast populations, there was a significant excess of heterozygosity compared with mutation-drift equilibrium under both the sign and standard difference statistical tests ($P < 0.000$). In addition, all West Coast populations exhibited the mode-shifted allele frequency distribution expected due to a loss of alleles during a bottleneck. There was also an excess of loci with high heterozygosity in the DE population, but this population still exhibited a normal distribution of allele frequencies according to the mode-shift test. We determined that there is significant signal for inbreeding in the DE population as well as OR and IRI (Table 2). Other populations did not have significant inbreeding coefficients. The inbreeding coefficient calculated from AMOVA across all populations is not significant.

There is low pairwise F_{ST} exhibited between all of the West Coast populations (pairwise F_{ST} for LB-SF = 0.040, LB-OR = 0.044, SF-OR = 0.040). The genetic distances between the West Coast populations are lower than the genetic distance between the DE and West Coast populations (pairwise F_{ST} for LB-DE = 0.066, OR-DE = 0.057, SF-DE = 0.10, $P < 0.01$), which is consistent with a pattern of secondary spreading after an initial introduction. Assuming equal variances in a one-tailed, paired t -test, there is a significant difference ($P = 0.035$) between the two sets of F_{ST} values. A Mantel test demonstrated that there was no IBD on the West Coast. We found that DE had a smaller effective number of alleles than the

introduced populations (Table 2). Also, the observed and expected heterozygosity were equal or greater in the introduced populations than in DE (Table 2).

Discussion

We find that there is a large amount of population genetic structure present in the native East Coast range of *Streblospio benedicti* (F_{ST} of 0.28, $P < 0.001$ excluding outliers), which is much larger than that previously reported for cytochrome oxidase I (COI) (Schulze *et al.* 2000). This structure is driven primarily by geographical population differentiation rather than larval type. In fact, there is little genetic distinction between larval types further indicating that this is a developmental dimorphism, rather than an incipient speciation event or a case of cryptic species, as has been found in some other cases of poecilogony (Hoagland & Robertson 1988). This is further supported by drift-migration equilibrium conditions (strong IBD) along the East Coast, which would be unlikely to persist if secondary contact occurred between evolutionarily divergent lineages. This IBD pattern is maintained when the Northern region—populations on the other side of a major phylogeographic transition—is removed, demonstrating that this is a true equilibrium pattern and not an artefact of other demographic or phylogeographic factors (Wares & Cunningham 2005).

Interestingly, in these analyses, the removal of the 20 outlier loci increased the amount of IBD detected on the East Coast. There are a number of sampling factors that may lead to recovery of strong IBD in marine populations (Selkoe & Toonen 2011). The increase in IBD may be because some of the outlier SNPs are adaptive and are acting to differentiate populations. For example, SNPs under divergent selection can increase population differentiation by having a nonlinear pattern of adaptation between populations such as a phylogeographic break. Also the eight low F_{ST} outliers were probably driving the overall reduction of IBD. The decrease in F_{CT} with an increase in F_{SC} that occurs when the outlier loci are removed from the AMOVA also demonstrates that these loci are influencing the strong regional structure, which can diminish a signal of IBD.

It is apparent that this species has more genetic structure across geographic populations than would be expected for a species with a predominately long-dispersing pelagic larval type (reviewed in Whitlock & McCauley 1999; Weersing & Toonen 2009; but see Faurby & Barber 2012) and little divergence at COI (Schulze *et al.* 2000). Other factors may be biasing estimates of gene flow: outbreeding depression from long-dispersing larvae into a distinct breeding pool may decrease the effective migration rate (Barton & Bengtsson 1986). Alternatively, F_{ST} could also underestimate migration if offspring distribution is skewed such that only a few parents contribute to the gene pool (sweepstakes-style recruitment; Eldon & Wakeley 2009). However, neither of these scenarios seems probable given the pattern of IBD and the reproductive biology of *S. benedicti*, which is not a broadcast spawner.

Outlier analyses

Outlier loci, based on their extremely high or low F_{ST} values, can bias estimates of gene flow, population size and structure (Luikart *et al.* 2003). We identified 20 of our 84 loci (23.8%) to be outliers in the total population. This number was reduced to 12 (14%) when

only the East Coast populations were used. This is probably because the inclusion of the West Coast populations, which have likely undergone a significant recent bottleneck, is probably leading to a higher rate of false positives (Foll & Gaggiotti 2008). Generally, studies report 5–10% of loci to be outliers (Nosil *et al.* 2009). There are difficulties in determining if outlier loci are truly due to selection, as they potentially occur due to genotyping error (Xue *et al.* 2009), false positives (Pritchard & Rosenberg 1999; Price *et al.* 2006; Zhang *et al.* 2008; Excoffier *et al.* 2009), or even variation in mutation rate (Hedrick 2005). In this study, it is unlikely that the SNPs are exhibiting genotyping error due to the relatively high frequencies of each allele in multiple populations (Table 3). We assume that the SNPs used here are in linkage equilibrium with each other because linkage disequilibrium decays within a few kilobases (Rockman 2012) and they occur in different contigs of the *S. benedicti* transcriptome, although that is almost certainly not the case for all SNPs (see Zakas *et al.* 2012). These 20 outlier loci do not exhibit more extreme behaviour (high/low F_{ST} or a greater excess of heterozygosity) in the inbred or bottleneck populations (DE, IRI, OR). Despite the potential problems with including outlier loci in estimating population demographic history (reviewed in Allendorf & Seeb 2000), we did not find a significant effect of these outliers in any of the analyses we performed. These outlier loci were useful for assignment tests for introduced individuals, but they were less definitive than using the entire SNP data set.

Geographic population structure

Barriers to gene flow have been detected in some coastal invertebrate species along the Northwestern Atlantic Coast, generally centred around Cape Cod, MA (reviewed in Wares 2002). The location of this regional break is occurring between Delaware and Maine, which is consistent with many other East Coast invertebrates (Franz *et al.* 1981; Dillon & Manzi 1992; Bastrop *et al.* 1998; Lee 1999). Species that have low-dispersal ability generally tend to have a genetic break in this region more often than species with highly dispersive larvae. For example, the bamboo worm *Clymenella torquata* has a larval phase of only a few days and demonstrated a break around Cape Cod (Jennings *et al.* 2009), but the polychaete *Marenzelleria viridis* has long-dispersing larvae and also shares this break (Bastrop *et al.* 1998). Because there are long-dispersing species that have a genetic break in this region and short-dispersing species that do not, it is difficult to make predictions about the expected genetic structure in this area for *S. benedicti*, especially because it is not clear whether this species should be treated as a long-disperser, a short-disperser, or a combination of both.

Larval population genetic structure

In *S. benedicti*, Levin *et al.* (1991) found that most traits associated with egg size and larval development have a strong additive genetic component and are highly heritable, indicating genetic differentiation between the larval morphs. However, compared with the observed geographic population structure, we identify very little genetic differentiation between predominately Small and Large populations. The genetic differentiation between developmental types within BR is comparatively low, although this could be influenced by the SNP loci that were chosen from a transcriptome of Large individuals (Zakas *et al.* 2012). While we were limited in our ability to choose SNPs that represent fixed differences between Large and Small populations, the SNPs used are polymorphic in both populations

(there are no cases of a SNP being fixed in Small populations and polymorphic in Large populations). We feel this potential bias towards underestimating genetic structure is minimal, and the results of our Powsim analysis show that we have the ability to detect low F_{ST} values with our loci. Because the SNPs were chosen from a Large, recently introduced population, we could expect a bias where less genetic structure is detected than what is actually present in the total population. However, we find a large amount of population genetic structure between geographic populations both within and across coasts, and comparatively less structure across larval types. Also, we detected no outlier loci when individuals are grouped based on larval type, demonstrating that none of the loci show adaptive differences between the two morphs (even though 12 of them rejected the neutral model when grouped by geographic populations). These data suggest that frequent mating between larval types within a population occurs in nature, as has been reported in laboratory mating studies (Levin 1984; Schulze *et al.* 2000).

There is, nevertheless, some evidence of genetic distinction between larval types. The small but significant within-population F_{ST} for BR suggests the potential for assortative mating; we note that although intermediate-sized larvae may be produced in laboratory crosses (Levin *et al.* 1991), such cases are not recovered in the field. It is also notable that when the degree of population differentiation (pairwise F_{ST}) was compared between all East Coast populations, a consistently greater and significant amount of genetic structure was found between Small and Large populations than between Small populations. Because this trend is consistent across the full geographical range, it suggests that there may be low-level gene flow inhibition between larval types, which warrants further investigation. A larger sample size of Small individuals from BR, and the incorporation of additional sites where both larval types coexist, will be necessary before we can definitively categorize the amount of gene flow between individuals of different larval morphs. While there is evidence of some genetic structure across larval types, it does not suggest that the larval types are separate evolutionary units.

It has been proposed that Small and Large larval morphs evolved in allopatry and these populations have recently come into secondary contact on the East Coast (Levin *et al.* 1991), which would explain the strong larval dimorphism. However, our results do not support this. The tendency for individuals to group more strongly by geographic region rather than larval type shows there is no evidence that a recent introduction of Large morph has occurred. (If, however, successful mating across previously isolated populations was occurring, we would expect an increase in additive genetic variation in traits related to larval type, where selection ultimately would act to reduce this variation (Ellstrand & Schierenbeck 2000; Ayres *et al.* 1999; Gillespie 1976). Under this scenario, we may predict the eventual loss of one larval morph). The East Coast populations appear to be in migration-drift equilibrium, as shown by the pattern of IBD, which supports that these morphs may have historically coexisted and may continue to do so without the loss of either larval type. While we cannot easily estimate when this lineage became poecilogonous (and had both larval types present) with this data set, the presence of a predominately Large population in the Northern region suggests that the Large larval morph could pre-date the New England phylogeographic break discussed above (see Wares 2002). The long-term coexistence of both larval types

throughout the species range is also supported by theoretical modelling efforts, which demonstrate the possible equilibrium conditions under which both types can persist (Zakas & Hall 2012).

Presumably, the Small morph is the plesiomorphic state for *S. benedicti* (discussed in Pernet & McArthur 2006). The lack of genetic differentiation between the larval types at neutral loci suggests that either (i) poecilogony (the addition of the Large morph) has arisen recently and independently in multiple populations on the East Coast, or (ii) poecilogonous populations have historically coexisted on the East Coast for many generations and differences in the larval types have only occurred at key developmental gene regions.

Work in diverse model systems indicates that the expression of alternative phenotypes does not depend on extensive genetic decoupling. Rather, morphological differences can be caused by mutations in a few key regulatory genes or differences in gene expression (Brakefield *et al.* 1996; Abouheif & Wray 2002; Moczek & Nagy 2005). Dimorphic offspring often demonstrate a similar level of developmental decoupling as that found between sexes (Bull 1983; Williams & Carroll 2009). This is true for the male offspring dimorphism seen in the beetle *Onthophagus taurus*, where horned and sneaker male morphs are phenotypically divergent, but patterns of gene expression are highly correlated between the morphs (Snell-Rood *et al.* 2010), which suggests that only a small proportion of development is decoupled. A similar developmental decoupling could exist in *S. benedicti*, in which case we would not expect to recover genetic differentiation in larval morphs outside of key developmental gene regions. Such decoupling could reconcile the additive genetic variance in larval traits detected by Levin *et al.* (1991) with the low amount of genetic differentiation described here.

Introduction to the West Coast

The presence of *S. benedicti* on the West Coast was first reported within the past century (Blake 1965). From 1907 to 1920 the Chesapeake oyster, *Crassostrea virginica*, was transplanted into San Francisco Bay, and it is probable that *S. benedicti* accompanied these introduction attempts in the surrounding sediment (Cohen *et al.* 1995; Wasson *et al.* 2001). This prediction is strongly supported by our genetic data. Not only are the West Coast populations most similar to DE, which is the closest population we sampled to the Chesapeake area, there are only Large individuals present in the West Coast and DE populations. However, there are known Small populations of *S. benedicti* in the Chesapeake area as well (IRI, Assawoman and Montego Bay, MD: Marsh & Fielman 2005), but there is no evidence to support that the mostly Small IRI population contributed to the West Coast introduction, despite its proximity to DE. The lack of population genetic differentiation on the West Coast supports that there was a single introduction location that has since spread, but it is not clear from the genetic data alone that San Francisco was the original introduction site. The largest pairwise F_{ST} comparison between the West Coast and DE is actually DE-SF, but this could be biased by the very small sample size of SF individuals.

We detect evidence for a recent bottleneck in West Coast populations, which is generally predicted for a long distance, single colonization event (Lee 2002). We do not find a reduction in the effective number of alleles or heterozygosity in the introduced populations

compared to the source, but this may be because the SNP loci used were chosen from loci that were polymorphic in the West Coast population of LB. While such a reduction may be expected in a recently introduced population that has presumably lost some genetic diversity due to a bottleneck, Wares *et al.* (2005) report that there are many known species introductions that do not experience a reduction in number of alleles or heterozygosity. It is possible that this introduction included a large number of colonizing individuals or high propagule pressure, in which case high levels of heterozygosity would be maintained in the introduced populations (Nei *et al.* 1975).

The invasion success of *S. benedicti* is due, in part, to the primary colonization ability of the species in disturbed and nutrient enriched areas. Consequently, areas such as ports that are frequently disturbed and polluted are also the most likely areas for foreign introductions to occur, which has been reported in a number of locations worldwide: Europe, including the Mediterranean and Black Sea, Venezuela (Carlton 1979, 1985) and Hawaii (Ward 1981). It is interesting, however, that only the Large populations appear to be invasive. An introduced population in Oahu, Hawaii appears to only be of the Large morph as well (B. Pernet, personal communication). It seems unlikely that only the Large morph was originally introduced into San Francisco Bay; *C. virginica* was transplanted repeatedly from areas where both larval morphs are found. Although it appears that the Small populations have not contributed to the current populations on the West Coast, we speculate that both larval morphs were included in the sediment transport but that only the Large morph was able to successfully establish. While the Small morph larvae are better dispersers and they may have greater colonization success (Small larvae are up to 20% more likely to naturally recolonize disturbed habitats than Large individuals; Levin & Huggett 1990), there are a number of reasons why the Large morph could be a more successful invader. As Schulze *et al.* (2000) suggest it is possible that in the case of human-mediated introductions, Large larvae are favoured. Schulze *et al.* (2000) mentioned that the limited dispersal ability of the Large morph and the recent timing of introduction argue against natural dispersal from San Francisco Bay, and instead the distribution along the West Coast could be due to the transport of larvae in ballast water (Carlton & Geller 1993). The Large larvae may be at an advantage in this situation because they do not require planktonic food to survive during transport. The invasive success of Large individuals may also be attributed to higher larval settlement densities in a restricted area, which will lead to greater future mating potential and propagation (Johannesson 1988). In addition, Zakas & Hall (2012) demonstrate that in environments where dispersal is subject to strong asymmetric currents, the Large morph is more likely to become fixed. Compared with the East Coast, West Coast estuaries are more dispersed and are subject to persistent unidirectional coastal currents (Bertness *et al.* 1996; Sotka *et al.* 2004), which are environments where the Large morph can be favoured (Zakas & Hall 2012).

Conclusion

From the SNPs used to genotype both larval morphs of *S. benedicti*, we further support that the larval dimorphism is a true case of poecilogony. Even though traits associated with larval type are highly heritable and must have some genetic differentiation (Levin *et al.* 1991), we find no SNPs in this data set that may differentiate the larval morphs, although

this is unlikely given the resource from which the SNPs were developed. We recover some small but significant F_{ST} values between larval morphs, which may be evidence for low-level assortative mating in populations where both types co-exist, but do not suggest the larval morphs constitute separate lineages. We also find that there is greater geographic population structure than would be expected for this species along its native East Coast range considering the abundance of high-dispersing Small larvae. Instead, it appears that this species has limited gene flow between nearby populations, which is a result more likely to be associated with the low-dispersal Large morph. The large amount of gene flow between larval morphs and the low gene flow across geographic populations is an unexpected and interesting result upon which we can shape our expectations about the population genetic dynamics of poecilogonous species.

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References

- Abouheif E, Wray GA. Evolution of the gene network underlying wing polyphenism in ants. *Science*. 2002; 297:249–252. [PubMed: 12114626]
- Allendorf FW, Seeb LW. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution*. 2000; 54:640–651. [PubMed: 10937239]
- Arndt A, Smith MJ. Genetic diversity and population structure in two species of sea cucumber, differing patterns according to mode of development. *Molecular Ecology*. 1998; 7:1053–1064.
- Ayres DR, Garcia-Rossi D, Davis HG, Strong DR. Extent and degree of hybridization between exotic (*Spartina alterniflora*) and native (*S. foliosa*) cordgrass (Poaceae) in California, USA determined by random amplified polymorphic DNA (RAPDs). *Molecular Ecology*. 1999; 8:1179–1186. [PubMed: 10447858]
- Barton N, Bengtsson BO. The barrier to genetic exchange between hybridizing populations. *Heredity*. 1986; 57:357–376. [PubMed: 3804765]
- Bastrop R, Jurss K, Sturmbauer C. Cryptic species in a marine polychaete and their independent introduction from North America to Europe. *Molecular Biology and Evolution*. 1998; 15:97–103. [PubMed: 9491608]
- Bertness MD, Gaines SD, Wahle RA. Wind-driven settlement patterns in the acorn barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series*. 1996; 137:103–110.
- Blake, JA. Masters Thesis. Fresno State College; Fresno, California: 1965. Spionid Polychaetes from Morro Bay, California, A Taxonomic and Biological Study.
- Blake, JA. PhD Thesis. University of Maine; Bangor, Maine: 1969. Systematics, Reproduction and Larval Development of *Polydora* from Eastern North America (Polychaeta, Spionidae).
- Blake JA, Kundenov JD. Larval development, larval nutrition and growth for 2 *boccardia* species (polychaeta, spionid) from Victoria, Australia. *Marine Ecology Progress Series*. 1981; 6:175–182.
- Bonnet E, Van de Peer Y. Zt, a software too for simple and partial Mantel tests. 2002
- Bouchet P. A review of poecilogony in gastropods. *Journal of Molluscan Studies*. 1989; 55:67–78.
- Brakefield PM, Gates J, Keys D, et al. Development, plasticity and evolution of butterfly eyespot patterns. *Nature*. 1996; 384:236–242. [PubMed: 12809139]
- Bull, JJ. Evolution of Sex Determining Mechanisms. Benjamin Cummings Pub. Co; Menlo Park, California: 1983.

- Byrne, M. Developmental diversity in the starfish genus *Patiriella* (Asteroidea, Asterninidae). In: Yanigasawa, T.; Yasumasu, I.; Oguro, C.; Suzuki, N.; Motokawa, T., editors. Biology of Echinodermata. Balkema, Rotterdam, The Netherlands: 1991. p. 499-508.
- Caley MJ, Carr MH, Hixon MA, Hughes TP, Jones GP, Menge BA. Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics. 1996; 27:477-500.
- Carlton, JT. PhD Thesis. University of California; Davis, California: 1979. History, Biogeography and Ecology of The Introduced Marine and Estuarine Invertebrates of the Pacific Coast of North America.
- Carlton JT. Transoceanic and interoceanic dispersal of coastal marine organisms, the biology of ballast water. Oceanography and Marine Biology. 1985; 23:313-371.
- Carlton JT, Geller JB. Ecological roulette, the global transport of nonindigenous marine organisms. Science. 1993; 261:78-82. [PubMed: 17750551]
- Cohen AN, Carlton JT, Fountain MC. Introduction, dispersal and potential impacts of the Green crab *Carcinus maenas* in San-Francisco Bay, California. Marine Biology. 1995; 122:225-237.
- Collin R. The effects of mode of development on phylogeography and population structure of North American *Crepidula* (Gastropoda, Calyptraeidae). Molecular Ecology. 2001; 10:2249-2262. [PubMed: 11555267]
- Collin R. Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. Evolution. 2004; 58:1488-1502. [PubMed: 15341151]
- Cornuet JM, Luikart G. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics. 1996; 144:2001-2014. [PubMed: 8978083]
- Dillon RT, Manzi JJ. Population genetics of the hard clam, *Mercenaria mercenaria*, at the northern limit of its range. Canadian Journal of Fisheries and Aquatic Sciences. 1992; 49:2574-2578.
- Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 1987; 19:11-15.
- Duda TF, Palumbi SR. Developmental shifts and species selection in gastropods. Proceedings of the National Academy of Sciences. 1999; 96:10272-10277.
- Eldon B, Wakeley J. Coalescent times and F_{ST} under a skewed offspring distribution among individuals in a population. Genetics. 2009; 181:615-629. [PubMed: 19047415]
- Ellstrand NC, Schierenbeck KA. Hybridization as a stimulus for the evolution of invasiveness in plants. Proceedings of the National Academy of Sciences. 2000; 97:7043-7050.
- Excoffier L, Lischer HEL. Arlequin suite ver 3.5, a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 2010; 10:564-567. [PubMed: 21565059]
- Excoffier L, Hofer T, Foll M. Detecting loci under selection in a hierarchically structured population. Heredity. 2009; 103:285-298. [PubMed: 19623208]
- Faurby S, Barber PH. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Molecular Ecology. 2012; 21:3419-3432. [PubMed: 22574811]
- Foll M, Gaggiotti O. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers, A Bayesian perspective. Genetics. 2008; 180:977-993. [PubMed: 18780740]
- Foltz DW, Hrinevich AW, Rocha-Olivares A. Apparent selection intensity for the cytochrome oxidase subunit I gene varies with mode of reproduction in echinoderms. Genetica. 2004; 122:115-125. [PubMed: 15609571]
- Franz DR, Worley EK, Merrill AS. Distribution patterns of common seastars of the middle Atlantic continental shelf of the northwest Atlantic (Gulf of Maine to Cape Hatteras). Biological Bulletin. 1981; 160:394-418.
- Gillespie JH. Role of migration in genetic structure of populations in temporally and spatially varying environments (2) Island models. Theoretical Population Biology. 1976; 10:227-238. [PubMed: 982331]
- Hart M. Phylogenetic analyses of mode of larval development. Seminars in Cellular and Developmental Biology. 2000; 11:411-418.

- Hart MW, Byrne M, Smith MJ. Molecular phylogenetics of life-history evolution in Asterinid starfish. *Evolution*. 1997; 51:1848–1861.
- Hedrick PW. A standardized genetic differentiation measure. *Evolution*. 2005; 59:1633–1638. [PubMed: 16329237]
- Hellberg M. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution*. 1996; 50:1167–1175.
- Hoagland KE, Robertson R. An assessment of poecilogony in marine invertebrates, phenomenon or fantasy? *Biological Bulletin*. 1988; 174:109–125.
- Jeffery CH, Emlet RB. Macroevolutionary consequences of developmental mode in temnopleurid echinoids from the Tertiary of Southern Australia. *Evolution*. 2003; 57:1031–1048. [PubMed: 12836821]
- Jennings RM, Shank TM, Mullineaux LS, Halanych KM. Assessment of the Cape Cod phylogeographic break using the bamboo worm *Clymenalla torquata* reveals the role of regional water mass in dispersal. *Journal of Heredity*. 2009; 100:86–96. [PubMed: 18790728]
- Johannesson K. The paradox of Rockall – Why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*Littorina littorea*). *Marine Biology*. 1988; 99:507–513.
- Krug PJ. Bet-hedging dispersal strategy of a specialist marine herbivore, a settlement dimorphism among sibling larvae of *Alderia modesta*. *Marine Ecology Progress Series*. 2001; 213:177–192.
- Krug PJ. Patterns of speciation in marine gastropods, a review of the phylogenetic evidence for localized radiations in the sea. *American Malacological Bulletin*. 2011; 29:169–186.
- Lee CE. Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*. 1999; 53:1423–1434.
- Lee CE. Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*. 2002; 17:386–391.
- Levin LA. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biological Bulletin*. 1984; 166:494–508.
- Levin LA, Bridges TS. Control and consequences of alternative developmental modes in a poecilogonous polychaete. *American Zoologist*. 1994; 34:323–332.
- Levin LA, Creed EL. Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. *Marine Biology*. 1986; 92:103–113.
- Levin LA, Huggett DV. Implications of alternative developmental reproductive modes for seasonality and demography in an estuarine polychaete. *Ecology*. 1990; 71:2191–2208.
- Levin LA, Zhu J, Creed E. The genetic basis of life-history characters in a polychaete exhibiting planktotrophy and lecithotrophy. *Evolution*. 1991; 45:380–397.
- Liebert AE, Gamboa GJ, Stamp NE, et al. Genetics, behavior and ecology of a paper wasp invasion: *Polistes dominulus* in North America. *Annales Zoologica Fennici*. 2006; 43:595–624.
- Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics, from genotyping to genome typing. *Nature Reviews Genetics*. 2003; 4:981–994.
- Marsh AG, Fielman KT. Transcriptome profiling of individual larvae of two different developmental modes in the poecilogonous polychaete *Streblospio benedicti* (Spionidae). *Journal of Experimental Zoology B: Molecular and Developmental Evolution*. 2005; 304B:238–249.
- McEdward L. Adaptive evolution of larvae and life cycles. *Seminars in Cellular and Developmental Biology*. 2000; 11:403–409.
- Meyer CP. Molecular systematics of cowries (Gastropoda, Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*. 2003; 79:401–459.
- Moczek AP, Nagy LM. Diverse developmental mechanisms contribute to different levels of diversity in horned beetles. *Evolution and Development*. 2005; 7:175–185. [PubMed: 15876190]
- Moyle LC. Correlates of genetic differentiation and isolation by distance in 17 congeneric *Silene* species. *Molecular Ecology*. 2006; 15:1067–1081. [PubMed: 16599967]
- Narum S, Hess JE. Comparison of F_{ST} outlier tests for SNP loci under selection. *Molecular Ecology Resources*. 2011; 11:184–194. [PubMed: 21429174]

- Nei M, Maruyama T, Chakraborty R. Bottleneck effect and genetic-variability in populations. *Evolution*. 1975; 29:1–10.
- Nosil P, Funk DJ, Ortiz-Barrientos D. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*. 2009; 18:375–402. [PubMed: 19143936]
- Paetkau D, Slade R, Burden M, Estoup A. Genetic assignment methods for the direct, real-time estimation of migration rate, a simulation-based exploration of accuracy and power. *Molecular Ecology*. 2004; 13:55–65. [PubMed: 14653788]
- Palumbi S. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*. 1994; 25:547–572.
- Palumbi, S. Using genetics as an indirect estimator of larval dispersal. In: McEdward, L., editor. *Ecology of Marine Invertebrate Larvae*. CRC Press; Boca Raton, Florida: 1995. p. 369–387.
- Peakall R, Smouse PE. Genalex 6, genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources*. 2005; 6:288–295.
- Pechenik J. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. *Marine Ecology Progress Series*. 1999; 177:269–297.
- Pernet B, McArthur L. Feeding by larvae of two different developmental modes in *Streblospio benedicti* (Polychaeta, Spionidae). *Marine Biology*. 2006; 149:803–811.
- Perron FE. Life history consequences of differences in developmental mode among gastropods in the genus *Conus*. *Bulletin of Marine Sciences*. 1986; 39:485–497.
- Piry S, Luikart G, Cornuet JM. BOTTLENECK, a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*. 1999; 90:502–503.
- Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. GeneClass2, a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*. 2004; 95:536–539. [PubMed: 15475402]
- Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA. Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*. 2006; 38:904–909. [PubMed: 16862161]
- Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *American Journal of Human Genetics*. 1999; 65:220–228. [PubMed: 10364535]
- Qian PY, Chia FS. Effects of diet type on the demographics of *Capitella* sp. (Annelida, Polychaeta), Lecithotrophic development vs. planktotrophic development. *Journal of Experimental Marine Biology and Ecology*. 1992; 157:159–179.
- Rockman M. Looking for poecilogony in the *Streblospio benedicti* genome. *Integrative and Comparative Biology*. 2012; 52:173–180. [PubMed: 22659203]
- Rousset F. Genetic differentiation and estimation of gene flow from F-statistic under isolation by distance. *Genetics*. 1997; 145:1219–1228. [PubMed: 9093870]
- Ryman N, Palm S. Powsim: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes*. 2006; 6:600–602.
- Schulze SR, Rice SA, Simon JL, Karl SA. Evolution of poecilogony and the biogeography of North American populations of the polychaete *Streblospio*. *Evolution*. 2000; 54:1247–1259. [PubMed: 11005292]
- Selkoe KA, Toonen RJ. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecological Progress Series*. 2011; 436:291–305.
- Snell-Rood EC, Cash A, Han MV, Kijimoto T, Andrews J, Moczek AP. Developmental decoupling of alternative phenotypes, insights from the transcriptomes of horn-polyphenic beetles. *Evolution*. 2010; 65:231–245. [PubMed: 20731717]
- Sotka EE. Local adaptation in host use among marine invertebrates. *Ecology Letters*. 2005; 8:448–459.
- Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Molecular Ecology*. 2004; 13:2143–2156. [PubMed: 15245390]
- Strathmann RR. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual review of Ecology and Systematics*. 1985; 16:339–361.

- Strathmann RR. Why life histories evolve differently in the sea. *American Zoologist*. 1990; 30:197–207.
- Todd C, Lambert J, Thorpe J. The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages. Are pelagic larvae “for” dispersal? *Journal of Experimental Marine Biology and Ecology*. 1998; 228:1–28.
- Vermeij GJ. Phenotypic evolution in a poorly dispersing snail after arrival of a predator. *Nature*. 1982; 299:349–350.
- Ward, LA. PhD thesis. Dept. of Zoology, University of Hawaii; Honolulu: 1981. A taxonomic study of the Spionidae (Annelida, Polychaeta) from the Hawaiian Islands and Johnston Atoll with notes on their ecology and biogeographical distribution; p. 224
- Wares JP. Community genetics in the Northwestern Atlantic Intertidal. *Molecular Ecology*. 2002; 11:1131–1144. [PubMed: 12074721]
- Wares JP, Cunningham CW. Diversification predates glaciation in *Balanus glandula*. *Biological Bulletin*. 2005; 208:60–68. [PubMed: 15713813]
- Wares, JP.; Hughes, RA.; Grosberg, RK. Mechanisms that drive evolutionary change. In: Sax, DF.; Stachowicz, JJ.; Gaines, SD., editors. *Species Invasions, Insights into Ecology, Evolution, and Biogeography*. Sinauer Associates, Inc; Sunderland, Massachusetts: 2005. p. 229–255.
- Wasson K, Zabin CJ, Bedinger L, Diaz MC, Pearse JS. Biological invasions of estuaries without international shipping, the importance of intraregional transport. *Biological Conservation*. 2001; 102:143–153.
- Weersing K, Toonen RJ. Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*. 2009; 393:1–12.
- Whitlock MC, McCauley DE. Indirect measures of gene flow and migration, F_{ST} ($4Nm + 1$). *Heredity*. 1999; 82:117–125. [PubMed: 10098262]
- Williams TM, Carroll SB. Genetic and molecular insights into the development and evolution of sexual dimorphism. *Nature Reviews Genetics*. 2009; 10:797–804.
- Wray, GA. Evolution of larvae and development modes. In: McEdward, L., editor. *Ecology of Marine Invertebrate Larvae*. CRC Press; Boca Raton, Florida: 1995. p. 413–448.
- Xue Y, Zhang H, Huang N, et al. Population differentiation as an indicator of recent positive selection in humans, an empirical evaluation. *Genetics*. 2009; 183:1065–1077. [PubMed: 19737746]
- Zakas C, Hall DW. Asymmetric dispersal can maintain larval polymorphism: a model motivated by *Streblospio benedicti*. *Integrative and Comparative Biology*. 2012; 50:E195.
- Zakas C, Schult N, McHugh D, Jones KL, Wares JP. Transcriptome analysis and SNP development can resolve population differentiation of *Streblospio benedicti*, a developmentally dimorphic marine annelid. *PLoS ONE*. 2012; 7:E31613. [PubMed: 22359608]
- Zhang F, Wang Y, Deng H. Comparison of population-based association study methods correcting for population stratification. *PLoS ONE*. 2008; 3:e3392. [PubMed: 18852890]

Fig. 1.

Map of sampled populations on the East and West Coast of North America. Predominately Large populations are designated with triangles and predominately Small populations are designated with a diamond. BR is designated with a star. There are three possible regional groupings for AMOVA analysis. Region 1 is the North East shown with the dotted black circle. Region 2 is the South East shown with a grey dotted circle. When the West Coast is included a third region is added including DE.

Fig. 2. Histogram of F_{ST} values for all SNPs calculated across all populations. Outlier SNPs are shown in black.

Fig. 3. Unrooted neighbor-joining tree for pairwise F_{ST} values between populations. The third regional grouping, including the West Coast, is shown with a dotted line.

Fig. 4. Plot of pairwise F_{ST} values between East Coast populations vs. the distance (kilometres) between populations. Points are grouped based on the larval types in each comparison: S is a predominately Small population and L is a predominately Large population.

Table 1
Collection sites and dates including the total number of definitive small and large larvae from each population

Population name	Abbrev.	Known Small	Known Large	Total	Date collected	Location
West Marsh, Nova Scotia	NS	0	7	19	June 2011	N 44.65 W -63.37
Darling Marine Center, ME	ME	11	0	23	May 2011	N 43.93 W -69.58
Lewes Beach, DE	DE	1	5	46	June 2010	N 38.79 W -75.17
Delaware Sea Shore State Park, DE	DNS	0	0	7	June 2010	N 38.64 W -75.07
Indian River Inlet, DE	IRI	0	0	28	June 2010	N 38.62 W -75.07
Morehead City, NC	MC	11	0	37	May 2010	N 34.73 W -76.68
Baruch Institute for Marine Sciences, SC	BR	5	17	79	March, May 2010, March 2011	N 33.34 W -79.20
Grice Marine Lab, SC	CH	4	0	38	March 2010	N 32.75 W -79.89
Sapelo Island LTR site 6, GA	SP6	9	1	51	May 2010	N 31.52 W -81.22
Sapelo Island LTR site 3, GA	SP3	15	0	47	June 2010	N 31.38 W -81.278
Oregon Institute for Marine Biology, OR	OR	0	44	44	July 2010	N 43.34 W -124.32
Steger Marsh, CA	SF	0	14	14	June 2010	N 37.90 W -122.32
San Pedro, CA	LB	0	47	47	July 2010	N 33.71 W -118.28
Total		56	135	480		

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Table 2

Summary statistics for each population

Population	N_A	H_O	H_E	F_{IS}	P
NS	1.400	0.237	0.281	—	
ME	1.231	0.223	0.202	—	
DE	1.397	0.242	0.270	0.112	0.000
DNS	1.291	0.219	0.220	—	
IRI	1.346	0.217	0.245	0.134	0.000
MC	1.351	0.239	0.227	—	
BR	1.384	0.246	0.236	—	
CH	1.210	0.171	0.166	—	
SP6	1.182	0.173	0.168	—	
SP3	1.281	0.228	0.205	—	
OR	1.448	0.249	0.284	0.088	0.010
SF	1.413	0.236	0.271	—	
LB	1.513	0.321	0.329	—	

N_A is the mean number of effective alleles and H_O and H_E are the observed and expected heterozygosity. Significant inbreeding coefficients (F_{IS}) are reported.

Table 3

Single nucleotide polymorphisms (SNPs) that are identified as outliers from BayeScan.

SNP name	P	α	F_{ST}	SNP	MAF	Top BLAST organism	BLAST gene region	e-value	Contig length	# SNPs	Avg. depth
#5*	1.00	1.62	0.48	T/A	0.22	<i>Troglophilus neglectus</i>	Mitochondrial	4.58E-25	1490	191	389.2
#9*	1.00	1.70	0.50	A/G	0.24				1320	42	10.7
#14	1.00	1.44	0.44	C/G	0.31				786	10	7.7
#29*	1.00	1.62	0.48	A/G	0.23				752	30	10.7
#41*	1.00	1.81	0.53	T/G ^N	0.42	<i>Sthenelanelia uniformis</i>	Efl-alpha	6.28E-53	2029	170	92.8
#60*	1.00	1.56	0.47	A/G	0.38				479	13	20.8
#71*	1.00	1.81	0.53	G/A ^N	0.02	<i>Saccostrea kegaki</i>	Beta-tubulin	0	1395	148	31.1
#34	0.99	1.37	0.43	T/G	0.12				1112	56	21.3
#58	0.99	1.38	0.43	A/G	0.33				1546	76	17.9
#32	0.99	1.06	0.36	T/A	0.23				2055	92	22.9
#52*	0.99	1.09	0.37	T/C	0.34				803	23	13.6
#72*	0.99	1.04	0.36	T/C	0.23		Mitochondrial	1.00E-09	1266	31	14.7
#21*	0.99	-2.06	0.03	G/C	0.27				1077	38	6.5
#46*	1.00	-2.15	0.03	A/C	0.32				1586	142	45.5
#1	0.99	-1.79	0.04	T/C ^N	0.23	<i>Strongylocentrotus purpuratus</i>	Ribosomal protein S9	6.91E-42	928	79	32.9
#53	0.99	-1.55	0.05	T/G	0.3	<i>Platynereis dumerilii</i>	EST transcription factor	1.28E-16	1174	16	9.3
#22	0.99	-1.55	0.05	A/G ^N	0.18	<i>Salmo salar</i>	Adenosylhomocysteinase B	5.85E-69	2054	93	18.1
#26	0.98	-1.42	0.06	T/C	0.4				1475	85	20.0
#43	0.98	-1.45	0.05	T/C	0.42				614	16	18.2
#4	0.97	-1.50	0.05	G/A ^S	0.16	<i>Aplysia californica</i>	Actin	0	1961	125	15.3
#77 [†]	1.00	1.53	0.37	G/A	0.45				914	1	5.8
#6 [†]	0.99	-1.81	0.29	A/T	0.42				786	3	18.2

* SNPs that are outliers when both the total population and only the East Coast populations are used

[†] SNPs that are only outliers when using the East Coast populations.

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The posterior Bayes probability and α value is shown for each loci, where negative α values indicate purifying or balancing selection and positive α values indicate diversifying selection. The best BLAST hit to NCBI's nucleotide database is given for SNPs (if it has an e -value less than $10E-6$). The length and average coverage depth of the contig that the SNP appears in, as well as the number of SNPs in the contig are also given. SNPs that could be identified as nonsynonymous (N) or synonymous (S) are designated. The minor allele frequency (MAF) for each SNP is for the total genotyped sample.

Table 4

AMOVA results for two groupings: (a) excludes the West Coast populations from the analyses and includes DE in the southern region. (b) AMOVAs include the West Coast populations and a third region consisting of DE and the West Coast populations. AMOVAs with and without outlier loci are included for both analyses

Source of variation	d.f.	Sum of squares	Variance component	% variation
(a1): AMOVA of two regions excluding outlier F_{ST} loci and the West Coast [*]				
Among groups	1	249.15	1.374 Va	17.93
Among pops within groups	8	523.08	0.789 Vb	10.3
Within pops	740	4070.35	5.50 Vc	71.77
Total	749	4842.57	7.66	
(a2): AMOVA of two regions with all loci excluding the West Coast [†]				
Among groups	1	403.63	2.297 Va	21.87
Among pops within groups	8	723.37	1.099 Vb	10.46
Within pops	740	5259.67	7.108 Vc	67.67
Total	749	6386.68	10.503	
(b1): AMOVA of three regions excluding outlier F_{ST} loci and including the West Coast populations [‡]				
Among groups	2	600.3	0.971 Va	12.6
Among pops within groups	10	527.1	0.639 Vb	8.29
Within pops	948	5776.3	6.099 Vc	79.12
Total	959	6903.8	7.71	
(b2): AMOVA of three regions with all loci and including the West Coast populations [§]				
Among groups	2	1155.9	2.002 Va	18.47
Among pops within groups	10	660	0.794 Vb	7.33
Within pops	947	7614.4	8.041 Vc	74.2
Total	959	9430.3	10.837	

^{*} $F_{ST} = 0.282$, $P < 0.001$; $F_{SC} = 0.126$, $P < 0.001$; $F_{CT} = 0.179$, $P = 0.018$.

[†] $F_{ST} = 0.323$, $P < 0.001$; $F_{SC} = 0.134$, $P < 0.001$; $F_{CT} = 0.219$, $P < 0.001$.

[‡] $F_{ST} = 0.201$, $P < 0.001$; $F_{SC} = 0.095$, $P < 0.001$; $F_{CT} = 0.126$, $P < 0.001$.

[§] $F_{ST} = 0.258$, $P < 0.001$; $F_{SC} = 0.089$, $P < 0.001$; $F_{CT} = 0.185$, $P < 0.001$.