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Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster *Crassostrea virginica*

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ABSTRACT: We examined the role of local adaptation in structuring the stable genetic step-cline of the eastern oyster *Crassostrea virginica* along an environmental gradient in the lagoon system of eastern Florida, USA. Reciprocally transplanted progeny, produced by a 10 × 10 genetic cross of wild brood stock from northern and southern genetic lineages yielded significant evidence of local adaptation (interaction of genes and environment) in variables related to fitness, including survival, wet meat weight, and reproductive maturation. The strength of local adaptation was asymmetric, with greater effects on the northern compared to the southern genetic lineage. To a lesser extent, we found evidence of both the role of environment (in particular, adverse effects on both genetic crosses in the southern region), and the role of genetic differences between the 2 crosses independent of environment, with higher initial growth of the southern genetic lineage and higher condition of the northern lineage. These differences suggest that maintenance of the genetic step-cline involves natural selection. We discuss the potential role of temperature and phytoplankton community composition between the northern and southern regions. Our study is the first to determine the genetic basis for fitness-related phenotypes, and to relate this to local adaptation of the eastern oyster. Understanding the role of the environment in structuring the eastern oyster throughout its range is critical for effective management, and the results of this study also suggest that small environmental changes may have significant effects on conservation of the eastern oyster, particularly in the northern genetic lineage.

KEY WORDS: Reciprocal transplant · Local adaptation · *Crassostrea virginica* · Growth rate · Survivorship · Reproductive maturation · Genetic cline · Life history · Marine invertebrate

INTRODUCTION

Understanding how species adapt to their environment is a fundamental question in biology, a question posed by Darwin (1859) in 'The origin of species', further addressed by Mayr (1963) in the context of geographic evolution of animals, and an area of active research (Bohonak 1999, Schluter 2000, Tobler et al. 2008). In 'open' systems, where new members may come from outside the local population and with species that have a high dispersal potential during part of their life history, such as the eastern oyster *Crassostrea virginica*, there are several challenges to understanding the role natural selection plays in the adaptation of populations to the local environment. Specifically, with a high dispersal potential and with-

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out any apparent physical barriers to dispersal (as in numerous marine systems), it is difficult to predict how the homogenizing effects of gene flow will limit local adaptation. Moreover, while researchers may find substantial genetic structure in marine species, without apparent morphological variation that is recognized as adaptive (Knowlton 1993, 2000) it is difficult to conclude whether any observed genetic differences among these populations or strong genetic breaks within the species range were due to local adaptation (selection) or to random processes such as genetic drift. Despite both of these challenges, several studies have shown genetic differences and potential local adaptation among populations for marine species with a long evolutionary history, large population sizes, a high potential for dispersal, and widespread distributions (e.g. Avise 1992, Sotka et al. 2004, Burford et al. 2011), but relatively few have clearly demonstrated local adaptation. Thus, to better relate observed genetic divergences to evolutionary processes, studies must distinguish whether the observed genetic structure is due to genetic drift or to natural selection. The challenge of addressing natural selection in these 'open' systems is an area of active research using molecular and modeling tools (Kirkpatrick & Barton 1997, Sanford & Worth 2010, Irwin 2012). These studies suggest that the evolutionary history, the historic events that caused population separations or range expansions, and contemporary events such as changes in environmental conditions that may influence local adaptation should be considered when defining the mechanisms responsible for the genetic structure of these populations and in determining their capacity for adapting to new conditions.

One approach for testing local adaptation that provides critical understanding of the process of divergent selection across environments is through the use of reciprocal transplant experiments (Schemske 1984). Reciprocal transplants offer a statistically powerful method of analyzing the variation in fitness measures both within and between environments in a species' range, and can tease out local adaptation that is genetically based (Schemske 1984, Angert & Schemske 2005). While this approach is typically used in terrestrial systems with limited dispersal (particularly with plants), it is gaining traction in marine systems for species that are easily manipulated, to ensure accurate phenotypic measurements (Janson 1982, Bertness & Gaines 1993, Hays 2007, Sherman & Ayre 2008, reviewed in Sanford & Kelly 2011). Several studies that directly measured local adaptation using reciprocal transplants showed individuals did better in their natal habitats even in cases where there was no strong barrier to dispersal between habitats (e.g. marine algae, Hays 2007; sticklebacks, Schluter 2000; Atlantic molly, Tobler et al. 2008). However, a limitation of many reciprocal transplants is that the organisms were field-caught, eliminating control for the genetic characteristics or maternal effects, and limiting the ability to assign genetically based local adaptation. By controlling for genetic characteristics of the 'outplanted' progeny (progeny reared in a common garden in aquaculture and then move to natural settings), we can better understand the genetic basis of local adaptation in marine systems. Using reciprocal transplants with marine species that have an expansive and continuous distribution within which there are strong environmental changes (e.g. biogeographic shifts along linear coastlines), and measuring the effects of abiotic and biotic conditions on growth, morphology, survival and reproduction (Sanford & Kelly 2011) may tell us how contemporary and historical factors that enhance local adaptation or limited gene flow influence adaptation of species to their environment (Hice et al. 2012). Understanding the influence of environmental factors on the genetic structure of species is particularly important for those species that provide important resources for other species, such as the eastern oyster *C. virginica.*

The eastern oyster is a reef-building, eurythermal species that has a widespread and mostly continuous distribution from New Brunswick, Canada to the Yucatan Peninsula, Mexico (Galtsoff 1964), which encompasses large environmental variation. It is a keystone species, creating a natural, hard substrate in estuaries and lagoons throughout its range which provides shelter and settlement habitat for juvenile oysters, many species of small invertebrates, and fishes (Wells 1961, Zimmerman et al. 1989, Nestlerod et al. 2007). In addition, this commercially important species has suffered large population declines due to increased environmental degradation (Futch 1967, De Freese 1991, Grizzle et al. 2002, Wall et al. 2005), disease outbreaks of *Perkinsus marinus* (Bushek & Allen 1996, Reece et al. 2001) and *Haplosporidium nelson* (Ford & Haskin 1982), and years of poor resource management throughout its range (De Freese 1991, Nestelrod et al. 2007). Prior to settlement on hard substrate in estuaries and lagoons, the eastern oyster has a several-week long planktonic larval stage (Korringa 1952), which is the only dispersive phase in its life history. Despite the high dispersal potential, the eastern oyster has a stable and steep genetic step-cline in eastern Florida, USA, centered

near Cape Canaveral (Reeb & Avise 1990, Karl & Avise 1992, Hare & Avise 1996, 1998, Zhang & Hare 2012). This genetic cline is coincident with a relatively sharp environmental and biogeographic cline between the temperate and subtropical waters, and the Carolinian and Caribbean zoogeographic provinces (Briggs 1974), respectively. There are also similar genetic breaks centered at or near Cape Canaveral in eastern Florida within many co-distributed species (e.g. black sea bass, killifishes, horseshoe crab, shore birds; reviewed in Avise 1992). The stability of the step-cline of the eastern oyster over multiple decades suggests that this secondary contact zone is not very recent (Reeb & Avise 1990, Hare & Avise 1998, Zhang & Hare 2012), and that contemporary factors prevent introgression of the 2 genetic populations north and south of this zone. A better understanding of how the eastern oyster is adapted to its environment and how adaptation factors into the stability of the step-cline is critical for conservation of this species with impending environmental change.

Researchers have proposed several hypotheses to explain this steep and stable cline of the eastern oyster, including limited dispersal in the secondary contact zone between the northern and southern genetic lineages, and local adaptation of populations in the northern temperate versus southern subtropical estuarine environments (Briggs 1974, Hare & Avise 1996). The possibility of dispersal limitation in this region (which would limit the continued expansion of the northern genetic population south of, and the southern genetic population north of Cape Canaveral) could be caused by low population sizes and loss of suitable habitat in the vicinity of Cape Canaveral (Futch 1967, Hare & Avise 1996), eroding stepping-stone connections between the north and south. Previous genetic data also indicated that there was some introgression at certain loci from the north to the south, but little from the south to the north (Reeb & Avise 1990, Hare & Avise 1996, 1998), and genetic data from related species suggest larval mediated gene flow around Cape Canaveral is sufficient to homogenize populations (Zhang & Hare 2012). Therefore, dispersal constraints are unlikely to fully explain the stable step-cline of the eastern oyster, and it is possible that both limited dispersal and local adaptation in conjunction contribute to the stability of the present genetic cline.

Our goal was to test the role of local adaptation in maintaining the stable step-cline between the northern and southern genetic populations of the eastern oyster. To achieve this goal, we analyzed fitness-related phenotypic differences between reciprocally transplanted populations of the northern and southern genetic stock along the north–south environmental cline in eastern Florida. The design of the reciprocal transplant allowed us to test several hypotheses: (1) the outplanted individuals from both the northern and southern population have higher survival or growth in 1 of the 2 environments (e.g. both genetic populations do better in the north than in the south, suggesting environment is the primary driver of the observed phenotypic differences); (2) 1 of the 2 genetic populations does better than the other in both environments (e.g. northern genetic progeny outperform southern progeny in both regions, suggesting genetic differences as the primary driver); or (3) an interaction between the genetic populations and environment such that one genetic population does better in one environment and the opposite in the other environment (e.g. northern progeny does better than southern progeny in the north, but worse in the south, suggesting reciprocal local adaptation to their respective home environments). A critical component for understanding the speciation process in marine environments and in other high gene flow, terrestrial environments, is to identify how natural selection contributes to the structuring of widely distributed species. Whether dispersal limitation or local adaptation alone or in combination are responsible for maintaining this stable step-cline, a better understanding of mechanisms important in structuring this commercially and ecologically important species will help develop predictive models for the response of oyster populations to anticipated environmental changes.

MATERIALS AND METHODS

Experimental design

Brood stock

We collected brood stock from 2 representative sites for both the northern and southern genetic populations of the eastern oyster in Atlantic Florida, USA during February and March 2008 (Fig. 1, Table 1). The northern brood stock was from Saint Augustine (SA) and Marineland (ML) and the southern brood stock was from MacWilliams Park (MP), Vero Beach and North Bridge Sunset Dock (FP), Fort Pierce (Table 1). Previous genetic surveys demonstrated that genetic differences on either side of Cape Canaveral were much greater than any heterogeneity within regions (Hare & Avise 1996).

Fig. 1. Reciprocal transplant locations and sampling locations of the eastern oyster *Crassostrea virginica* in 2008. The map shows 4 experimental locations in north (N), central (C), and south (S) Florida for the transplant experiment, but only the north and south are analyzed here. ☆: location of brood stock collections; ★: location of Harbor Branch Oceanographic Institute aquaculture facility; inset: photo of one of the PVC pipe slate holders

We held the adults in tanks with filtered seawater at an average of 31.5 ppt salinity and 21 to 23°C at the Harbor Branch Oceanographic Institute, Fort Pierce, Florida (Fig. 1) for a minimum of 28 d. To eliminate maternal effects, it is standard practice to produce and raise F_1 brood stock in a common environment, make crosses, and then outplant F_2 oysters (Schemske 1984, Sanford & Worth 2010). However, accomplishing this in captivity could involve strong domestication selection (Christie et al. 2012), and hatchery-produced F_2 individuals would likely have reduced genetic diversity compared to the F_1 generation due to uneven parental contribution (Boudry et al. 2002, Zhang et al. 2010). Therefore, conditioning of adult and F_1 oysters in hatchery conditions minimized maternal effects while producing cohorts that better maintained the genetic diversity of wild oysters.

Table 1. Brood stock collections of eastern oyster *Crassostrea virginica* along the east coast of Florida; N: number of individual oysters collected

Location	Latitude (°N)	Longitude (°W)	N
Saint Augustine	29° 53′ 48.38″	81° 18′ 39.58″	100
Marineland	29° 40′ 13.64″	81° 12′ 57.06″	200
Vero Beach MacWilliams Park	27° 39′ 15.73″	80° 22′ 8.45″	200
Fort Pierce North Bridge Sunset Dock	27° 28′ 25.31″	80° 19′ 20.94″	100

Genetic cross production and hatchery culture

On 14 April 2008, we performed genetic crosses both within and between the northern and southern genetic stocks of the eastern oyster using northern and southern adult females and males from previously collected brood stock. We conducted a 10 × 10 male:female mating after strip-spawning adults (Scarpa & Allen 1992) and quantifying sperm and eggs of each individual to equilibrate parental contribution. We chose 20 adults from each brood stock based on feasibility and previous genetic analyses, and to ensure less than 5% inbreeding (Hare & Avise 1996, 1998, Tave 1999). After examining many oysters (200+ ind.), we chose the 10 male and 10 female oysters from each genetic stock with the highest quality and quantity of gametes to make the crosses (north: 10 ML males and 10 ML females; south: 8 MP and 2 FP males, and 7 MP and 3 FP females). The F_1 progeny we produced were from (1) 10 northern females and 10 northern males (N×N), (2) 10 northern females and 10 southern males (N×S), (3) 10 southern females and 10 northern males (S×N), and (4) 10 southern females and 10 southern males (S×S). We produced each of the resulting progeny groups, barring differences in fertilization success, with equal gametic contribution from 20 individuals in each cross by pooling equal quantities of eggs, then dividing them equally to allow separate fertilization by each male. We pooled zygotes within each cross and cultured the larvae separately in four 400 l tanks located at the Harbor Branch Oceanographic Institute. These crosses remained in separate tanks from hatching, through larval development until they were outplanted. There were 8 tanks in total, and progeny populations were rotated daily among the tanks to minimize any tank effects. We controlled daily tank temperature and salinity, which ranged from 22 to 30°C and from 31 to 32 ppt. Daily changes of filtered seawater were accompanied by daily counts and size measurement throughout the larval development phase of approximately 2 wk. As soon as we observed eyed-larvae (indicating competence to settle; Thompson et al. 1996), we placed 12 settlement plates (roof-slate, provided by Camara Slate Products) in each of the tanks that contained progeny populations. We rotated the slate daily to achieve even settlement across both sides of the slates.

Approximately 2 wk after initial settlement and just prior to outplanting, we culled settled oysters down to 10 or 20 individuals per slate side, such that for each genetic cross there was one slate with 20 and one with 10 individuals per side at each location (DENSITY treatment). The juvenile oysters had an average shell area size of 0.217 cm^2 at outplant. To check that progeny populations resulted from multiple parents, and to analyze regional differences in parental contribution for each progeny population, we conducted a microsatellite parentage analysis on a small subset of individuals at the end of the experiment (see Supplement 1 at www.int-res.com/articles/suppl/m505p161_supp.pdf).

Experimental locations

In choosing experimental locations, we used the following criteria: confirmed presence of adult oysters nearby, suitable depth that facilitated submergence for most of the experiment and that corresponded to the adjacent adult oyster bed at that location. After hatchery culling, we outplanted approximately 2 slates of progeny from each genetic cross at each of 12 different experimental sites located in the lagoon system of eastern Florida on 19 May 2008 in the north, and 20 May 2008 in the south (Fig. 1, Table 2). We also deployed YSI SONDE 600 series instruments at 10 of 12 sites and collected bihourly temperature, salinity, dissolved oxygen (DO), and water depth data at each site.

For this study on local adaptation, we present results from the 2 pure-cross (parental) progeny populations (N×N and S×S) and the 2 non-central regions (north and south) to ascertain any negative effects the northern and southern genetic populations may experience in either 'home' or 'away' environments. These 2 regions corresponded to the northern temperate and southern sub-tropical zoogeographic regions (Briggs 1974). In both regions, we had 4 replicate experimental sites along the lagoon system (total of 8 experimental sites; Fig. 1). These experimental sites were treated as random samples of regional habitats on either side of the genetic cline, with northern sites within the nonclinal northern genetic population and southern sites below the steepest part of the genetic cline (Hare & Avise 1996). Within the 2 regions we had 4 experimental sites, with 2 PVC frames per site and 4 slates per frame, resulting in 2 slates per genetic cross at each site (Fig. 1, Table 2). We switched the orientation for each frame weekly to avoid orientation bias, and we submerged frames to approximately 30 cm off the bottom so that the frames remained submerged for all but the lowest low tides. Due to our permit requirements (Florida Special Activity License #07SR-702), we terminated the experiment at 11 wk after outplanting, to ensure that the experimental oysters did not spawn in the field.

Phenotypic data collection and analysis

Reciprocal transplant data collection

After outplanting the slates at each of the experimental sites, we monitored growth and survival on a weekly basis. Each week, we pulled the slates out of the water, removed any fouling organisms (mostly barnacles) and new oyster recruits, counted the original outplanted oysters for survival estimates, and photographed each slate for digital size and growth analyses. We also collected environmental data from the data logger at 6 of the 8 experimental sites. At one site (Fort Matanzas Dock) we accessed data collected from an YSI SONDE (600 series) deployed as part of a monitoring project by NOAA and US Fish

Table 2. Outplant locations and number of individuals for the reciprocal transplant of the eastern oyster *Crassostrea virginica* along the east coast of Florida. No. of N×N (S×S) is the number of individuals from the N×N (S×S) cross that were outplanted at each location

Region	Location	Latitude (°N)	Longitude (°W)	No. of N×N	No. of S×S
North	Fort Matanzas Dock	29° 44.302′	81° 14.701′	33	50
North	Whitney Dock	29° 40.209′	81° 12.940′	45	52
North	Princess Place Preserve Dock	29° 39.532′	81° 14.289′	39	51
North	Bruce's Dock	29° 29.667′	81° 8.552′	49	52
South	Harbor Branch Dock	27° 31.794′	80° 21.160′	32	50
South	Carribe Colony Dock	27° 29.414′	80° 20.281′	44	57
South	Jack Island Dock	27° 30.895′	80° 19.199′	37	53
South	Walton Dock	27° 18.892′	80° 15.759′	50	50

and Wildlife in the Guana Tolomato Matanzas National Estuarine Research Reserve. In addition, we took weekly instantaneous measures of salinity, DO and temperature at one site in the southern region (Jack Island Dock). We also collected phytoplankton samples every other week at each of the experimental sites to estimate differences in food sources (e.g. dinoflagellates versus diatoms) between the 2 regions (see Supplement 1). As the individual oysters continued to grow, we monitored when their shells came into contact with each other, and in a few cases removed the individual that was closer to the edge of the slate (i.e. individuals that were likely to grow over the edge), which allowed us to continue to measure individual oysters' radial growth within the slate, and presumably diminished competitive interactions. We did not count culled individuals in the final survival analysis or in any growth analyses. After 11 wk of monitoring, we removed the slates from the field and processed the samples in the laboratory at Harbor Branch Oceanographic Institute.

We analyzed size and growth of the outplanted oysters by digitally measuring the oyster shell area (size) using ImageJ (v1.40g; Abramoff et al. 2004). We analyzed measurements at Week 0 (outplant) to account for any hatchery effects, at Week 2 (initial), Week 6 (midpoint), Week 11 (endpoint), and total size change (between Weeks 2 and 11). We also analyzed the growth rate as the shell area difference divided by the number of days in the interval between Weeks 1 to 2 (initial), Weeks 5 to 6 (midpoint), and Weeks 10 to 11 (endpoint), and total growth rate (Weeks 2 to 11). For the total growth rate analysis, we analyzed growth rate from Week 2 for only those oysters that survived the entire experiment. We also analyzed oyster survival, including Weeks 1 to 2 (initial), Weeks 2 to 6 (midpoint), and Weeks 2 to 11 (endpoint); the latter was equivalent to total survival as those individuals survived the entire experiment. We averaged the environmental data by week, and in order to match the size and growth rate data analyses were done at Weeks 2, 6 and 11.

In addition to the weekly data collection, at the end of the experiment we destructively sampled approximately 10 individuals per cross from each of 8 experimental sites and collected data on whole body weight, shell weight, wet meat weight (square root transformed data), and condition (wet meat weight/whole body weight, after removing the entire oyster from the slate), and then preserved tissue in 95% ethanol for DNA extraction. From the same individuals, we also sampled a cross section of oyster gonadal tissue for histological determination of sex (male, female, hermaphrodite) and reproductive stage. We analyzed reproductive stage using the protocol developed by Loosanoff (1942), and classified the oyster gonads as reproductive stages 1 through 8 (Kim et al. 2006). For a subset of individuals (5 ind. site^{-1} and cross^{-1}) we removed whole bodies for determining average *Perkinsus marinus* body burden of sampled individuals (including samples without cysts). We also estimated prevalence (proportion infected) and infection intensity (average body burden of infected oysters). We processed sampled individuals using alternative Ray's fluid thioglycollate medium (ARFTM, Sigma #A-0465) and following a modified method to isolate and enumerate *P. marinus* (Bushek et al. 1994, La Peyre et al. 2003, Ragone Calvo et al. 2003).

Statistical analyses

The experimental variables were REGION (NORTH and SOUTH), DENSITY (HIGH and LOW), and CROSS (N×N and S×S). We used SITE (experimental location, n = 8; Fig. 1) as a random effect to account for the correlations between oysters at a given site. As neither starting nor experimental DENSITY was a significant factor for any outcome, either alone or in combinations of factors (including the full model), it was dropped from all subsequent models reported here (see also Fig. S1 in Supplement 2 at www.int-res.com/articles/suppl/m505p161_supp.pdf). For all models, we used a generalized linear mixed model with SITE as a random effect and REGION and CROSS as fixed effects (size, growth, survival, reproductive stage, etc. = region + cross + region × cross). We used 2 main procedures in SAS (v9.2): the MIXED procedure for the endpoint size, reproductive stage, condition, parasite load, and growth data, and the GLIMMIX procedure using a logistic model for the binary survival data (assumes a binomial distribution of error; Floyd 2001). Several researchers have used the GLIMMIX procedure to analyze data with a binomial distribution of error in ecological and epidemiological studies (Brown & Prescott 1999, Hanninen & Vuorinen 2001, Verween et al. 2007, Melody et al. 2008) and adapted both procedures (MIXED and GLIMMIX) for growth and survival data, respectively, for oyster species (Dégremont et al. 2012). Our *a priori* assumption was of local adaptation, and we did not expect to see genetic lineages maladapted to their environment of origin. Therefore, for hypothesis testing and to further examine the pattern, we conducted planned contrasts, cor-

recting for multiple comparisons, including N×N in the north versus south, S×S in the north versus the south, N×N versus S×S in the north, and N×N versus S×S in the south. For the results of the GLIMMIX procedure, we report the maximum likelihood odds ratio, which can be interpreted as the relative odds of an oyster of a given progeny population dying during the test interval. For comparisons between crosses, the odds are relative to the S×S CROSS, and for comparisons between regions, the odds are relative to the SOUTH REGION. For example, an odds ratio of 2.5 in the north would mean that N×N progeny individuals were 2.5 times more likely to survive as the S×S progeny individuals in the north. Likewise, an odds ratio below 1 would indicate a higher likelihood of S×S progeny surviving. For analysis of the environmental data, we also used MIXED procedure to first analyze regional differences in salinity, temperature, and DO, and then an ANCOVA to analyze growth and survival with environmental covariates at the 3 different time points (initial, midpoint, and end). Because we had 2 crosses at each site and 2 performance measures associated with these crosses, and a single data point for each environmental variable at each site, we ran ANCOVAs for each cross separately.

RESULTS

Environmental characterization

We found a trend of consistently lower temperatures and higher DO in the north compared to the south throughout the experiment. However, due to low statistical power, temperature differences in the north versus south were only significant in the final week (Week 11, approx. 5 August 2008), after corrections for multiple comparisons in the ANCOVA (temperature Week 11, $p < 0.05$). Weekly temperatures ranged from 31 to 25°C and 32 to 26°C in the north and south, respectively (overall mean of 28 and 29°C, respectively; Fig. 2). Salinity was similar among all sites and over time during the experimental season in 2008, with a weekly range from 35.4 to 29.1 ppt and 36.9 to 29.4 ppt in the north and south, respectively (overall mean 33.5 and 32.6 ppt in the north and south; Fig. 2). We did not find any significant patterns of averaged temperature extremes, high or low, between regions (data not shown). Dinoflagellates outnumbered diatoms in the southern region during 2 of 5 sampling periods, and diatoms consistently exceeded dinoflagellate counts in the north (Fig. 2, Supplement 1).

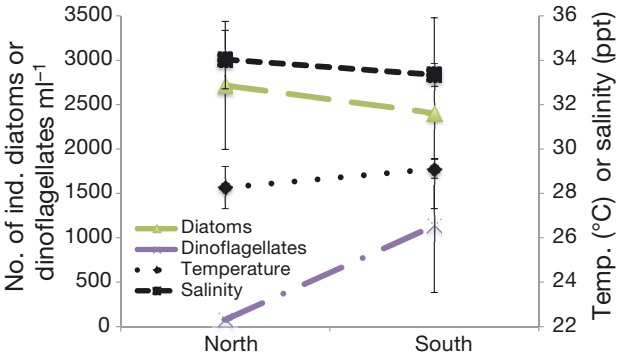

Fig. 2. Environmental conditions between the northern and southern genetic regions of the reciprocal transplant of the eastern oyster *Crassostrea virginica*. Data include mean (±SE) of phytoplankton composition, average temperature, and salinity

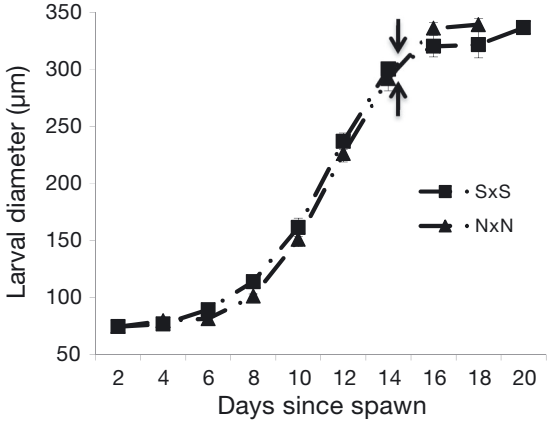

Fig. 3. Larval progeny growth mean (±SE) of N×N and S×S *Crassostrea virginica* progeny populations in the hatchery prior to the reciprocal transplant in 2008. The arrow indicates the first appearance of eyed-larvae for each of the progeny populations

Crosses and larval culture

Early oyster development

We found similar growth between the 2 progeny populations for the first 18 to 20 days since spawn (DSS) before the larvae began to metamorphose and settle on the roof slate in their respective tanks (Fig. 3). For both progeny groups, the greatest change in size was between 6 and 14 DSS. The percent mortality from 2 to 14 DSS was not significantly different between the 2 progeny populations (2-tailed paired *t*-test, $p = 0.91$).

We outplanted a total of 744 N×N and S×S oysters amongst the regions (Table 2) We found a significant

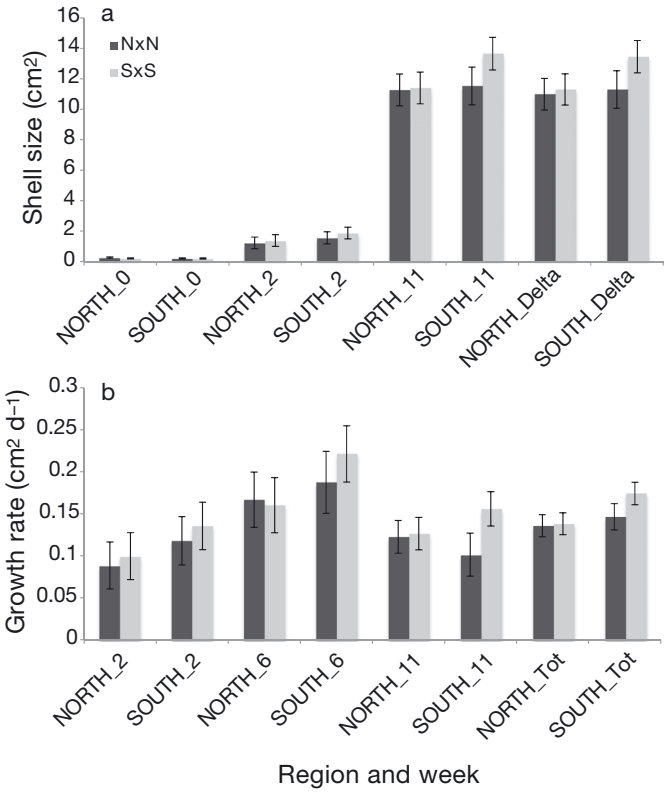

Fig. 4. Mean (±SE) shell area and growth of *Crassostrea virginica* during the 2008 reciprocal transplant for N×N and S×S progeny populations in the northern and southern regions: (a) shell area at outplant, Week 2, Week 11, and overall (delta Week 2–11); (b) growth rate at Week 1–2, Week 5–6, Week 10–11, and from Week 2–11 (total)

difference in size between the N×N and S×S progeny populations outplanted in the northern region (N×N versus S×S in the north, p = 0.0001, Week 0; Fig. 4a). However, within one week we found no significant difference in oyster size between genetic crosses in the northern region. Because estimates of size were prone to measurement error when the oysters were very small (average of 0.217 cm^2), we focused on more accurate estimates of size from Week 1 forward (Fig. 4a). We found no regional differences in the parental contribution for the 2 progeny crosses (see Supplement 1).

Reciprocal transplant performance measures

Survival

We found significant REGION-by-CROSS interaction effects on the probability of survival for all 3 time periods (initial p = 0.014, midpoint p < 0.001, final p < 0.001). We report the maximum likelihood odds ratio, an estimate of the reciprocal transplant selection gradient for survival, with respect to the planned contrasts. For example, the odds ratio between N×N and S×S in Wk 2 is a 1.7 higher probability of survival of the N×N progeny compared to the S×S progeny in the North (Table 3). Comparing the 2 parental crosses to each other within each region, there was significantly higher probability of survival of N×N progeny compared to S×S progeny in the north, and the opposite in the south for all 3 time periods (p < 0.05; Table 3), with the exception of the northern comparison in the initial period. Over the experimental time period, there was an approximately 2-fold higher probability of individual survival of N×N progeny over S×S progeny in the north, while in the south there was an approximately 3-fold higher probability of individual survival of the S×S progeny over N×N progeny (Table 3). Comparing individual progeny crosses between regions, the probability of survival of N×N progeny was significantly greater in the north than the south at all 3 time periods (p < 0.01), with an approximately 6-fold higher probability of survival of N×N progeny in the north than in the south over the experiment. The S×S progeny population had a significantly greater probability of survival in the north than in the south at the initial time period (p = 0.05), but there was no significant difference at the midpoint or endpoint time periods. In the outplant week, both crosses had significantly better survival in the north than in the south (data not shown, p < 0.05), but subsequent weeks (Week

Table 3. Results of survival from the maximum likelihood odds ratios of the reciprocal transplant of the eastern oyster *Crassostrea virginica* during 3 different time intervals

Genetic cross odds ratio	Time interval	North	South
N×N:S×S	Week 1–2	1.70	0.49[a]
N×N:S×S	Week 2–6	1.99[a]	0.37[a]
N×N:S×S	Week 2–11	1.77[a]	0.37[a]
Region odds ratio	**Time interval**	**N×N**	**S×S**
North:South	Week 1–2	9.96[a]	2.89[a]
North:South	Week 2–6	6.58[a]	1.23
North:South	Week 2–11	6.52[a]	1.37

[a]Significant difference in planned contrasts in the mixed model logistic after corrections for multiple comparisons; see text for specific p-values for overall survival

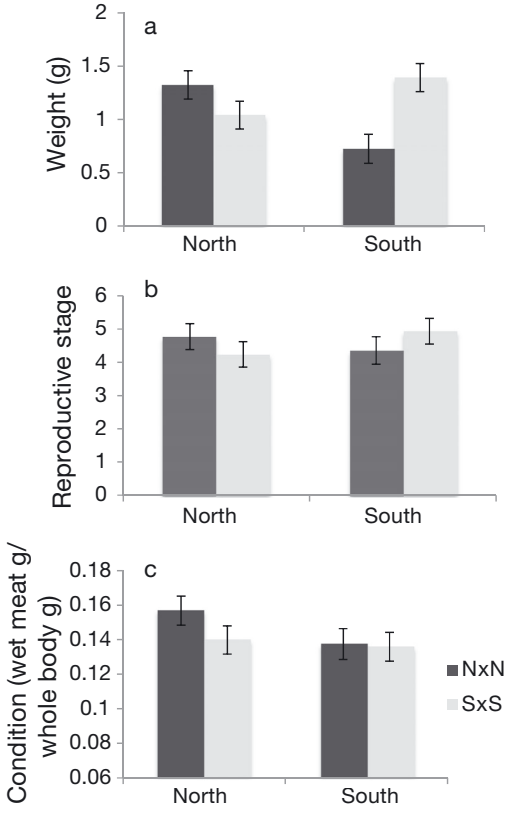

Fig. 5. Mean (±SE) final measures for N×N and S×S *Crassostrea virginica* progeny populations including (a) wet meat weight, (b) reproductive stage, and (c) condition (wet meat weight/whole body weight)

2 onwards) were not significantly different, and mortality in outplanted oysters leveled off between Week 4 to the end of the experiment (see Fig. S2 in Supplement 2).

Reproduction, weight, & condition

We found significant REGION-by-CROSS interaction effects for measures of final reproductive stage and square-root transformed wet meat weight in the mixed model ($p = 0.014$ and $p = 0.014$, respectively; Fig. 5). For both response variables, N×N progeny were more advanced and heavier than S×S progeny in the north, and S×S progeny were more advanced and heavier than N×N progeny in the south. However, the only significant contrast was the comparison square-root transformed wet meat weight of N×N versus S×S in the south (wet meat weight: $p = 0.31$ north, $p = 0.018$ south; reproductive stage: $p = 0.08$ for both). There were no significant effects of CROSS, REGION, or the REGION-by-CROSS for sex (male versus female; data not shown).

We found a significant CROSS effect in the mixed model for oyster condition ($p = 0.048$; Fig. 5). In the planned contrasts, the N×N progeny had significantly greater condition than the S×S progeny in the north ($p = 0.006$), and no significant difference in the south ($p = 0.82$). The contrast comparisons for progeny populations across regions showed a trend in which N×N progeny were heavier and had a higher condition index in the north than in the south, and S×S progeny were heavier and had a lower condition index in the south than in the north.

Growth rate

The total growth rate (change d^{-1} starting at Week 2 to end of experiment) and change in size (delta size; size at Week 2 to end of experiment), showed a significant CROSS effect ($p = 0.033$ and 0.037, respectively; Fig. 4) and a trend of REGION-by-CROSS interaction ($p = 0.074$ and 0.082, respectively). We found a higher growth rate of S×S progeny than N×N progeny in both regions, and this was significant in the south at Week 2 ($p = 0.02$; Fig. 4b). In addition, we found greater growth rate of S×S progeny in the south than in the north ($p = 0.056$). Breaking down growth rate into initial, midpoint, and final periods, we found a significant CROSS effect ($p = 0.017$) in the initial period and no significant effect of growth rate in the midpoint period. The final period (Week 11) was similar to the total growth rate with a trend towards a REGION-by-CROSS interaction ($p = 0.07$; Fig. 4b) and a significant cross effect ($p = 0.035$). The statistical results for oyster shell area (Fig. 4a) were similar to oyster growth (Fig. 4b).

Parasite load

We did not find significant effects of CROSS, REGION, or interactions for *Perkinsus marinus* prevalence, body burden, or infection intensity (data not shown). The average body burden (number of parasitic cysts per gram tissue) of progeny populations in the north was 3.25 and 4.61 (N×N and S×S, respectively) and of progeny populations in the south was 3.99 and 2.88 (N×N and S×S, respectively). Average prevalence of northern progeny populations was 0.85 and 0.65 (N×N and S×S, respectively) and of southern progeny populations was 0.48 and 0.85 (N×N and S×S, respectively).

Environmental correlations

There were no significant correlations between weekly growth rate for each cross and weekly averages of salinity, temperature, or dissolved oxygen during the initial, midpoint, and final time periods. The degree of among-site variation of environmental measurements, and in some cases a lack of significant difference in the measures between regions (salinity), was higher than our statistical power to detect significant patterns given only 4 sampling locations per region. We also did not find any significant correlations using temperature extremes between the regions (data not shown).

DISCUSSION

We found evidence of local adaptation in the eastern oyster by comparing northern and southern genetic lineages in a reciprocal transplant across approximately 300 km of eastern Florida coastline. In the 4 southern experimental locations, northern genetic oysters fared poorly compared to the southern genetic oysters, showing lower probability of survival and poorer total growth and condition. There were trends in the data suggesting that growth rate was similarly affected. In the 4 northern locations, oysters of both lineages did relatively well, although the northern genetic lineage outperformed the southern lineage, demonstrating local adaptation in the north as well. These experimental results demonstrate local adaptation by showing that oysters have better fitness in their home environment.

The results of this study support an ecological role in the maintenance of the genetic step-cline of the eastern oyster in Florida by the following factors, in order of greater versus less prevailing: (1) interactions between genes (G) and environment (E) (i.e. local adaptation, G × E), (2) environmental differences along the ecotone in eastern Florida lagoon system (E), and (3) genetic differences between the northern temperate and southern subtropical genetic populations of the eastern oyster (G). In particular, the G × E interaction was significant with the response variables of reproductive maturation and survival, demonstrating that if oyster larvae migrate and settle in the opposite regions they would experience, on average, lower survival and delayed reproduction than the local larvae. While outplanting progeny from genetic crosses minimized the influences of phenotypic plasticity, the evidence we found for local adaptation should be interpreted with caution for 3 reasons. First, we used F_1 oysters, which may suffer from unknown maternal effects. Second, the relatively small number of crossed individuals for each lineage might lead to family effects. Finally, variation in early mortality might affect density, which may bias the results.

Carry-over maternal effects from field-collected brood stock individuals could bias our results by providing the progeny crosses with an unknown advantage or disadvantage. We addressed this by holding the adult brood stock under standardized conditions for approximately 1 month before strip-spawning, forcing them to finish gonad conditioning under a uniform environment. In addition, we produced progeny that metamorphosed and settled within hatchery tanks of similar environment (common garden) before exposing them to the environmental cline. Therefore, if maternal effects wane during development (maximum effects on survival before larval day 6; Newkirk et al. 1977), then outplanting settled juveniles helped minimize the potential for maternal effects. Finally, by using F_1 rather than F_2 progeny, we minimized issues with tank selection that might have confounded the experiment (Christie et al. 2012).

Given the potential for sperm competition in oysters that may affect parentage contribution (Boudry et al. 2002) and the genetic diversity of progeny populations, our 10-by-10 male:female matings were an effort to capture substantial genetic diversity of each genetic population by equalizing contributions of 20 individuals. While the progeny populations do not completely represent the genetic diversity of either genetic population, our methods did provide a logistically feasible representation of the genetic populations given the limitations of conducting successful genetic crosses in the laboratory. Due to the large number of individuals contributing genetically to the progeny populations at the end of the experiment, and the lack of significant differences in parental contributions between regions, the significant difference in outplanted oyster response likely reflect regional population difference rather than family effects.

In reciprocal transplants, any changes in density of outplanted organisms which may be due to early mortality unrelated to the experiment (e.g. transport mortality or transplant shock) that favors growth or survival of one of the genetic crosses may confound the interpretation of local adaptation. However, the design of this experiment avoided density effects, and this was supported by the empirical results. As part of the experimental design we culled settled oysters in the hatchery prior to outplant, which established spacing that prevented most oysters from

growing into direct contact; we culled the few that did make contact. Therefore, interference competition was minimal or non-existent in the early part of the experiment, and perhaps overall. We also outplanted 2 densities of oysters and observed no impact of initial density on any measure of oyster response. Finally, while N×N and S×S oysters had a similar early decline in the south (Fig. S2 in Supplement 2 at www.int-res.com/articles/suppl/m505p161_supp.pdf), we did not find any significant correlation between density and growth or size for outplanted oysters in either region (Fig. S1 in Supplement 2). We were unable to identify the cause of early mortality in the southern region, but these results demonstrate that differences in density did not confound evidence of local adaptation.

Based on the relative performance of the outplanted eastern oyster progeny populations in the reciprocal transplant, and ruling out maternal, family size, and density dependent effects as important confounding factors, we conclude that local adaptation (G × E) is an important mechanism contributing to the maintenance of the 2 genetic populations. We also found differences between the environment north and south of the step-cline (E) and between the 2 genetic populations (G). All of these factors, both interactive (local adaptation) and individual effects (environment and genes), have important implications for the evolution of the step-cline and for management and conservation of the eastern oyster, to the extent that the experimental treatments were representative of natural oyster conditions.

Interaction of genes and environment: local adaptation

Given the inherent limited statistical power of most reciprocal transplants conducted over large geographic areas, it was compelling that we found a signature of the interaction between genetic cross and region (G × E) in multiple response variables, even with only 4 replicate outplant locations per region. This significant evidence of local adaptation in response variables was directly related to fitness: survival and reproductive maturation (reproductive stage and wet meat weight). We also found a trend towards G × E for 3 related endpoint measures of growth: growth rate, oyster shell surface area, and whole body weight.

The finding of local adaptation fits in the context of the historical demography and both historic and contemporary evolutionary processes. Based on the genealogical patterns of contemporary phylogeography, the northern and southern populations were separated into different environmental regions during the Pleistocene and earlier (Reeb & Avise 1990, Avise 1992). Their nearly continuous distribution along eastern Florida today is hypothesized to result from post-Pleistocene secondary contact, producing a step-cline maintained by some combination of differential selection and barriers to gene flow (Hare & Avise 1996). At every point in time, the large effective size of eastern oyster populations (Rose et al. 2006, He et al. 2012) is predicted to limit the random effects of genetic drift counteracting local adaptation (Lande 1976). For these reasons, oyster populations subjected to reduced gene flow should have the capacity to adapt to relatively subtle environmental distinctions. Several previous reciprocal transplant studies found individuals do better in their home environment or niche in a variety of systems that lack strong physical barriers to dispersal (e.g. Hays 2007, Tobler et al. 2008, Brady 2012). For example, a similar pattern in a study of the intertidal organism *Silvetia compressa* (Hays 2007) showed evidence of local adaptation between populations growing at different tidal heights within the same location, despite the highly dispersive reproductive strategy. While Hays' (2007) study suggested that high gene flow will not preclude local adaptation within small geographic areas, the strong environmental differences in tidal heights and subsequent exposure duration and potential for desiccation for populations of *S. compressa* also provides evidence of a strong selective force. This suggests that high genetic exchange will limit the effect of local adaptation without strong selective differences (Hendry & Taylor 2004, Hereford 2009). In comparison to these studies, the eastern oyster has high potential for gene flow, due to the 2+ wk pelagic larval phase. The high potential for gene flow combined with the lack of genetic structure within large portions of the northern and southern regions individually, suggests high gene flow within regions is often realized (Karl & Avise 1992, Hare & Avise 1996, Hare et al. 2005, Varney & Gaffney 2008). Nonetheless, the evolutionary history of isolation, large effective population sizes, and restricted gene flow in the contact zone support local adaptation in the eastern oyster, demonstrating that even with small measured environmental differences, these differences were sufficient to drive local adaption. Results from this study support the hypothesis that local adaptation in this system was enhanced by the historic separation between 2 large genetically diverse populations and recent limita-

tions in gene flow between the northern and southern oyster populations.

Individual effects, environment and genes

The role of environment, regardless of genetic lineage, was particularly important in the first few weeks of the reciprocal transplant based on the regional pattern of low oyster survival in the south. In addition, the greater average differences of several endpoint response variables between the progeny populations in the south suggested that environmental factors influenced either post-settlement development or survival, and that perhaps the environment in the southern locations was more stressful in general. Environmental differences between the north and south, such as differences in temperature, current circulation patterns, higher disease prevalence, or food quality may be important in early larval development or survival of the outplanted oysters. The southern study region is at the edge of both genetic lineages' distributions along the eastern coastline of the USA, and may be a suboptimal region for both genetic lineages. Recently, water circulation and usage changes in the lagoon system in the Cape Canaveral region and boating activity (which adversely affects oyster survival and condition in general) resulted in large losses of adult oyster beds throughout the central and southern regions (De Freese 1991, Grizzle et al. 2002, Wall et al. 2005). In particular, changes in water circulation or water usage that lower water quality could cause increase stress, resulting in a higher frequency of disease outbreaks or lower abundance or quality of food sources for the oysters. While we did not find significant differences in the prevalence or parasite load of *Perkinsus marinus* between regions, other parasites or regionally discrete blooms of toxic dinoflagellates (described by Badylak & Phlips 2004) could adversely affect oyster survival. The results from the phytoplankton survey confirmed that the progeny oysters in the southern region were exposed to higher numbers of dinoflagellates throughout most of the experimental period, which may mean that the recently outplanted oysters in the south had lower food quality or greater exposure to toxins from dinoflagellates. While this ecological factor may contribute to the significant environmental effects, the G × E results of this study suggested that southern oysters were better able to handle these and other stressors in the southern region and, therefore, environment alone was not driving this pattern.

In addition to the role of environment, we found significant differences in life history characteristics between the 2 progeny populations (i.e. a genetic effect). The combination of faster early growth in the S×S progeny (in both regions) and higher condition of the N×N progeny suggests different life history strategies. A similar pattern was documented in a congener, the Pacific oyster *Crassostrea gigas*, as Ernande et al. (2003) found genetic polymorphisms associated with 2 early life history strategies: fast growing and developing versus slow growing and developing phenotypes. Perhaps space limitations of settling oysters are accentuated in the south where there are warmer conditions or higher crowding rates, and selection for fast growth to stake their spatial claim outweighs investment in condition. The results of the significant cross or gene effect in this study and the resulting differences in characteristics of early growth or condition between the 2 progeny populations suggest a link between trade-offs and local adaptation in the eastern oyster. To better define and address the consistency of the environmental and genetic effects on oysters requires additional reciprocal transplants that include regions deeper into both the southern and northern genetic populations' ranges, and investigates additional environmental roles such as the regularity of toxic phytoplankton blooms, while evaluating differences in life history trade-offs between the progeny populations.

Mechanism maintaining distribution of the eastern oyster

One of the fundamental questions in both evolutionary and conservation biology is how populations adapt to their environment, and how this plays a role in geographic structuring of populations. We found support for local adaptation and the role of environment in fitness-related differences between the 2 genetic populations of the eastern oyster. For some of the response variables, there were stronger G × E effects in the south, suggesting additional mechanisms were important in maintaining the geographic cline of the eastern oyster. Dispersal of genotypes may be limited across the genetic step-cline, as suggested by multi-locus assignment tests comparing oyster populations on either side of the step-cline at Cape Canaveral in 2007 and 2009 (M. P. Hare et al. unpubl. data). While a longer-term data set might shed light on whether low dispersal is uniform across time, these patterns are consistent with dispersal constraints that could contribute to the evolution of

local adaptation, and may be important in maintaining the geographic cline. In previous phylogenetic and phylogeographic studies along the eastern Florida ecotone (Karl & Avise 1992, Hare & Avise 1996), alleles characteristic of the northern populations were found south of the step-cline at a low frequency, but the opposite was not true in the north, suggesting asymmetrical porosity in the cline. It was hypothesized that the asymmetric shape of the cline was due to genes flowing primarily from north to south, although historical events may have also caused the asymmetry (Hare & Avise 1996). Given the pattern of local adaptation found in this study, northern migrants will experience a more severe filter, which suggests that historic gene flow or admixture may no longer be realized.

Much of the previous phylogenetic work assessed populations prior to recent warming trends in the northeastern USA (1990s and beyond; e.g. Cook et al. 1998). In the 1980s, there was higher annual frequency of cooler years that may have favored the northern genetic lineage, allowing leakage across the hybrid zone from north to south. Indeed, we may be capturing a turn in conditions from those that allowed some asymmetric gene flow from north to south, to conditions that limit it. This mechanism was proposed in another marine system to explain geographic changes in frequencies of genetic lineages with years that were colder versus warmer than average along the nearshore regions of western North America (e.g. Burford et al. 2011). Understanding the role of the environment in maintaining genetic diversity and shaping the future evolutionary trajectory of these regionally adapted populations is critical for the eastern oyster. The environmental thermal cline represents an approximately 2°C temperature gradient, which is at the low end of 'worst-case' predictions of temperature change (0.3 to 7.5°C surface-air temperature, 2 to 6°C sea-surface temperature in North America; Najjar et al. 2000, IPCC 2001, Zwiers 2002). Changes in temperature will likely affect growth and reproduction of the eastern oyster, but could also increase the risk of disease in oyster populations (Cook et al. 1998, Harvell et al. 1999). In this study, we were limited in our ability to decipher the degree to which differences in temperature affected the outplanted oysters, with the exception of significant differences between the regions towards the end of the outplant. Therefore, a more controlled component to test the temperature shifts on growth and survival, or a reciprocal transplant deeper into either genetic population's range would be important.

This study provides the first phenotypic comparison of oyster populations on either side of the genetic step-cline, demonstrating that clinal patterns, observed in a small percentage of the genome (Murray & Hare 2006), are associated with regionally adapted populations. Given the regionally concordant pattern of genetic breaks for many species in this region (Avise 1992), information gained from this analysis of local adaptation in the eastern oyster can be applied to better help us understand how both historical and contemporary processes shaped, promoted, and in some cases maintain the genetic structure upon secondary contact. Indeed, with the insights provided here and the growing knowledge of functional genetics relating to disease and physiology, the eastern oyster is becoming a more valuable looking-glass on the dispersal biology and evolutionary dynamics of lagoon populations along eastern Florida.

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