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ECOLOGICAL GENETICS IN THE NORTH ATLANTIC: ENVIRONMENTAL GRADIENTS AND ADAPTATION AT SPECIFIC LOCI

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Abstract. The North Atlantic intertidal community provides a rich set of organismal and environmental material for the study of ecological genetics. Clearly defined environmental gradients exist at multiple spatial scales: there are broad latitudinal trends in temperature, meso-scale changes in salinity along estuaries, and smaller scale gradients in desiccation and temperature spanning the intertidal range. The geology and geography of the American and European coasts provide natural replication of these gradients, allowing for population genetic analyses of parallel adaptation to environmental stress and heterogeneity. Statistical methods have been developed that provide genomic neutrality tests of population differentiation and aid in the process of candidate gene identification. In this paper, we review studies of marine organisms that illustrate associations between an environmental gradient and specific genetic markers. Such highly differentiated markers become candidate genes for adaptation to the environmental factors in question, but the functional significance of genetic variants must be comprehensively evaluated. We present a set of predictions about locus-specific selection across latitudinal, estuarine, and intertidal gradients that are likely to exist in the North Atlantic. We further present new data and analyses that support and contradict these simple selection models. Some taxa show pronounced clinal variation at certain loci against a background of mild clinal variation at many loci. These cases illustrate the procedures necessary for distinguishing selection driven by internal genomic vs. external environmental factors. We suggest that the North Atlantic intertidal community provides a model system for identifying genes that matter in ecology due to the clarity of the environmental stresses and an extensive experimental literature on ecological function. While these organisms are typically poor genetic and genomic models, advances in comparative genomics have provided access to molecular tools that can now be applied to taxa with well-defined ecologies. As many of the organisms we discuss have tight physiological limits driven by climatic factors, this synthesis of molecular population genetics with marine ecology could provide a sensitive means of assessing evolutionary responses to climate change.

Key words: adaptation; climate; cline; endogenous selection; hybrid zone; intertidal; latitude; polymorphism.

Introduction

Replicated clinal variation along parallel environmental gradients provides some of the most convincing evidence for natural selection in the wild (Endler 1986). These patterns bring together the fundamental processes of evolution in action: differential survival or reproduction of genetically based phenotypes in response to environmental challenges. Clinal variation is particularly appealing because it provides a predictive relationship between specific environmental factors and the phenotypic traits in question. This can help dissect long-standing questions on the role of genetic variation in adaptation to environmental heterogeneity. Parallel clines on different continents in *Drosophila* are some of the most studied examples of this replicated natural

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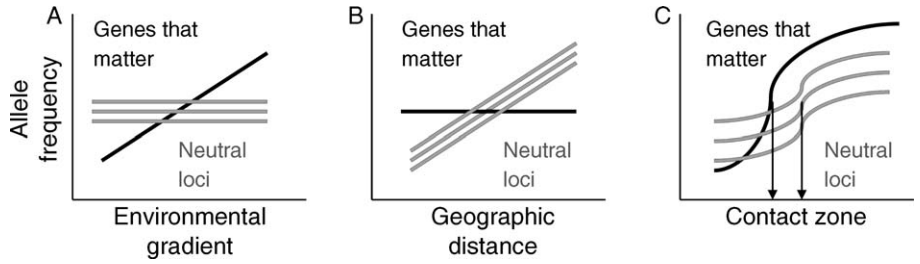

FIG. 1. Models of selection on specific traits in ecological genetics. Three different neutral models illustrate how genes that matter in ecology will show variation across gradients that are distinct from neutral loci. (A) Directional selection is implied at loci that track environmental gradients while neutral markers do not. (B) Stabilizing or balancing selection is implied at loci that fail to show isolation by distance when many neutral loci do. (C) Loci that show stronger clinal variation than neutral loci sampled across contact zones can imply habitat-specific selection, or selection against genetic incompatibilities. Statistical methods for identifying these loci are provided by Lewontin and Krakauer (1973) and Beaumont (2005).

selection, such as the alcohol dehydrogenase (*Adh*) polymorphism (Oakeshott et al. 1982) and clines for body size (Huey et al. 2000, Zwaan et al. 2000). *Drosophila*, and other model genetic organisms, are powerful systems for dissecting the genetic bases of complex traits, but often lack the necessary understanding of ecological factors that might be driving patterns of genetic variation in natural populations.

In this paper, we will suggest that the intertidal organisms of the North Atlantic offer a rich set of material to test hypotheses of selection along environmental gradients. Many of the species in the North Atlantic are recent colonists, having arrived from the Pacific after the opening of the Bering Strait 5 Ma (million years ago), with most arriving during the great invasion 3.5 Ma (Marincovich and Gladenkov 1999). This introduced a number of species onto both coasts of the Atlantic, offering an opportunity to study parallel aspects of ecological and evolutionary adaptations within the same set of species. A particularly attractive aspect of these organisms is the extensive ecological literature describing patterns and processes governing recruitment, reproduction, and community structure. A further strength of these communities is the multiple spatial scales over which clinal variation can be examined: there are parallel gradients spanning latitude, estuaries, and tidal height on both coasts of the North Atlantic. While most intertidal organisms are poor models for genetic and genomic analyses, progress in comparative genomics will facilitate the application of molecular methods to many non-model genetic organisms (e.g., Stillman et al. 2006, Teranishi and Stillman 2007). Moreover, statistical methods in population genetics have provided some powerful approaches to the study of adaptive genetic variation that can be used in any organism (McDonald 1994, Kreitman 2000, Yang and Bielawski 2000, Beaumont and Balding 2004, Beaumont 2005). When these tools can be applied to organisms whose ecology is both well understood and readily manipulated, deeper insight into the biological significance of genetic polymorphisms can be obtained. Such a synthesis is a goal of this paper.

USING CHANGES IN ALLELE FREQUENCY TO IDENTIFY "GENES THAT MATTER TO ECOLOGY"

It is the organisms that place gene products into interesting ecological contexts. More than 30 years ago, Clarke (1975) proposed a research agenda to investigate the mechanistic bases of genetic polymorphism along environmental clines. He proposed manipulative experiments to the biochemical, physiological, and performance characteristics of individuals with different alleles at the same locus. Before these important functional assays can be carried out, candidate "genes that matter" must be identified.

Identifying genes that matter through a posteriori comparisons of multiple loci

Selection is implicated, but by no means demonstrated, when alleles at a given locus co-vary along with a particular environmental factor along a geographical or environmental cline. For example, the "94" allele at the *Lap* locus in *Mytilus edulis* exhibits a cline from the base to the mouth of Long Island Sound (Koehn and Hilbish 1987), an estuary with a strong salinity gradient. Further evidence for selection is needed, because a clinal shift in allele frequency can be generated by chance, history, or other factors (Nei and Maruyama 1975, Robertson 1975, Vasemägi 2006). If a single locus is affected, as seems to be the case in Long Island Sound, a hypothesis of selection can be tested by investigating replicated salinity gradients elsewhere in the species' range (Koehn et al. 1976). It is unlikely that drift or historical accidents would generate similar genetic clines at one locus across independent environmental gradients. Of course, the Lap^{94} example is compelling precisely *because* other allozyme loci do not show similar shifts in frequency. Fig. 1 describes neutral expectations in three circumstances:

1) Environmental gradient. Neutral genes should show little change across an environmental gradient, while genes that matter, such as *Lap*, show a sharp change in allele frequency.

2) Geographic transect. In this case, isolation by distance across large geographic distances might reduce

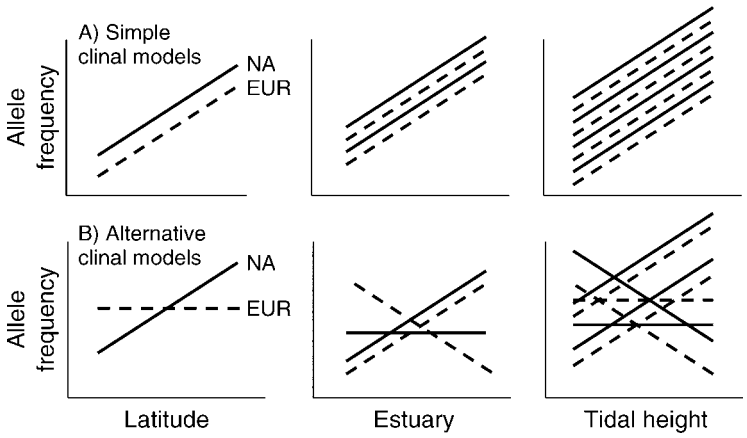

FIG. 2. (A) A "simple clinal model" where candidate genes or traits show parallel clinal variation on three spatial scales: across latitudes, across estuarine gradients, and across tidal gradients. North American (NA, solid lines) and European (EUR, dotted lines) transects show parallel responses at each spatial scale. (B) "Alternative clinal models" where the parallel models break down. Dissecting those cases where the non-parallel patterns emerge as repeatable phenomena should provide as much insight into the mechanisms of selection as those cases that show the simple clinal model. Explanations for deviation from the simple clinal model could be in three general categories: (1) environmental contingencies, (2) genetic contingencies, and (3) interactions between genetic and environmental contingencies.

migration between populations, so that neutral loci will gradually change in frequency due to genetic drift. An allele that does not show variation across great distances may be under positive or stabilizing selection.

3) Contact zone. In this case, populations that have diverged in isolation come into secondary contact. The figure shows how relatively sharp clines in neutral loci can result either from the recency of contact, or due to negative selection against hybrid genotypes (e.g., Barton and Hewitt 1989). The figure shows that genes that matter can still differ significantly from neutral expectations (see the example of *Fundulus heteroclitus* described below).

In the multi-locus comparative approach illustrated in Fig. 1, it is essential to establish neutral expectations. These can be derived from a random sample of loci across a genome (Lewontin and Krakauer 1973, Beaumont and Balding 2004, Beaumont 2005). This can be a random sample of protein-coding loci, such as comparisons across many allozyme loci (as seen below in *Fundulus heteroclitus*). Inferences of selection can be strengthened when candidate genes are compared to polymorphisms that are expected a priori to behave in a neutral manner. For example, the neutral expectation can be established by investigating clinal variation in putatively neutral polymorphisms such as non-coding single nucleotide polymorphisms (SNPs) or microsatellites. The expectation is that non-coding polymorphisms are more likely to be neutral so any patterns of clinal variation or population structure they exhibit can provide a control for the effects of drift, historical population subdivision, or linkage with selected loci. These neutral patterns can then be compared to patterns in functionally relevant markers such as allozymes, polymorphisms in expressed genes, or genetically based phenotypes identified from common-garden experiments.

A priori identification of candidate genes

A second approach focuses on specific genes or traits that are expected a priori to mediate organismal performance and fitness in distinct environments, such as glycolytic enzymes that influence energy balance and associated phenotypes (Watt 1977, Schmidt 2001). Candidate locus-specific approaches have provided some of the empirical foundations for evaluating the adaptive significance of molecular polymorphisms, including the afore-mentioned case of *Lap* in the blue mussel (Hilbish and Koehn 1985, Koehn and Hilbish 1987). Other examples include *Gpi* in *Colias* butterflies (Watt 1977, 1983), *Adh* in *Drosophila* (Oakeshott et al. 1982, Kreitman and Hudson 1991), and *Ldh* in *Fundulus* (Crawford and Powers 1989).

Considering "genes that matter" across multiple scales

Before describing the North Atlantic model system, it is useful to consider that environmental gradients can occur across multiple scales, from the largest scale (e.g., latitude), intermediate scales (e.g., estuary), or fine scale (e.g., tidal height). If changes in allele frequency are driven by selection across environmental gradients, then the same patterns should be observed repeatedly in distinct geographic regions. If both sides of the North Atlantic show the same general pattern of allelic change with latitude (as in Fig. 2A), this is powerful evidence for the generality of this pattern. Yet environmental gradients are just as powerful across smaller scales. Fig. 2A shows the simple expectation that alleles under selection should behave the same on both sides of the

North Atlantic, wherever those parallel environmental gradients are found.

As described above, the "simple clinal model" in Fig. 2A, where similar patterns across replicated gradients are found on both sides of the North Atlantic, is only a first step towards identifying genes that matter to ecology. What, then, if alleles behave differently on both sides of the North Atlantic, as in Fig. 2B? What if a change in allelic frequency is predictably different between American and European populations across the same clines? Dissecting those cases where non-parallel patterns across the North Atlantic emerge as repeatable phenomena should provide as much insight into the mechanisms of selection as those cases that follow the simple clinal model. Explanations for deviation from the simple clinal model fall into three general categories: (1) environmental contingencies where habitats vary in unpredictable ways, (2) genetic contingencies where genetic backgrounds affect the behavior of combinations of loci, and (3) interactions between genetic and environmental contingencies, such as genotype-by-environment interactions. In the following section, we describe the advantages of considering both sides of the North Atlantic as a model system to study genes that matter in ecology.

The North Atlantic as a Model System

Replicated environmental gradients on both sides of the North Atlantic

The marine North Atlantic presents an environment with replicated gradients of ecologically important abiotic factors at many geographic scales. At the broadest level, the coastlines of North America and Europe represent replicated (but not identical) gradients of factors associated with latitude (Fig. 2A, B and Fig. 3A–C). Sea surface temperatures exhibit a steeper cline on the northwest Atlantic coast than on the northeast Atlantic coast, suggesting potential differences in the spatial scale of temperature-mediated selection. Within each coastline, there are hundreds of estuaries (gradients of salinity and correlated abiotic factors), and thousands of kilometers of intertidal coastlines (again, with gradients in desiccation, temperature, and other correlated abiotic factors; see *Comparing closely related species on both sides of the North Atlantic*).

Thus, the environment of the North Atlantic provides nested examples of abiotic gradients over various geographic scales, from meters to thousands of kilometers. It may be that clines for particular genes are evident at all scales, or at only one scale. If a particular allele or phenotype is not clinal at a latitudinal scale but is strongly clinal across the intertidal, this helps dissect the relevant environmental and physiological factors that may govern natural selection in the wild. The patterns of variation for temperature or tidal range across the North Atlantic suggest points of departure for new experimental analyses (see Fig. 3A–C).

Comparing closely related species on both sides of the North Atlantic

In many cases, the species themselves are found on both sides of the North Atlantic. This similarity in species composition can provide similar physical structure to ecosystems on both sides of the North Atlantic, especially in the rocky intertidal. As described by Jenkins et al. (2008), many of the major players are found across the North Atlantic, including seaweeds (*Fucus*, *Ascophyllum*, *Chondrus*), mussels (*Mytilus*), barnacles (*Semibalanus*, *Chthamalus*), and snails (*Nucella*, *Littorina*). Although there is substantial overlap in species and ecosystem composition, the historical response to Pleistocene climate changes can be very different on the two sides of the North Atlantic, even in the same species (Maggs et al. 2008). Specifically, certain populations may have experienced bottlenecks due to reduced habitat during glaciation. Other species may have gone extinct altogether on one coast and have been recolonized from the other (Maggs et al. 2008).

Therefore, even if a species' distribution includes both coastlines, the North American and European populations are not necessarily equivalent and may be genetically distinct. Hence two general patterns may be observed: (1) "parallel" clines may exist on both coasts suggesting similar underlying dynamics or (2) clines may be non-parallel or discordant and provide a system to dissect the differences in ecological and evolutionary forces (see Fig. 2A, B).

Although other ocean basins also present examples of paired coastlines, with many properties similar to the North Atlantic, the North Atlantic has been the focus of extensive research into the ecology and evolution of intertidal organisms. This background information enhances the ability to identify replicated environments and populations and to evaluate competing hypotheses across macro, meso, and micro scales (Fig. 2). Here we review a few examples that illustrate clinal variation at each of these scales, and examine how well they fit the simple and alternative models outlined in Fig. 2. Some of the clearest examples have data from only one coast of the Atlantic and thus motivate further studies that could test the generality of the connection between pattern and process.

Latitudinal Clines

The latitudinal gradient is the largest spatial scale of analysis considered here. Such a broad gradient can be very coarse with respect to environmental grain size, which has the potential to obscure complex neutral and non-neutral dynamics within nested environmental gradients. Adding to the complexity of analyzing variation across a geographic scale, a number of environmental factors covary with latitude (e.g., community composition and history, climate, and multiple aspects of temperature); furthermore, latitude may not be an accurate predictor of the actual temperature experienced by an organism (e.g., Helmuth et al. 2006).

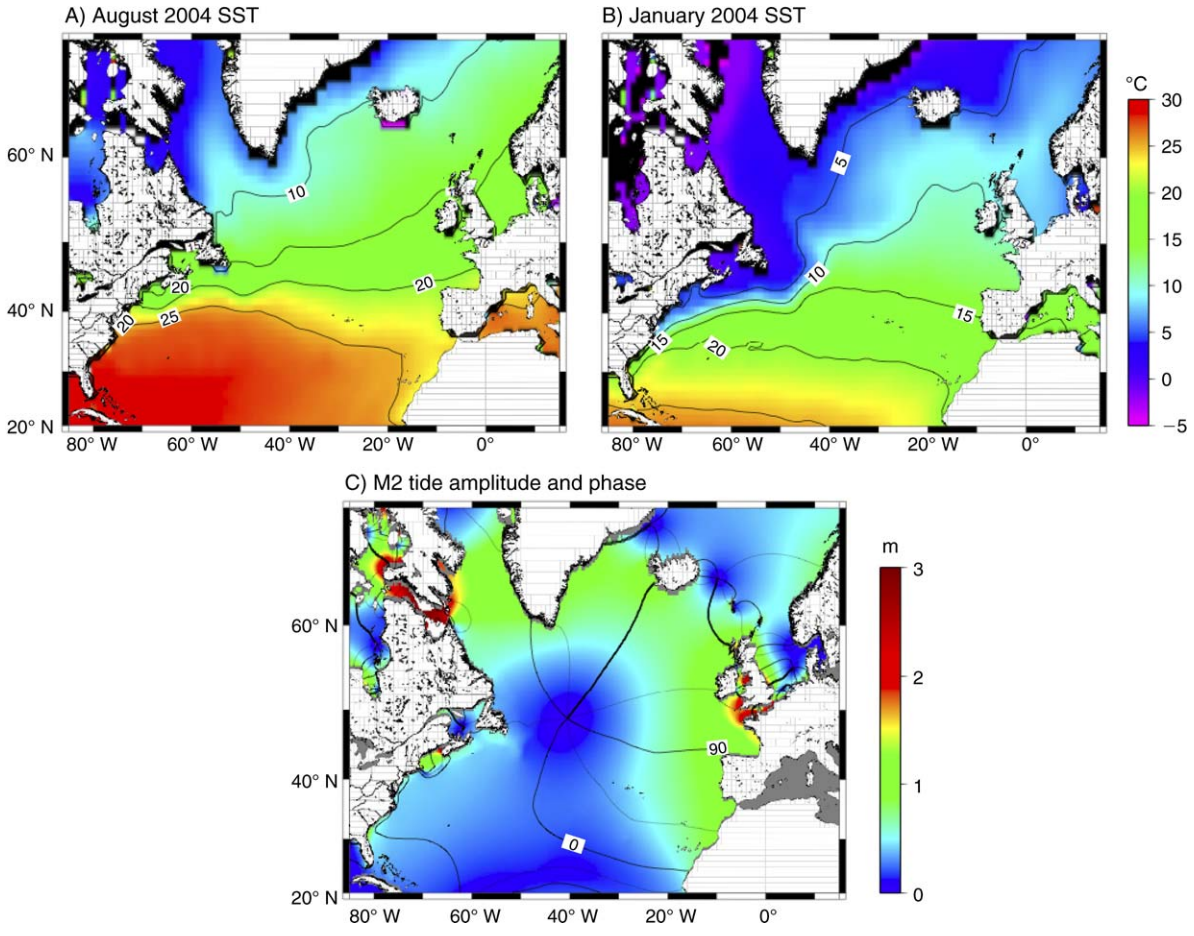

FIG. 3. Sea surface temperature and tidal amplitude in the North Atlantic. Sea surface temperature (SST) for (A) August 2004 and (B) January 2004. Data were obtained from the Hadley Centre HadISST data set (Rayner et al. 2003). Both graphs include contours at 5°, 10°, 15°, 20°, and 25°C. Note that in winter the geographic range between Mauritania and Lisbon is compressed on Florida–South Carolina, and the range between Lisbon and Northern Ireland is compressed between South Carolina and Cape Hatteras, North Carolina, and Northern Ireland to Iceland is compressed between Cape Hatteras and Cape Cod. In summer, there is similar compression on the U.S. coast. For instance, the 20°C and 25°C isotherms are separated by 25 degrees of latitude in Europe/Africa, and by 5 degrees of latitude in North America. This map says that southern Europe is like Cape Cod to the Washington, D.C., area, and UK/Brittany/mid Norway is like Cape Cod to the southeast tip of Newfoundland. Norway to Iceland is compressed to the east coast of Newfoundland. (C) The amplitude and timing of the semidiurnal tides in the North Atlantic are illustrated using color gradients (color bar represents amplitude in meters). Data were obtained from the Oregon State Tidal Prediction System (Egbert and Erofeeva 2002). Note that the amplitude in most of Europe is much higher than in most places in North America. The UK/Channel coast has amplitudes more like northern Maine than anywhere else. South of Cape Cod on the North American side, the amplitudes are smaller than most European sites with the exception of Denmark–southern Norway. The spider lines on the image are the phase angles of the tide in degrees (there are 15 degrees per hour). The lines are three hours apart, so tides in southern Morocco are three hours before central Morocco, which are three hours ahead of northwestern Spain, which is three hours ahead of western Ireland.

However, the existence of a latitudinal cline for a trait of interest can be informative with regard to the roles of gene flow and selection in generating patterns of variation in natural populations. As described above, the null hypothesis that a cline reflects genetic drift and isolation by distance (vs. selection, be it direct or indirect) can be tested by comparing patterns of variation among loci across replicate populations and/or spatial scales (Figs. 1 and 2). The utility of this inter-locus contrast has been effectively demonstrated in *Drosophila* where latitudinal clines for candidate genes appear to be the rule rather than the exception (Berry and Kreitman 1993, Schmidt et al. 2000*b*, Gockel et al. 2001, Sezgin et al. 2004, Tauber et al. 2007).

In marine systems, the impact of temperature variation on organismal performance has been well studied (e.g., Somero 2005). Coupled with the latitudinal gradients on both sides of the Atlantic, the examination of multi-locus patterns of variation in North Atlantic marine communities provides a framework in which to examine selection and drift at a broad geographic scale. The killifish, *Fundulus heteroclitus*, is an example of such

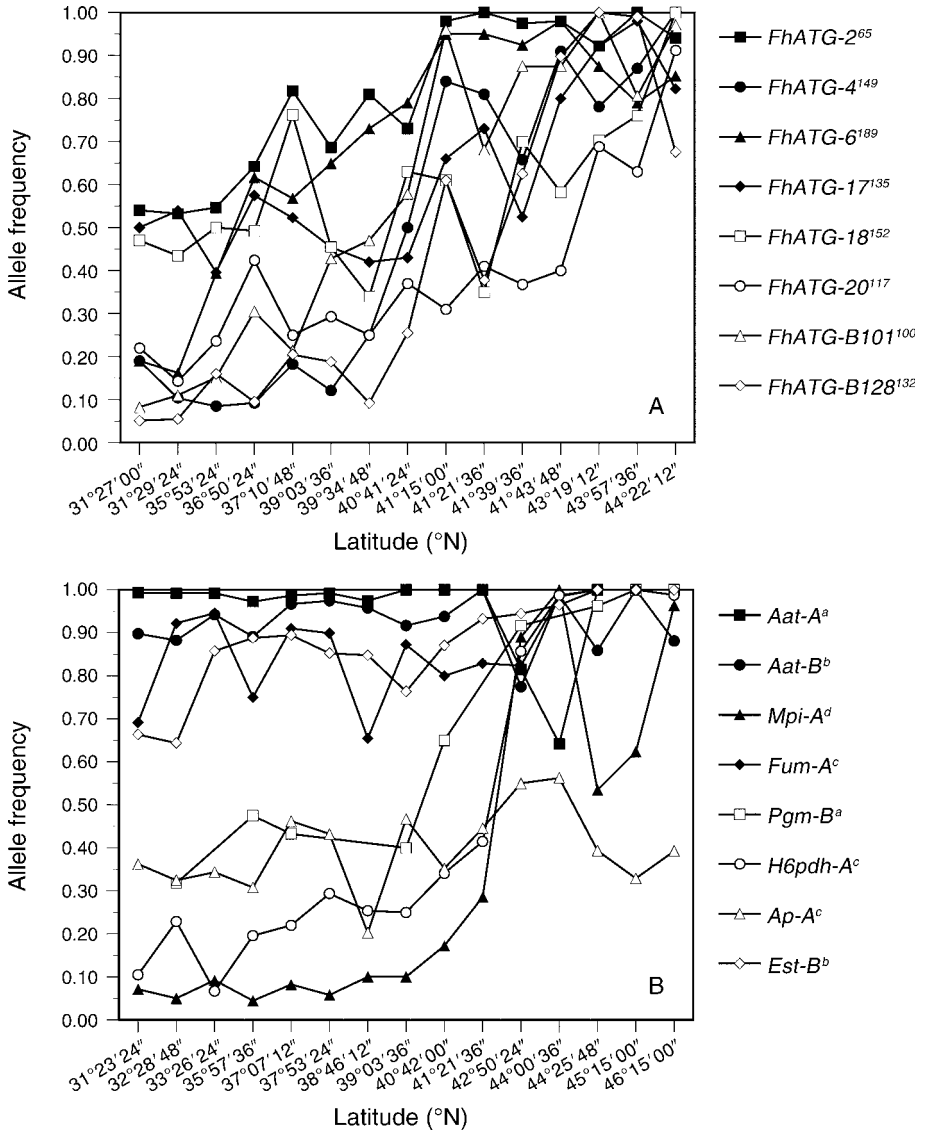

FIG. 4. The frequency of the most common allele for (A) eight microsatellite (from Adams et al. 2006) and (B) allozyme loci (from Ropson et al. [1990]) in sampled *Fundulus heteroclitus* populations along a latitudinal gradient in the western North Atlantic. Note that, for presentation, the *x*-axis is categorical rather than a value axis. Performing simple regressions of allele frequencies on latitudinal origin, each of the eight microsatellite loci exhibits a significant cline (mean slope = 0.05121, SD = 0.020, upper and lower 95% CI are 0.0686 and 0.0338, respectively). In the analysis of Ropson et al. (1990), 13 of 16 allozyme loci in *F. heteroclitus* exhibit a significant cline based on cline shape. However, of the eight allozymes shown in panel B, only four are clinal (*Mpi-A*, *Pgm-B*, *H6pdh-A*, *Est-B*) based on the regression of allele frequency on latitude (mean slope = 0.0271, SD = 0.0268, upper and lower 95% CI = 0.0495, 0.00464).

an approach (e.g., Burnett et al. 2007). *F. heteroclitus* is an estuarine teleost with a broad geographic range in the western north Atlantic. Heterozygosity is high within populations, and 13 of 16 polymorphic allozyme loci exhibit latitudinal clines among sampled populations (Powers and Place 1978, Ropson et al. 1990; see Fig. 4). However, mtDNA haplotypes also demonstrate frequency breakpoints between northern and southern regions, suggesting that the previously observed allozyme clines may also be affected by secondary intergradation between genetically subdivided populations (Gonzalez-Villasenor and Powers 1990). This pattern is also seen for sequences of the nuclear encoded *Ldh* locus (Bernardi et al. 1993). Similarly, a series of microsatellite loci all exhibit striking clines when the frequency of the northern allele is plotted against latitudinal origin of the analyzed populations (Adams et al. 2006) (see Fig. 4). The concordant clines among functional classes of genetic markers clearly demonstrate a strong historical and demographic component to the observed patterns of variation, which is further supported by the comprehensive analysis of microsatellite allele frequency vari-

ation among populations (Adams et al. 2006). As discussed by Ropson et al. (1990), the divergent shapes, widths, and midpoints of various allozyme clines indicate that selection as well as genetic drift may play a role in the maintenance of specific clines (see analyses described in Fig. 4).

For example, the association between water temperature and allele frequencies in *F. heteroclitus* has been well documented (Mitton and Koehn 1975), and the mechanistic dissection of the *Ldh-B* cline remains one of the most comprehensive functional analyses of a polymorphism in natural populations (Crawford and Powers 1989, 1992).

While *Fundulus* provides an example of clinal contrasts between presumably neutral (e.g., microsatellites) and selected markers that are strikingly more clinal (e.g., *Pgm-B*), it does not occur in Europe so the cline cannot be replicated geographically. However, a comparable situation exists on the European coast for the eelpout, *Zoarces viviparus*. *Z. viviparus* is ecologically distinct from *F. heteroclitus*, as it ranges down to 40 m depth, grows to ~50 cm, and frequents rocky shorelines and tide pools, while *F. heteroclitus* is a small (~7 cm) salt marsh species that is restricted to shallow estuaries (Abraham 1985). Samples of the eelpout collected from the Danish coast of the North Sea around the Jutland peninsula into Kattegat in the Western Baltic show a clear clinal variation at two protein markers (hemoglobin I and esterase III), and the complete absence of clinal patterns at two other allozyme loci (Pgm I and Pgm II; Christiansen and Frydenberg 1974; see Fig. 5). This clinal contrast is at the interface between a latitudinal and a meso-scale cline, but is the closest European example of clinal variation in a teleost, relative to *Fundulus*. There have been no recent follow-up studies of this system using putatively neutral markers, which would be a valuable contribution to the understanding of selection in this species.

The intertidal snail *Littorina obtusata* is a promising example of clinal variation, as it is distributed on both the American and European coasts and has a well studied ecology. Moreover, it provides an example of a "candidate" gene approach to clinal variation. Many other marine species show evidence for selection at *Mpi* (mannose phosphate isomerase; see *Fundulus* example above; Fig. 4; Schmidt and Rand 1999), so this marker has been widely evaluated in species where ecological selection is well understood. Recent work has uncovered a latitudinal cline for the *Mpi* polymorphism in *L. obtusata* (Schmidt et al. 2007). Although this species lays egg masses on algae and is therefore not predicted to have widespread gene flow among populations, the analysis of allele frequency variation at three variable microsatellite loci reveal little evidence of genetic subdivision among sampled populations across the Gulf of Maine. Similarly, allele frequencies for two polymorphic allozymes are homogeneous among the populations

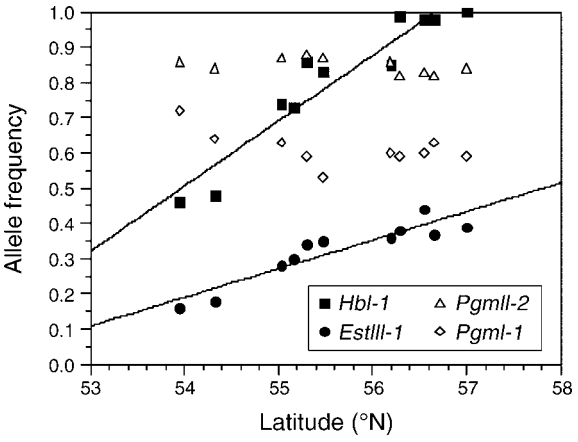

FIG. 5. Latitudinal clines for allozyme polymorphisms in *Zoarces viviparus*. From the data presented in Christiansen and Frydenberg (1974), a representative latitudinal transect was extracted from the 46 sampled *Z. viviparus* populations. The localities presented here are 10, 12, 13, 17, 18, 20, 26, 27, 28, 35, and 37 from the original data set; this represents a latitudinal gradient from Kattegat through the Little Belt and into the Western Baltic. No populations from Zealand or the Baltic Sea were included in the present analysis. The frequency of the most common electrophoretic allele for each of the four allozyme loci is plotted against latitudinal origin of the selected subset of populations sampled. For both the *HbI* and *EstIII* loci, a significant latitudinal cline is evident (*HbI*, $R^2 = 0.8996$, latitude $= 0.1855$, SE $= 0.0207$, $P < 0.0001$; *EstIII*, $R^2 = 0.8549$, latitude $= 0.0813$, SE $= 0.0112$, $P < 0.0001$). Neither of the *Pgm* allozymes demonstrates a significant association with latitude (*PgmI*, $R^2 = 0.3107$, latitude parameter $= -0.0265$, SE $= 0.0140$, $P > 0.09$; *PgmII*, $R^2 = 0.2711$, latitude parameter $= -0.0111$, SE $= 0.0064$, $P > 0.12$).

sampled. In contrast, the a priori candidate allozyme, MPI, exhibits a significant latitudinal cline (Fig. 6).

These patterns of clinal variation at the candidate and lack of clines for reference loci are also replicated across a temperature gradient at the meso-spatial scale, further suggesting that the observed cline is generated in part by the action of spatially variable selection. As in other systems, however, it is likely that multiple alleles with distinct genealogies are segregating within each allozyme class (e.g., Verrelli and Eanes 2001). This emphasizes the need for direct sequencing of candidate genes in order to inform subsequent functional analyses (e.g., Eanes 1999). As with the examples discussed above and below, the observation of a locus or trait-specific cline merely represents a point of departure for subsequent investigations.

Despite the patterns evidenced in *F. heteroclitus*, and *L. obtusata*, most comparisons between allozyme and presumably neutral DNA markers across large spatial scales in marine organisms demonstrate relative spatial homogeneity of the allozymes (e.g., Pogson et al. 1995). Again, this may simply reflect the widespread homoplasy that plagues the use of allozymes as candidate markers. Aside from the complex dynamics associated with hybrid zones or cryptic species whose distributions

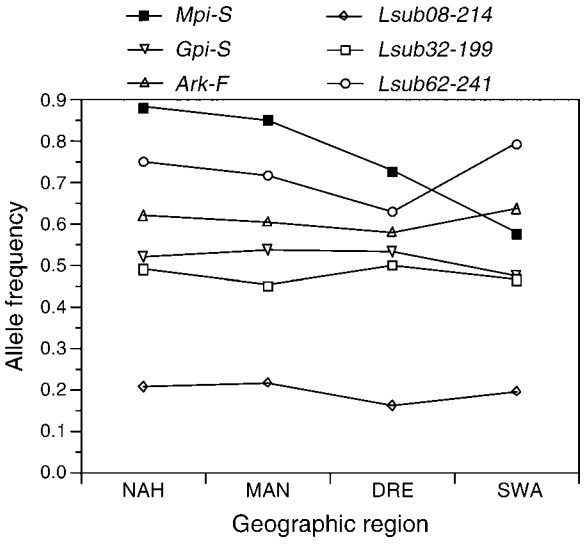

FIG. 6. Geographic variation in the frequency of the most common allele for three allozyme and three microsatellite loci in *L. obtusata*. Two replicate sites were sampled from each region, with a sample size of >50 individuals per site. Regions are listed on the *x*-axis in latitudinal order (NAH, Nahant, Massachusetts, USA; MAN, Manchester, Massachusetts, USA; DRE, Damariscotta River Estuary, Maine, USA; SWA, Swans Island, Maine, USA). Nominal logistic regression was used to evaluate allozyme genotype frequency variation among regions: the effect of geography was significant only for *Mpi* (6 df, Wald chi square = 67.771, $P < 0.001$). For the three microsatellite loci (Tie et al. 2000), global F_{st} values were non significant (*Lsub08*, $F_{st} = 0.00377$, $P < 0.214$; *Lsub32*, $F_{st} = 0.003$, $P < 0.199$; *Lsub62*, $F_{st} = 0.0143$, $P < 0.09$).

vary with geography (see *Mesoscale clinality*) latitudinal clines for candidate genes are relatively infrequent in marine systems. This lack of observed clines at large spatial scales may be associated with the widespread gene flow and long distance dispersal potential that characterize many taxa. Alternatively, the environmental gradients that have the strongest impact on organismal performance and fitness may exist at spatial scales smaller than a latitudinal gradient. A case in point is the *Mpi* and *Gpi* polymorphisms in the acorn barnacle *Semibalanus balanoides*. Characterization of allele frequency variation across a large latitudinal gradient in the western North Atlantic reveals shallow clines, but no detectable latitudinal pattern in the eastern North Atlantic on either British or continental shores for either locus (Flowerdew 1983). This contrast between patterns of variation on North American and European coasts may reflect the differences in sea surface temperature on the two coasts (see Fig. 3A, B); nevertheless, the latitudinal patterns are very weak even on the American coast. However, the dynamics of both polymorphisms are affected by selection at both the meso- and intertidal spatial scales (e.g., Schmidt and Rand 2001, Rand et al. 2002), indicating that environmental variation at large latitudinal scale may be less relevant to the ecological genetics in *S. balanoides*.

MESOSCALE CLINALITY

Many areas within the marine realm experience fluctuating or stable variation along salinity gradients. In the northwestern Atlantic, the estuaries in Chesapeake Bay, the Long Island Sound, the rivers along the coast of Maine, and the Gulf of St. Lawrence vary widely in freshwater influence associated with seasonal variation in rainfall and runoff. This in turn alters associated factors such as sedimentation and turbidity. In the northeastern Atlantic, the estuaries flanking the Bay of Biscay, the English Channel, the Wadden Sea and the many fiords in Scandinavia are the counterparts to estuaries along the coast of North America. In contrast, the brackish and non-tidal Baltic Sea is characterized by a stable gradient of salinity reduction with distance from the connection to the fully marine North Sea through the Kattegat and Skagerrak. The Baltic has undergone a well-studied history of salinity changes, with the last marine period between 7500–3000 yr BP (the *Littorina* Sea period), when species richness was higher (Ignatius et al. 1981). Baltic species are subject to stable, lowered salinity relative to North Sea conspecifics, as well as loss of immersion–emersion cycles for intertidal species. Thus, strong clinal gradients in abiotic stress (salinity), and a sharp transition from intertidal cycles of emersion to submerged non-tidal conditions exist within the Baltic. These two kinds of estuaries (fluctuating and stable) provide useful contrasts to the problems of detecting selection from clinal variation.

Mesoscale variation in Mytilus

The canonical example of mesoscale clinal variation is that of the *Lap* locus in the blue mussel, *Mytilus edulis* (Koehn et al. 1980, Koehn and Hilbish 1987). *Lap* encodes *leucine amino peptidase* (LAP, E.C. 3.4.1.1), an enzyme that cleaves peptide bonds adjacent to free amine groups, with high activity for leucine containing peptides. In *M. edulis*, the Lap^{94} allele occurs at a frequency of about 0.55 in coastal population near Cape Cod and Long Island. Inside Long Island sound the Lap^{94} allele decreases in frequency sharply to less than 0.20 over the first 20 miles of shoreline. This is observed on both the Long Island and Connecticut shores, and in Cape Cod Bay, Massachusetts (Koehn et al. 1976), providing independent evidence for selection. While some other allozyme loci show variation in allele frequencies, none show the repeated change in frequency inside estuaries, implicating selection at *Lap* (Koehn et al. 1976). The mechanism underlying fitness differences at the *Lap* locus have been addressed in a number of biochemical, physiological and population genetic approaches. The Lap^{94} allele has higher activity than other alleles, and cleaves proteins more efficiently. This effectively increases the osmolarity inside the cell, but using protein fragments or amino acids as osmotic particles is a metabolically expensive way to regulate osmotic pressure as protein synthesis requires considerable ATP expenditure. In lower salinity seawater, such

as that found inside the Long Island sound estuary, the Lap^{94} allele is at a selective disadvantage (Koehn and Hilbish 1987).

The mode of selection was examined by comparing the departures from Hardy-Weinberg equilibrium (HWE) for the *Lap* locus. Selection against the Lap^{94} allele in low salinity environments could result from a dominant phenotype of the allele (Lap^{94} heterozygotes have the same fitness as Lap^{94} homozygotes), an additive phenotype (Lap^{94} heterozygotes are intermediate between Lap^{94} homozygotes and homozygotes for an alternative allele), or a multiplicative relationship between genotype and fitness. Each of the models predicts a distinct departure from Hardy-Weinberg equilibrium, and the observed patterns from natural populations clearly support the dominance model (Hilbish and Koehn 1985). This is a textbook case of the connection between a molecular polymorphism and the probable mechanistic processes that maintain the variation; its strength lies in the direct biochemical evidence for allelic effects on phenotype. However, this remains essentially a single-locus case study, and the relative roles of gene flow and multilocus epistasis in maintaining the less fit allele in either environment are unresolved. Data from a series of coding and noncoding loci in samples from these estuarine gradients would shed more light on the exact nature of the selective pressures exerted on *Lap* and other loci.

In Europe, *Mytilus* shows further evidence suggestive of selection at specific loci on a meso scale that is associated with changes in salinity. However, *Lap* does not show the strongest pattern of apparent selection, indicating that the European populations do not provide simple replication of the meso scale clines that exist in North America. This lack of replication in Europe is most easily explained by the existence of hybrid zones between *Mytilus* species, making the genetic context rather distinct from the Long Island cline within a single species. For example, the salinity gradient between the North Sea and the Baltic traverses a hybrid zone between *M. edulis* and *M. trossulus* (Vainola and Hvilsom 1991, Bierne et al. 2003*b*, Riginos and Cunningham 2005). A further complicated example is the hybrid zone between *M. edulis* and *M. galloprovicialis* on the Atlantic coast of Europe (Hilbish et al. 2002, Bierne et al. 2003*a*). As previously discussed, when the middle of a hybrid zone is coincident with the environmental transition it may not be clear whether selection is driven by external environmental conditions (e.g., salinity) or internal genetic interactions that cause selection against specific alleles in different genetic backgrounds (such as linkage or epistasis). Comparisons of multiple unlinked loci may aid in distinguishing these potentially confounding factors (Fig. 1), but these interlocus contrasts may not be unambiguous in resolving the relative contributions of environmental vs. intra-genomic selection.

In *M. edulis* and *M. trossulus*, alleles at the allozyme loci *Est-D*, *Gpi*, *Lap*, *Mpi*, *Odh*, and *Pgm* show clear clinal shifts in frequency from the North Sea into the low-salinity Baltic, with the *M. trossulus* genotypes being favored in low salinity locations. Initial analyses of mtDNA and ITS markers showed that Baltic populations had *M. edulis* haplotypes, which has been proposed as evidence for selection against the allozymes across this environmental transition (Rawson and Hilbish 1998, Riginos et al. 2002). Subsequent analyses discovered nuclear DNA markers that showed allele frequency shifts concordant with the allozyme transitions, suggesting that the change in frequencies could be a result of the secondary contact between the two species (Bierne et al. 2003*b*). Under various models of secondary contact in hybrid zones, large blocks of chromosomes may follow concordant patterns of introgression between species, generating a large variance in population structure across loci. The initial interpretation of apparently concordant clinal patterns for allozymes into the Baltic, with wider asymmetric gene flow implied for putatively neutral DNA markers (mtDNA and ITS) may have followed from the limited sample of apparently neutral DNA markers. Demonstration that some nuclear DNA markers have clinal patterns consistent with the allozymes provides a very clear example of how inter-locus contrasts can weaken the hypothesis of direct selection (see Fig. 1 for contact zones).

Additional geographic comparisons and functional experiments in the field indicate that the direction and mode of selection in this secondary contact zone is quite complex. Mussels were transplanted between high and low salinity locations, and the change in frequency for various markers was subsequently measured. Notably the *Gpi* and *Pgm* alleles that show the clear transition across the cline into the Baltic also show the strongest shift in frequency after experimental transplant between salinity regimes (Riginos and Cunningham 2005). A distinct hybrid zone exists between *M. edulis* and *M. trossulus* in the Canadian Maritimes, and the patterns of allele frequency shift are oddly reversed from that in the Baltic hybrid zone: *M. trossulus* alleles appear to be favored in more saline (and wave-exposed) environments. The simplest explanation invokes a combination of adaptive and historical factors. *M. trossulus* appears to be more adapted to low salinity in the Baltic, but to high salinity and/or wave exposure in the Canadian Maritimes. The ages of the two hybrid zones must also play a role in generating context-specific patterns of environmental and intra-genomic selection on the markers studied (Riginos and Cunningham 2005). Selection on the set of co-adapted genes that maintain, at least in part, the cohesiveness of the two genomes in this system is mediated by a complex genotype-by-environment interaction; further studies are needed in order to comprehensively test the relative roles of endogenous vs. exogenous selection.

The comparisons across geographic locales uncovered a further locus-specific pattern that also awaits explanation: the *Lap* locus is not a major factor in the putative salinity clines in the Baltic and Canadian Maritimes. The clear evidence of salinity selection on *Lap* in Long Island Sound may be unique to that system. Again, the simplest explanation is that locus-specific selection is crucially dependent on local genomic context and local environmental conditions. Further examples of shifting patterns of selection on strong candidate loci are apparent at other spatial scales, suggesting that genotype-by-environment interactions modulating selection are the central problem in testing adaptation at specific loci.

The hybrid zone between *Mytilus edulis and M. galloprovincialis* further illustrates the challenge of distinguishing meso scale selection on individual loci. In estuaries off the coast of Cornwall, there is a strong increase of the *Glu 5′-180* allele in the innermost part of the estuaries, while the *Glu 5′-126* allele is much more abundant at the mouth (Hilbish et al. 2002). It would be an error to interpret that as an evidence for direct selection on this locus, because almost any locus differentiating *M. edulis* from *M. galloprovincialis* will show the same pattern, whether neutral or not. Indeed, the two species interact in a complex mosaic hybrid zone all along the European coast (Bierne et al. 2003*a*), and it is difficult to decipher which genes and alleles affect fitness in a particular environment. In general *M. galloprovincialis* is more resistant to wave exposure, whereas *M. edulis* is more tolerant to low salinity water in estuarine conditions. These complex patterns result from the interaction of congealed co-adapted genomes occupying more or less differentiated patches of environment. This situation is likely not to be an exception for *Mytilus*, and other species need to be considered for studies at the meso scale.

Mesoscale variation in algae

Contrasting patterns of neutral vs. phenotypic traits have been documented in the main habitat-structuring seaweed of the Baltic Sea, *Fucus vesiculosus*. No significant differentiation along the North Sea-Baltic transition zone was detected using nuclear and chloroplast markers (microsatellites and RbcL spacer sequences) (Tatarenkov et al. 2005, Johannesson and Andre 2006). There are, however, genetically based phenotypic differences across this gradient, as Baltic individuals have lost the capacity to withstand abiotic emersion stresses (freezing and desiccation [Pearson et al. 2000]) and Baltic gametes perform better at achieving external fertilization than their North Sea counterparts (Serrão et al. 1996). Although the first stages of North-Sea-Baltic transition zone don't show change in neutral markers, farther into the Baltic there is a sharp transition zone for this species, where microsatellite allelic frequencies change sharply in concordance with an experimentally shown shift towards clonal reproduction (Tatarenkov et al. 2005), an important selective trait in a habitat where the species distribution reaches the salinity tolerance limit of the gametes to achieve sexual reproduction (Serrão et al. 1996, 1999).

Clines in reproductive mode towards loss of sexuality have been documented for other species in the Baltic Sea, although examples based on common garden experiments are rare. Clinal variation in RAPD marker frequencies was observed in the red alga *Ceramium tenuicorne*, which appeared to follow the salinity gradient Oslofjord (Skagerrak)–Kattegat–inner Baltic. This cline was accompanied by phenotypic variation in reproductive mode in cultured algae sampled along this gradient, with increasing trend towards asexuality at low salinities (Gabrielsen et al. 2002). These cases in algae provide a different approach to the neutral-vs.-selected marker paradigm that is implicit in DNA vs. allozyme comparisons. Any heritable phenotypic trait is more likely to be subjected to environmental selection than isolated molecular polymorphisms. Phenotypes are the composite expression pattern of many genes and thus provide a larger "target size" for environmentally relevant selection. Comparisons between molecular F_{st} and quantitative Q_{ST} are emerging as an effective approach for identifying selection (Storz 2002).

Marginal habitats such as the Baltic may impose certain obstacles for the unambiguous identification of a selective basis (indirect or direct) for clinal variation in genetic traits. First, marginal habitats often have reduced population sizes that inevitably result in some loss of diversity over time through genetic drift. This has been observed recently for many Baltic taxa when compared with their Atlantic counterparts (Johannesson and Andre 2006). Second, in smaller populations typically seen near margins, habitat subdivision can be exaggerated and selection may be less effective if new mutations occurring at low frequency are more prone to loss by drift. When allele frequencies covary with total allelic diversity, neutral forces may predominate and result in observed clines. Third, the very nature of marginal populations and the ecotone which differentiates them makes them good candidates to "trap" tension zones and hence congealed genomes that will persist by a combination of endogeneous counter-selection and local adaptation.

The Intertidal

This gradient represents the finest scale we consider for clinal variation. Over the distance of a few meters the habitat changes from fully marine to sub-aerial, an extreme physical change that necessitates certain physiological responses for survival. Species that exist in wide tidal gradients provide the best opportunity for detection of genotypic clines across the intertidal. Moreover, most tidal cycles affect wide stretches of coastline providing natural access to independent locations that experience similar environmental gradients. This offers a form of replication that can add power to marker

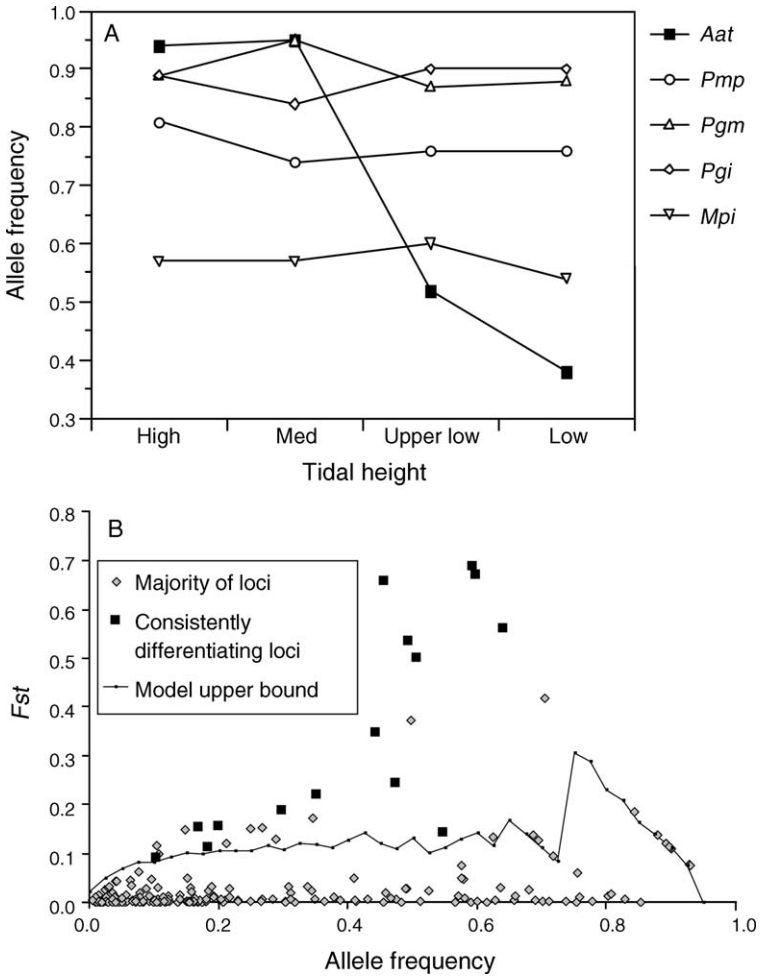

FIG. 7. (A) Among-microhabitat variation in the frequency of the most common allele for five allozyme loci in *L. saxatilis*. Data are taken from Johannesson et al. (1995) from the 1987 sample from crevice A. A sharp transition in *Aat* allele frequencies is evident between the mid to low shore microhabitats. (B) F_{st} values as a function of allele frequency for 306 AFLP loci in *L. saxatilis*. The solid line depicts the null distribution. Fifteen AFLP markers are significantly differentiated between the H and M shell morphotypes. Data are taken from Wilding et al. (2001).

analyses aimed at detecting selection (Fig. 1). While desiccation and temperature stress are obvious abiotic factors that vary with tidal height, there are other correlated factors, as well as gradients of exposure to predators and to competitors (Sanford et al. 2003).

Intertidal variation at allozyme loci

A well-studied example of an intertidal cline is the littorine snail *Littorina saxatilis*. Strong evidence for selection at a single locus comes from studies of aspartate aminotransferase (*Aat*) in *L. saxatilis*. *Aat* (E.C. 2.6.1.1), also known as aspartate transaminase, is a pyridoxal-phosphate protein that catalyzes L-aspartate + 2-oxoglutarate = oxaloacetate + L-glutamate. Thus, *Aat* plays an important role in amino acid metabolism. *L. saxatilis* has two major allozymes of *Aat*. In high shore areas of Northern Europe, Aat^{120} is most frequent, whereas Aat^{100} is more common in low shore areas and rock pools (Johannesson and Johannesson 1989). The *Aat* locus offers several examples of non-neutral clinal variation. First, *Aat* is more clinal than other loci; of five enzyme loci examined (Johannesson et al. 1995), only one (*Aat*) showed a clinal change across the intertidal (see Fig. 1 and Fig. 7). Some additional loci exhibit similar patterns of high to low shore frequency differences, although most loci are not genetically differentiated with respect to tidal height (Johannesson and Johannesson 1989, Johannesson and Mikhailova 2004). Second, the frequency differences are found in parallel across independent intertidal populations in Northern Europe (Johannesson and Johannesson [1989]; consistent with the simple model in Fig. 2A). Third, there are unique populations that violate the simple patterns just described: the *Aat* cline is reversed on the Atlantic coast of Spain (Galicia), Aat^{120} being more common in the lower shore (Johannesson et al.

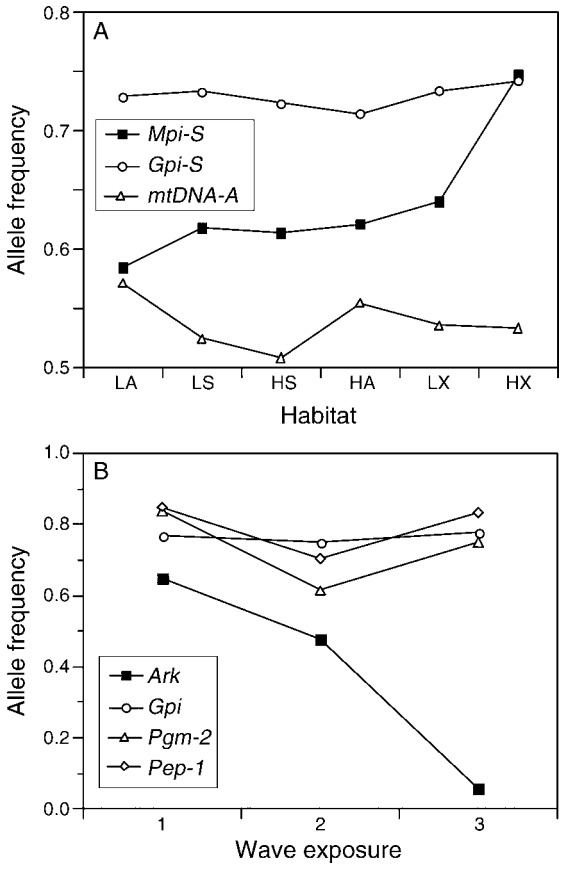

FIG. 8. Microhabitat variation in allele frequencies in (A) the northern acorn barnacle, *Semibalanus balanoides*, and (B) the periwinkle *Littorina mariae* (*fabalis*). In the top panel, data are taken from (Schmidt et al. 2000*a*). Barnacles were transplanted into six experimental treatment combinations, which are ordered on the *x*-axis by increasing degree of physiological stress; the physical distance among microhabitats is less than 2 m. *Mpi* allele and genotype frequencies diverged among treatments as a consequence of mortality events, whereas the frequencies of the other allozyme and mtDNA markers did not. In panel B, data are extracted from Tatarenkov and Johannesson (1994) and represent allele frequency data from Ursholmen. Sites are ordered on the *x*-axis by decreasing degree of wave exposure, which was a qualitative designation based on distribution and abundance of algal cover; the spatial scale is on the order of 20 m. Allele frequencies for arginine kinase (*Ark*) exhibit a pronounced cline, whereas other assayed loci do not.

1993). This provides an example of an alternative model in Fig. 2B, suggesting that the *Aat* cline is subject to genotype-by-environment interaction effects.

The *Littorina saxatilis* model has been used to address mechanistic questions related to the physiological and biochemical bases of the observed selection. High-intertidal snails are physiologically differentiated from low-shore animals and better able to withstand heat exposure and desiccation stress (Sokolova et al. 2000). In Northern Europe, Aat has higher activity in high-shore *L. saxatilis* individuals relative to low-shore individuals at high temperatures (Sokolova and Pörtner 2001), which may indicate that Aat among high shore snails is more thermally stable than in low shore populations. These catalytic properties are strongly correlated with genotype (Hull et al. 1999, Panova and Johannesson 2004), such that $Aat^{100/100}$ individuals (typical of the low shore) have the highest enzyme activity. Panova and Johannesson (2004) surmised that low-shore animals are under selection to maximize their efficiency, whereas high-shore animals are faced with a greater risk of desiccation and the low enzyme activity of $Aat^{120/120}$ genotypes is associated with a reduction in metabolic energy expenditure. This makes the apparent reversal of the cline in Galicia hard to explain in functional terms, unless it is the size and habit of the snails that are the key factors. In northern Europe, typically high-shore *L. saxatilis* are smaller and evidently more sedentary in habit than are low-shore snails, while in Galicia this is reversed. There is evidently more to be done in understanding the interrelationship between allele frequency, functional properties of the enzymes, and the phylogenetic constraints across often closely related species (Hull et al. 1999).

An analogous pattern of intertidal selection on an allozyme locus has been documented for the acorn barnacle, *Semibalanus balanoides*. The mannose phosphate isomerase (*Mpi*) locus shows strong differentiation between high and low tidal heights, while the glucose phosphate isomerase (*Gpi*) locus and an mtDNA RFLP show no differentiation (Schmidt and Rand 1999). This pattern is repeated across years and estuaries along the Maine coast, and experimental transplants have recapitulated the pattern of selection favoring the Mpi-fast (Mpi^F) allele in high tidal zones while favoring the Mpi-slow (Mpi^S) allele in low tidal zones and under algal cover (Schmidt et al. 2000*a*) (see Fig. 8A). In Maine, the selection that favors the Mpi^F allele in the high intertidal is restricted to a window of time in the late winter–early spring after metamorphosis when young barnacles begin filter feeding (Schmidt and Rand 2001). Notably, this selection interval has no statistical effect on the *Gpi* and mtDNA polymorphisms. When barnacles are subjected to factorial treatments of thermal stress and access to dietary mannose or fructose, thermal selection at *Mpi* is observed only when mannose is available (Schmidt 2001). If *Mpi* alleles are heat sensitive, excess mannose could lead to a fitness cost due to a negative ATP balance resulting from low flux through the glycolytic pathway (Schmidt and Rand 1999). This biochemical evidence implicates the *Mpi* locus itself as the target of selection, and not a linked locus, based on the mannose-dependent response of the alternative *Mpi* alleles. Mannose is a common sugar found in algae and marine phytoplankton available to barnacles in the water column, and thus appears to be an environmentally relevant substrate.

This system follows the paradigm of the *Lap* locus in *Mytilus* (Koehn and Hilbish 1987): a functional polymorphism is more clinal that other polymorphisms

and direct experimentation reveals a plausible physiological and biochemical mechanism for selection mediated by biotic and abiotic environmental factors. However, as we have seen for *Mytilus* and *Littorina*, the canonical patterns observed in initial studies are often reversed in other geographic locales. Such is the case for *Semibalanus* as well. In Narragansett Bay, Rhode Island, an estuary near the southern limit of the range for *S. balanoides*, the zonation patterns of *Mpi* and *Gpi* are reversed: Mpi^S allele is slightly more common at high tide zones, and *Gpi* is consistently zonated where it is statistically neutral in the Maine estuaries (Rand et al. 2002). In both regions mtDNA is not zonated across the intertidal, suggesting selection at the allozymes, but clearly direct sequencing of the genes and more putatively neutral markers are needed.

In The Gulf of St. Lawrence, both *Mpi* and *Gpi* are implicated in selection as inferred from a break in allele frequency across the mouth of the Mirimichi estuary (Holm and Bourget 1994). More recent work has further implicated selection at *Mpi* and *Gpi*, as microsatellite loci show somewhat less geographic variation than the allozymes (Dufresne et al. 2002). Notably, the Gulf of St. Lawrence data are somewhat more similar to the Rhode Island data in that *Gpi* appears to vary more across the mouth of the estuary (Dufresne et al. 2002). Moreover, the patterns of selection on *Mpi* and *Gpi* also shift with geographic location along the coastline (Veliz et al. 2004), suggesting that the highly repeatable selection on *Mpi* in Maine is not general for other populations. Again, the evidence for reversals of clinal patterns in different geographic of ecological contexts points to the pervasive nature of genotype-by-environment interactions governing selection on many polymorphisms, with the combined effects of diet and thermal stress likely to play important roles.

This method of inquiry illustrated with *Littorina* and *Semibalanus* can be considered a sort of *post hoc* "candidate locus" approach, with the qualification that the candidate status emerges after sufficient evidence arises, some of which is based on the biochemical properties of the allozyme locus in question. For example, the barnacle studies were motivated by findings of temperature-mediated selection at *Gpi* in Colias butterflies (Watt 1977, 1983). The initial findings that *Gpi* exhibited neutral patterns whereas *Mpi* did not presented a puzzle until it became clear that mannose is a more relevant sugar for marine filter feeders, while glucose is more relevant for nectar feeding insects. In Rhode Island, intertidal zonation of genotypes exists for *Gpi* while in Maine the zonation is observed for *Mpi*. This leads to the hypothesis that geographic variation in the diet of barnacles, with mannose-rich plankton being more common in Maine estuaries while glucose-rich plankton are more common in Rhode Island (Rand et al. 2002). Analogous arguments can be made regarding the *Aat* locus in *Littorina* and the physiology of amino acid metabolism (Hull et al. 1999, Panova and Johannesson 2004). As previously mentioned, the use of allozymes as a class of putatively selected loci is hampered by the likelihood of molecular heterogeneity within electrophoretic classes. However, allozymes do offer one advantage: they have substrates that can focus mechanistic experiments to elucidate allelic effects on performance and fitness.

Genomic scans for intertidal differentiation

A different approach to that of using putatively neutral or selected protein coding loci is to perform a genomic scan of populations from opposite extremes of a cline or environmental gradient (see Luikart et al. 2001). Marker loci that show exceptionally high levels of differentiation (as measured by F_{st}) become candidate markers presumably linked to the actual loci responsible for the adaptive differentiation. The challenges of this approach are to determine the distribution of F_{st} values that a random set of neutral loci might generate under mutation, migration and drift (Beaumont and Balding 2004, Beaumont 2005) and to eliminate single-locus false positives (Vasemägi 2006). This methodology, originally introduced by Lewontin and Krakauer (1973), has advanced considerably in recent years.

This approach has been applied using amplified fragment length polymorphism (AFLP) markers in *Littorina* (Wilding et al. 2001, Grahame et al. 2006). An AFLP scan comparing high vs. low intertidal samples of *L. saxatilis* revealed 306 polymorphic markers. Analysis of the null distribution of population differentiation (F_{st} values) for these markers identified several with significantly elevated F_{st} scores between tidal zones (see Fig. 7B). These AFLP markers that show high population differentiation (high F_{st}) become candidates for selection, or markers of selection at linked loci. While the anonymous nature of these bands precludes immediate functional analyses, a number of population genetic tests become apparent. For example, if just the neutral AFLP loci are considered, populations showed isolation by distance across a 45-km transect. When 15 high-F_{st} markers were included in such analyses, isolation by distance no longer held, suggesting that selection at these markers swamped the signal of neutral drift at the remaining markers (Wilding et al. 2001). A subsequent study showed that these same 15 loci were distributed in a clinal manner on two independent shores, and this cline was coincident with a cline in shell morphology (Grahame et al. 2006). Elevated levels of linkage disequilibrium among these 15 loci were also observed, which could indicate a zone of secondary contact (Grahame et al. 2006). It is also not unreasonable to observe elevated linkage disequilibrium under primary differentiation along a cline if selection is sufficiently strong.

There are additional examples of extreme and replicated tidal height variation at one marker relative to others in *Littorina* (Wilding et al. 2002). A clear advantage of this genomic scan approach is that any

marker linked to a potential target of selection could, in principle, be identified if the density of marker loci is sufficient. Protocols are widely available making it relatively straightforward to develop a large number of AFLP or microsatellite markers. In model genetic organisms, this has been extended to hundreds and even thousands of markers. The few examples we have summarized here have clear advantages over most of the genomic scan studies in *Drosophila* or other genetic model organisms: the ecology of the species involved and the level of environmental replication offered by the North Atlantic intertidal. As we outlined above, we suggest that a central problem for these kinds of marker-association studies of adaptation in the wild is finding a tractable system for identifying the selection.

Prospectus

Many of the classic papers describing clinal variation dealt with cases of a single locus that showed a strong clinal pattern in allele frequency. How is a researcher interested in studying clinal variation in a novel system to proceed, particularly when working with non-model organisms for which little or no molecular data are available?

As described at the beginning of this manuscript, the two approaches that have been successful in the past are to focus on (1) a posteriori comparisons of multiple loci, including genome-wide scans or (2) a priori candidate genes. As we have reviewed, some allozyme markers can serve as candidate loci, but there is an inherent post hoc component to this candidate status and also the issue of the molecular identity of allozyme alleles. One can construct a logical argument why, for example, mannose metabolism or amino acid metabolism is the key environmental factor explaining selection on the alternative alleles. A true candidate locus approach would identify the environmental stress and then go after the genes that are known to be affected by those stressors. Temperature variations might suggest profiling HSP genes and cofactors (Hofmann 2005), or variation in heavy metal levels might dictate analyses of metallothionein proteins (Jenny et al. 2004). De novo targeted searches for genes or transcripts in marine ecological systems are likely, in most cases, to be hampered by a lack of genomic data. However, as suggested by Thomas and Klaper (2004), available tools can be applied to the species that is of interest for the clinal gradient studied. Advances in comparative genomics have provided relatively easy access to genes in non-model organisms by exploiting conserved domains in proteins (Fredslund et al. 2006). This can be a useful approach particularly when the candidate gene sequences are available for related organisms or groups, allowing cross-taxon amplification using degenerate primers.

An intermediate approach between the candidate gene and genomic scan approach is to compare a series of markers from distinct functional classes, such as random markers from expressed sequence tags (ESTs), vs. random markers from non-coding loci cloned from genomic DNA (Stillman et al. 2006). Although fully representative EST databases are relatively expensive and time consuming to generate, for many cases one need not sample the majority of the genes in the genome. The occurrence of SNPs as well as highly polymorphic microsatellites in the untranslated regions of ESTs is proving to be a rich source of gene-associated genetic variation (Vasemägi et al. 2005). SNPs from random genomic clones, or microsatellites offer a putative class of neutral markers. An efficient means of identifying such SNPs is to sequence samples of individuals from the extreme ends of the cline under study. While sequencing costs have dropped significantly in recent years, this can still be a limiting factor if one seeks to sample many loci. A more cost effective approach to marker development is analysis of AFLP as we have described for *Littorina* (Wilding et al. 2001, Campbell and Bernatchez 2004). Similarly, newer techniques such as restriction site associated DNA (RAD) genotyping may be used to generate high-resolution genotyping of both individuals and bulk population samples in any species (Miller et al. 2007). In both cases, comparisons among loci across the genome can be used to test neutral expectations and infer selective processes (reviewed in Beaumont [2005]).

A polymorphism can affect the phenotype upon which selective forces operate in a variety of ways, such as by modifying the functional properties of a gene (i.e., causing non-synonymous substitutions that alter the amino acid residues in the gene product), by changes in the regulation of a gene (e.g., the transcriptional response), or by influencing regulation at the protein level (e.g., temporal stability). Therefore, patterns of allele frequencies, or transcriptional responses (transcriptional profiling) are two classes of methods that can be used to detect selection along environmental clines, with reference to a null (neutral) model. It has long been recognized that adaptive evolution of gene expression (i.e., gene regulatory variation) is an important potential source of variation (King and Wilson 1975, Wittkopp et al. 2004). The emergence of new technologies for post-genomic investigation of gene expression is revolutionizing studies of adaptive evolution. Transcript profiling is an attractive approach since it requires no prior knowledge of the physiological basis of a response, or the relative importance of specific gene families or metabolic pathways; rather, the candidate genes emerge as a result of the profiling technique employed. Microarray analysis has recently been applied to study gene expression divergence in populations of the teleost *Fundulus* (Whitehead and Crawford 2005) and to detect possible targets of selection (Whitehead and Crawford 2006). However, microarrays require extensive sequence data to design target cDNA or oligonucleotides (by definition something usually unavailable for non-model organisms). This, together with their high (although decreasing) cost puts microarrays beyond the scope of

most marine ecologists but these kinds of approaches are certainly going to be more common in the future (e.g., Teranishi and Stillman 2007). This is particularly true for the types of non-model species especially for "model" non-model species (for instance the one that attracts parallel studies by several groups of researchers on either side of the Atlantic such as reviewed above) since the costs for sequencing ESTs are dramatically being reduced with the current evolution of sequencing technologies.

While these methodological advances are certain to open up new opportunities for marine ecologists in identifying the genes that matter in natural populations, these advances go hand in hand with the ecological and evolutionary context of the environmental gradients under study. As reviewed in the limited number of cases above, there are several instances of polymorphisms showing strong clinal variation in one context that is reversed in a different context or geographic location. One interpretation of these reversals is that the markers are displaying an inherently chaotic pattern that gives the impression of selection, but may be sampling error. Another one is that they represent different outcomes of the interactions between differentiated gene pools, such as expected from the many secondary contacts and other admixtures brought by the Pleistocene cycles of climate change. However, the real strength of the systems we have discussed is the tradition of direct experimentation under field conditions that has a long history in marine ecology. While most of the organisms in marine contexts are bad genetic and genomic models, they are tractable ecological and evolutionary models. As the technologies from model systems become increasingly transferable to non-model organisms, the prospects for identifying the factors that connect genetic and ecological associations are promising.

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