Evolution, 59(12), 2005, pp. 2656–2668

ENVIRONMENTAL CORRELATES OF POPULATION DIFFERENTIATION IN ATLANTIC HERRING

DORTE BEKKEVOLD,[1,2] CARL ANDRÉ,[3] THOMAS G. DAHLGREN,[3,4] LOTTE A. W. CLAUSEN,[5] ELSE TORSTENSEN,[6] HENRIK MOSEGAARD,[5] GARY R. CARVALHO,[7] TINA B. CHRISTENSEN,[1] ERIKA NORLINDER,[3] AND DANIEL E. RUZZANTE[8]

[1]*Danish Institute for Fisheries Research, Department for Inland Fisheries, 8600 Silkeborg, Denmark*
[3]*Department of Marine Ecology, Göteborg University, Tjärnö Marine Biological Laboratory, S-452 96 Strömstad, Sweden*
[5]*Danish Institute for Fisheries Research, Department for Marine Fisheries, Charlottenlund, Denmark*
[6]*Institute of Marine Research, Flødevigen, N-4817 His, Norway*
[7]*School of Biological Sciences, University of Wales Bangor, Gwynedd LL57 2UW, United Kingdom*
[8]*Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada*

Abstract.—The marine environment is characterized by few physical barriers, and pelagic fishes commonly show high migratory potential and low, albeit in some cases statistically significant, levels of genetic divergence in neutral genetic marker analyses. However, it is not clear whether low levels of differentiation reflect spatially separated populations experiencing gene flow or shallow population histories coupled with limited random genetic drift in large, demographically isolated populations undergoing independent evolutionary processes. Using information for nine microsatellite loci in a total of 1951 fish, we analyzed genetic differentiation among Atlantic herring from eleven spawning locations distributed along a longitudinal gradient from the North Sea to the Western Baltic. Overall genetic differentiation was low ($\theta = 0.008$) but statistically significant. The area is characterized by a dramatic shift in hydrography from the highly saline and temperature stable North Sea to the brackish Baltic Sea, where temperatures show high annual variation. We used two different methods, a novel computational geometric approach and partial Mantel correlation analysis coupled with detailed environmental information from spawning locations to show that patterns of reproductive isolation covaried with salinity differences among spawning locations, independent of their geographical distance. We show that reproductive isolation can be maintained in marine fish populations exhibiting substantial mixing during larval and adult life stages. Analyses incorporating genetic, spatial, and environmental parameters indicated that isolating mechanisms are associated with the specific salinity conditions on spawning locations.

Key words.—*Clupea harengus*, hybrid-zone, isolation by distance, local adaptation, microsatellite DNA, migration, salinity.

Received April 1, 2005. Accepted September 22, 2005.

Although the long held view that abundant and widely distributed marine fish are unlikely to exhibit significant genetic structure at any but the largest of geographic scales has been shown to be largely inaccurate (e.g., Ruzzante et al. 1996; Nielsen et al. 2003; 2004; O'Reilly et al. 2004; Zardoya et al. 2004), the relative roles of migratory behavior and local differences in environmentally induced selective pressures in effecting such structure remain elusive. Structure has in some cases been explained by ''isolation by distance'' (Wright 1943), which may occur when the distribution of the species is larger than the dispersal range of individuals. Isolation by distance is evident in a number of pelagic fishes and crustaceans (e.g., Palumbi 1994; Pogson et al. 2001; O'Reilly et al. 2004) but not in others (Borsa 2002; Hoarau et al. 2002; Knutsen et al. 2003; Stamatis et al. 2004). Oceanographic processes and the topography of the ocean floor have been linked to population structure in a number of species (e.g., Ruzzante et al. 1998; Norris 2000; Knutsen et al. 2004), yet few studies conducted to date have tested specifically for relationships between environmental parameters of adaptive significance and population structuring in marine migratory fish, and even fewer have examined evidence of local adaptation (Billerbeck et al. 2001; Yamahira and Conover 2002). To gauge the relative importance of various potential mechanisms for population structure in marine pelagic fishes it is useful to combine information about levels of gene flow obtained from neutral genetic markers with knowledge about migratory behavior and environmental factors that are likely to be of adaptive significance. Correlation methods have been developed to assess associations between genetic, geographic, and environmental parameters (e.g., Smouse et al. 1986; Legendre and Legendre 1998; Yang 2004). Although such methods provide a means for assessing overall associations, they do not reveal if the strength of associations are uniform over the examined spatial range. An analytical approach based on computational geometry has therefore been developed recently to address this shortcoming (Manni et al. 2004). Here, we apply such an approach to examine population structuring in Atlantic herring, *Clupea harengus* (L.), in relation to environmental heterogeneity across a small geographic, but ecologically diverse, sea area (North Sea to Western Baltic).

Atlantic herring from the North Sea–Baltic Sea transition zone provide a model system for comparing patterns of migration and gene flow and for testing hypotheses of environmentally induced barriers to gene flow in a highly migratory pelagic fish. The Atlantic herring is an iteroparous clupeoid maturing at two to three years. Herring are abundant throughout the North Sea and the Baltic, occurring in feeding and wintering shoals of mixed origin during the majority of the year (ICES 1991). Spawning takes place on rock, gravel, and/or sandy substrates at 10–50 m, and individual population

[2] Corresponding author. E-mail: db@difres.dk
[4] Present address: Department of Zoology, Göteborg University, 405 30 Göteborg, Sweden.

components have traditionally been distinguished based on differences in spawning time.

The North Sea–Baltic Sea transition zone represents a major environmental gradient, as salinity changes from a full marine scale at 35‰ in the North Sea to close to zero in the inner parts of the Baltic Sea. Temperatures also differ substantially, as the North Sea confers a more stable climate than the Baltic which has shallower waters and more locally influenced temperature regimes with large annual variation. Both temperature and salinity regimes are expected to have selective impact as low temperatures impede development and survival during early larval stages (Johnston et al. 2001) and low salinity decreases fertilization and larval development success in the closely related *C. pallasi* (Griffin et al. 1998) and in other fishes from the North Sea–Baltic Sea transition area (Nissling and Westin 1997; Nissling et al. 2002).

Herring larvae are pelagic and may drift several hundred kilometers over a few months (Johannesen and Moksness 1991). Studies on tagging and morphometric character differences show that larvae spawned in autumn and winter along the British North Sea coasts drift east across the North Sea and into the Skagerrak, Kattegat, and inner Danish waters, where they feed for one to three years before returning to spawning locations in the western North Sea (Iles and Sinclair 1982). In the eastern North Sea, the Norwegian Sea, the Skagerrak, and the Baltic Sea spawning primarily takes place in spring, but with smaller autumn and winter spawning population components occurring locally (ICES 1991). The densest spawning aggregations in the area occur off the island of Rügen in the western Baltic, where spawning extends from March to May. Tagging studies have shown that Rügen spawners migrate to feeding areas in the Kattegat, Skagerrak, and the Eastern North Sea (Aro 1989). Both juvenile and adult spring spawning herring perform feeding migrations into the Skagerrak and Kattegat (Rosenberg and Palmén 1982; Johannesen and Moksness 1991), and spring spawning adults of nonlocal origin are encountered in high densities in the eastern North Sea (ICES 1991). Spawning components also occur throughout the Baltic Sea, but these and western Baltic components show no evidence of mixing (Aro 1989). However, studies also suggest that herring migratory behavior can be aberrant. Migration routes are observed to covary with changes in demography and thus appear to have some degree of social transmission (Dragesund et al. 1997).

In spite of the extensive mixing of individuals, herring population structure has been described largely from differences in morphology, growth, migration, and spawning behavior (Iles and Sinclair 1982). However, all of these traits are influenced by the physical and/or social environment that individuals experience early in life, and thus differences may be the result of phenotypic plasticity rather than of independent evolutionary trajectories. Molecular studies have provided some evidence for genetic differentiation among discrete spawning components (Shaw et al. 1999; Hauser et al. 2001; McPherson et al. 2001a; 2004; Jørstad et al. 2004; for a study employing protein markers, see also Ryman et al. 1984). In most cases levels of differentiation are estimated to be low and no study has reconciled patterns of differentiation with hypotheses about gene flow or with different selective pressures encountered locally.

The objective of this study was to use Atlantic herring sampled from spawning locations from the North Sea, the Skagerrak, and the western Baltic to (1) test the hypothesis that population structure can be maintained in a highly migratory marine fish with well described migratory patterns, and (2) to test the hypothesis that population structure correlates with environmental conditions at spawning locations. Based on highly polymorphic microsatellite DNA markers combined with detailed information about salinity and temperature regimes, we used partial Mantel tests (Legendre and Legendre 1998) and a computational geometry approach by Manni et al. (2004) to evaluate hypotheses about selectively induced barriers to gene flow. We discuss the implications of our results for predictions of local adaptation in Atlantic herring and other highly migratory marine fishes.

Materials and Methods

Sample Collection

Samples of herring (mostly) in spawning condition were collected from a total of 10 spawning locations from the eastern North Sea to the Western Baltic over a period of two years (2002 and 2003) with six of the locations sampled in both years (Table 1). One of these six locations (Rügen) was sampled repeatedly over three consecutive months in 2002 (March, April, May) and two months in 2003 (April–May). This was done to account for the temporally and presumably reproductively isolated ''spawning waves,'' which based on morphological analyses are reported to occur at this location (Rechlin 2000). No spawning herring were found in 2003 at one of the locations sampled in 2002 (Karmoy). A sample presumably representing the same Norwegian spring spawning population component, was therefore taken in 2003 at the more northerly location Møre (Fig. 1).

Individuals were aged on the basis of counts of otolith (sagitta) winter rings following the standard procedure detailed in ICES (2003). Aging was conducted by the Institute for Marine Research in Bergen, Norway, for samples from locations 6–10, and by the Danish Institute for Fisheries Research in Charlottenlund, Denmark, for samples from locations 1–5. Otolith central area microstructure was analyzed by the same laboratories to determine each individual's hatching season (spring, autumn, or winter) following a procedure described in Moksness and Fossum (1991). The percentage of ripe and running (spawning) herring was above 90% for 13 of the 20 samples (Table 1). Two samples, one from the Limfjord (2003) and one from the Kolding Fjord (2002), contained only 1% and 8% running fish, respectively. Nonrunning fish constituted a mix of fish that were about to mature and fish that had spawned recently. A sample of herring from Trinity Ledge, Nova Scotia, in the northwest Atlantic was included as an out-group for comparison (for further details about this sample, see McPherson et al. 2004).

Molecular Analyses

DNA was isolated from fin or muscle tissue using a Chelex technique (Walsh et al. 1991). For a small subset of the

TABLE 1. *Clupea harengus* samples. Locality numbers refer to Figure 1. The age distribution of each sample is shown by the numbers of individuals born in different years (1995+ indicates fish born in 1995 and earlier). Also given are mean age and percentages of spawning fish per sample. See McPherson et al. (2004) for further details on the outgroup sample from the northwest Atlantic.

Regional area	Locality	Latitude/longitude (decimal)	Sample date	Sample size	Age class							Age (years)	% ripe-and-running
					2000	1999	1998	1997	1996	1995+	Undetermined		
Western Baltic	Rügen (1)	54.23N/13.44E	22/03/02	100	—	2	16	24	37	21	—	5.69	100
			18/04/02	100	—	1	27	14	33	25	—	5.67	100
			06/05/02	100	—	1	39	9	23	28	—	4.55	100
			24/04/03	100	—	—	14	46	14	26	—	5.72	100
			06/05/03	100	—	3	31	43	9	14	—	5.12	100
Kattegat and inner Danish waters	Kolding Fjord (2)	55.49N/09.54E	12/04/02	100	—	32	36	24	2	5	1	4.10	8
			05/04/03	70	—	19	26	20	3	2	—	4.10	97
	Lillebælt (3)	55.66N/09.90E	07/04/03	100	—	1	21	18	19	40	1	5.95	50
	Kattegat (4)	55.95N/11.61E	06/05/02	44	—	11	29	2	1	1	—	3.91	74
			03/04/03	100	—	—	46	12	1	3	38	4.40	62
	Limfjord (5)	57.06N/10.06E	22/05/03	100	37	36	13	9	3	2	—	3.11	1
Skagerrak	Flatbrotten (6)	58.10N/11.33E	07/03/02	100	—	22	71	6	—	—	1	3.84	100
			19/03/03	100	—	42	30	25	—	3	—	3.99	93
	Måseskär (7)	58.34N/11.27E	19/03/02	100	—	24	67	5	1	—	3	3.82	96
			14/03/03	100	—	27	23	43	2	3	2	4.23	94
	Tjöme (8)	59.35N/10.55E	13/03/02	120	—	5	88	16	5	5	1	4.30	98
			04/03/03	120	—	2	13	62	20	9	14	5.22	100
North Sea	Karmøy (9)	59.25N/05.17E	14/03/02	100	—	—	3	3	6	80	8	7.96	100
	Møre (10)	62.78N/06.08E	17/02/03	78	—	—	—	10	5	60	3	8.31	32
Northwest Atlantic	Nova Scotia (11)	44.01N/66.31W	27/08/96	75	—	—	—	—	—	—	—	4.95	87

samples, DNA isolation was carried out using a HotSHOT technique (Truet et al. 2000). Nine tetranucleotide microsatellite loci Cha1017, Cha1020, Cha1027, Cha1202 (McPherson et al. 2001b), Cpa101, Cpa111, Cpa112, Cpa113 and Cpa114 (Olsen et al. 2002) were PCR amplified using standard reagents and annealing temperatures between 50° and 60°C (exact protocols are available on request). The loci had been chosen based on low expectations for the presence of null alleles and linkage disequilibrium (McPherson et al. 2001b; Olsen et al. 2002). An additional locus *Cpa106* (Olsen et al. 2002) was initially screened but could not be scored consistently due to presence of nonspecific peaks and was therefore omitted from the analysis. DNA fragments were visualized and genotyped using a BaseStation 51 fragment analyzer (MJ Research, Skovlunde, Denmark) in conjunction with the software Cartographer, 1.2.6 (MJ Geneworks Inc., Skovlunde, Denmark) (samples from locations 1–5), and a Pharmacia ALF-express automated sequencer in conjunction with the Fragment analyzer software (Amersham Pharmacia Biotech, Hillerød, Denmark) (samples 6–11), according to the recommendations of the manufacturers. Prior to these analyses, 10 individuals from each of four sampling locations were cross-analyzed using both methods to determine scoring consistency. No genotyping inconsistencies were observed for any of the nine loci analyzed in these 40 fish. The 40 cross-tested individuals were not included in the present study, as none was a ripe-and-running spawner, an important condition to ensure correct sampling of individual spawning components. For the present dataset, scoring consistency was continuously ascertained by double-analyzing subsets (ca. 10%) of individuals for both visualization methods. In addition, every gel included two standard individuals per locus. These standard individuals were chosen among the 40 cross-tested individuals so that their alleles together spanned the observed size ranges, which incidentally corresponded well with the size ranges observed in the full dataset.

Environmental Data

Ambient salinities and temperatures (average, maximum, and minimum) close to the sea floor (where spawning takes place and eggs are deposited) were obtained for each of the spawning locations for the month and year of sampling and for the two consecutive months. This was done to account for the time of spawning, the egg phase (7–14 days) and the larval phase (about two months). To examine the temporal stability of the hydrographic features on spawning locations, we obtained monthly average values over the years 1997–2003. Relationships between environmental parameters were examined by a series of correlation analyses using untransformed data, when Shapiro-Wilks tests indicated no deviation from normality. Environmental data were supplied by the Swedish Meteorological and Hydrological Institute, Göteborg, Sweden, by the Limfjordsovervågningen, Viborg, Denmark, and by the Institute for Marine Research, Bergen, Norway.

Statistical Analyses

Departure from Hardy Weinberg proportions (HWE) were tested for each locus and sample using GENEPOP (Raymond and Rousset 1995). The same software was used to analyze for departure from gametic phase equilibrium (linkage disequilibrium) between loci by means of ''exact tests.'' Population differentiation was estimated per sample pair and

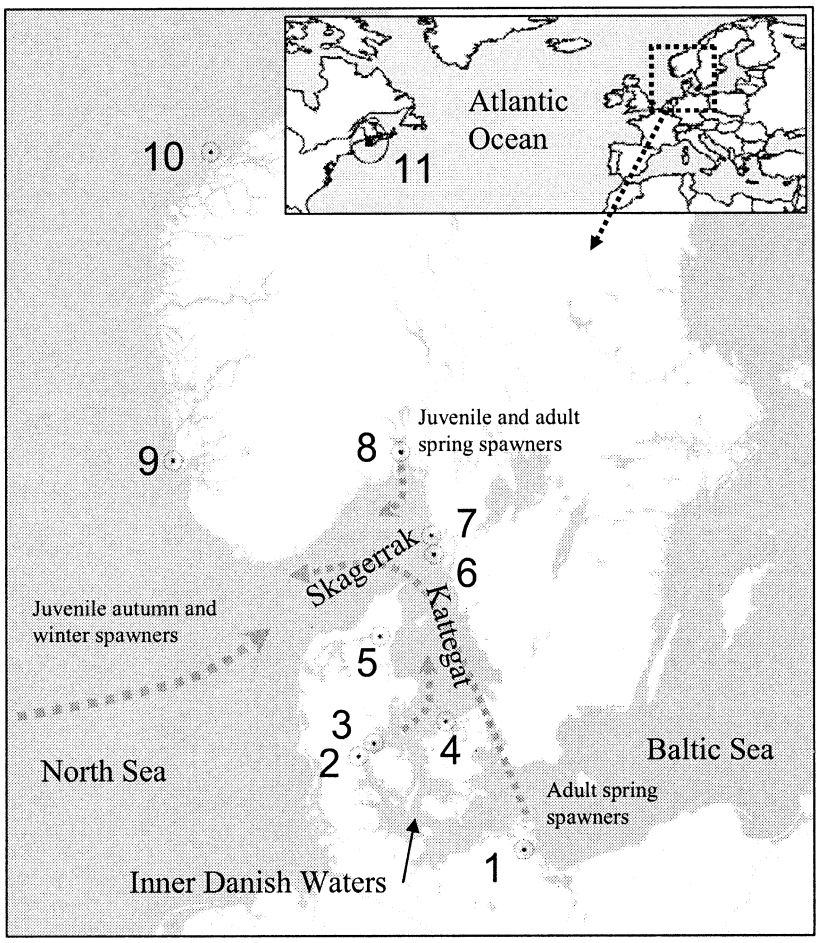

FIG. 1. Sampled *Clupea harengus* spawning locations (numbers refer to Table 1). Main migratory patterns are indicated by arrows (see text).

overall using the unbiased estimator θ (Weir and Cockerham 1984) and statistical significance was examined using permutation tests implemented in FSTAT (Goudet 2001). Temporal, within-location samples not exhibiting significant differentiation (at $\alpha = 0.05$) in these tests were pooled in subsequent analyses. FSTAT was also used to estimate allelic richness per locus and sample, using rarefaction, and to test for differences among groups of samples, using permutation tests.

In age-structured populations with overlapping generations, allele frequencies are predicted to differ among age classes due to drift, and genetic variance estimates based on varying contributions from different age classes may therefore be inflated (Jorde and Ryman 1995). To estimate potential age class effects on our results we applied hierarchical analysis of molecular variance (AMOVA, following Schneider et al. 2000) using allele frequency information from subsets of population samples, for which more than 20 multilocus genotypes were available per age class (cohort).

ViSta 5.6.3 (Young 1996) was used to perform multidimensional scaling (MDS) analysis of pairwise θ values, and to visualize genetic relationships among samples. We used the approach implemented in the software Barrier 2.2 (Manni et al. 2004) to identify shifts in genetic differentiation among spawning groups. Briefly, the method uses computational geometry and a Monmonier's maximum-difference algorithm to identify barriers to gene flow. First, a geometric map is obtained from a matrix of geographic coordinates by Voronoï tessellation, which represents the polygonal neighborhood for each sample (population) constructed so that the borders of each neighborhood are closer to the centroid (the sample coordinate) than to any other sample. A triangulation method (Delauney) is applied to connect neighboring samples into a network of triangles, and a Monmonier's algorithm is used to first determine which of the borders between neighboring populations exhibits the highest genetic differentiation. A barrier is then constructed by the algorithm continuously locating the largest genetic distance along adjacent borders and extending the barrier in a stepwise manner until reaching the outer edge of the network or another barrier. The user specifies the numbers of barriers drawn, with subsequent barriers decreasing in order of importance. We computed barriers, first by using a multi-locus θ matrix to represent genetic differentiation among samples, and second, by using θ for the nine loci separately to determine ''consensus barriers.'' The latter analysis allows for an evaluation of the robustness of barriers suggested based on the multilocus θ, by reporting

TABLE 2. *Clupea harengus* pairwise F_{ST}-values estimated by θ (below diagonal) and *P*-values (based on 1000 permutations) for pairwise significance (above diagonal). Significant F_{ST}-values following sequential Bonferroni correction ($k = 76$ tests) are indicated by asterisks (α: ***$P = 0.001$, **$P = 0.01$, *$P = 0.05$).

	Rügen March 02	Rügen Apr–May 02/03	Kolding Fjord 02	Kolding Fjord 03	Lillebælt 03	Kattegat 02/03
1. Rügen March 02		0.0061	<0.0001	0.0062	<0.0001	<0.0001
1. Rügen April–May 02/03	0.0045		<0.0001	<0.0001	<0.0001	<0.0001
2. Kolding Fjord 02	0.0097***	0.0056***		<0.0001	<0.0001	<0.0001
2. Kolding Fjord 03	0.0041	0.0047**	0.0048***		0.2085	<0.0001
3. Lillebælt 03	0.0068***	0.0025***	0.0030*	0.0016		0.0015
4. Kattegat 02/03	0.0066***	0.0029***	0.0053***	0.0033**	0.0054	
5. Limfjord 03	0.0118***	0.0032***	0.0055***	0.0064*	0.0005	0.0040***
6. Flatbrotten 02/03	0.0151***	0.0095***	0.0088***	0.0099***	0.0075***	0.0062***
7. Måseskär 02/03	0.0147***	0.0102***	0.0078***	0.0083***	0.0069***	0.0065***
8. Tjöme 02/03	0.0169***	0.0108***	0.0096***	0.0099***	0.0086***	0.0065***
9. Karmoy 02	0.0206***	0.0175***	0.0137***	0.0164***	0.0144***	0.0123***
10. Møre 03	0.0270***	0.0194***	0.0181***	0.0222***	0.0182***	0.0164***
11. Outgroup: Nova Scotia	0.0230***	0.0194***	0.0169***	0.0194***	0.0161***	0.0164***

the numbers of loci supporting the individual sections of the barriers.

Relationships among genetic, geographic, and environmental differences among spawning components were analyzed using partial Mantel tests (Legendre and Legendre 1998) implemented in the software IBD 1.5 (Bohonak 2002). Matrices of genetic (using $\theta/1 - \theta$), geographic (logarithmic shortest waterway distance, estimated using the GIS software ArcMap 8.2 supplied by ESRI (http://www.esri.com), and estimates of differences among sampling localities in each of eight environmental parameters were constructed for all pair wise population comparisons. Environmental parameters based on estimates over the years 1997 to 2003 were: (1) average salinity in spawning month, (2) minimum salinity encountered at site, (3) maximum salinity encountered at site, (4) daily variance in salinity during spawning month, (5) average temperature in spawning month, (6) minimum temperature, (7) maximum temperature, and (8) variance in temperature during spawning month. All tests were based on 10,000 randomizations and results were sequential Bonferroni corrected for multiple tests.

RESULTS

Otolith Analysis: Aging and Hatching Month

The age compositions of the sampled fish are given in Table 1. All fish in the analysis exhibited fidelity between the season they were caught as spawners and the month they had hatched (i.e., spring), as determined from the analysis of otolith structure.

Within Sample and Location Genetic Analysis

Nine microsatellite loci were analyzed in 1951 fish. Scoring success overall was high as 97% of all individuals were genotyped at all nine loci (see Appendix available online at http://dx.doi.org/10.1554/05-183.1.s1). Repeated analyses of subsets of individuals for each of the two sequencing methods demonstrated high genotyping consistency across methods, because across loci and samples 98.15% of the alleles were scored consistently. Following correction for 180 multiple tests, three Hardy Weinberg proportions tests in three different loci indicated significant deviations (see Appendix available online). If a less conservative correction factor of $k = 20$ was applied, significance was indicated for a total of eight deviations. Because the deviations were distributed across loci and samples and since none was associated with a large F_{is}, we do not attribute them to null alleles. No significant effects of gametic phase disequilibrium were found across loci and samples. Of the seven within-location temporal comparisons, only the samples from Kolding Fjord exhibited significant differentiation (θ estimated at 0.0048 [95% CI = 0.0023–0.0080]). Within the Rügen location, only the March 2002 sample exhibited low but significant differentiation from any of the other Rügen samples (θ ranging between 0.003 and 0.008 in four pairwise comparisons). This sample was therefore entered separately into subsequent analyses, whereas samples from April and May 2002 and 2003 were pooled.

Spatial Population Differentiation

Estimates of pairwise population differentiation generated for pooled temporal samples ranged between −0.0002 and 0.0270 (Table 2), and the overall θ was estimated at 0.008 (95% CI = 0.004–0.013, $P < 0.001$). The Nova Scotia outgroup sample was significantly differentiated from all samples, except the two geographically closest North Sea samples (Table 2). An AMOVA incorporating data for 1997, 1998, and 1999 age classes nested within the Rügen, Kolding Fjord, and Tjöme sampling locations did not indicate genetic differences among age classes within locations (−0.21% variance explained, $P = 0.998$), whereas a highly significant proportion of the genetic variation was explained by differentiation among spawning locations (0.93% variance explained, $P < 0.001$). Allelic richness varied among samples for all loci (Appendix 1) and (averaged over loci) decreased from the North Sea and Skagerrak to the Baltic (North Sea [two samples] $R_s = 14.93$; Skagerrak [three samples] $R_s = 15.31$, Kattegat and inner Danish waters [five samples] $R_s = 13.98$; Western Baltic Sea [two samples] $R_s = 13.44$; $P = 0.002$).

Samples grouped along the first dimension axis in the MDS analysis in general correspondence with their longitudinal

TABLE 2. Extended.

Limfjord 03	Flatbrotten 02/03	Måseskär 02/03	Tjöme 02/03	Karmoy 02	Møre 03	Nova Scotia
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.3520	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.0073***		0.0576	0.1306	0.1097	<0.0001	<0.0001
0.0083***	0.0002		0.2180	0.0257	<0.0001	<0.0001
0.0090***	0.0002	−0.0002		<0.0001	<0.0001	<0.0001
0.0158***	0.0005	0.0015	0.0022***		0.4866	0.0203
0.0170***	0.0033***	0.0046***	0.0035***	0.0000		0.0049
0.0173***	0.0033***	0.0033**	0.0036***	0.0017	0.0037	

position from the North Sea to the Baltic, except for the Northwest Atlantic ''outgroup'' sample which grouped between North Sea samples (Fig. 2A). The first axis explained 68% of the variation. The second axis, explaining 9% of the variation, indicated separation between Skagerrak and North Sea samples. Subsequent dimensions each explained less than 8% of the variance and were not considered further. In the BARRIER analysis the first reproductive barrier, which was supported by five to eight loci over different sections of the barrier, separated North Sea and Skagerrak populations from all other samples (Fig. 2B). The second barrier, supported by five and six loci, separated the Rügen March sample from all other samples. The third and fourth barriers, both supported by four to five loci, separated respectively, the Kolding Fjord 2003 sample from all other samples, and the Kattegat and Rügen samples from all other samples. Subsequent barriers were supported by only two to four loci and not considered further.

Hydrographic Environment and IBD

Across the ten sampling locations, strong positive correlations were found between average salinity in the first three months following spawning (all $r > 0.94$), indicating that hydrographic differences among locations remained constant during the egg- and first-larval stages. Lesser but similar trends were found for temperature (all $r > 0.56$). Positive correlations were also observed for both salinity and temperature in the year and month of sampling compared to averages over the years 1997-2003 ($r_{\text{salinity}} > 0.97$; $r_{\text{temperature}} > 0.85$), indicating that conditions in the years represented in our samples were similar to those encountered over a six to seven year period and presumably longer. Figure 3 shows trends in salinities and temperatures in the month of sampling for the ten locations. Differences among sampling locations in average salinity, maximum and minimum salinity were correlated with each other (all $r > 0.94$). No other correlations were detected between any of the other environmental parameters examined.

Mantel tests indicated significant correlations between genetic differentiation and average and minimum salinity at spawning locations, which persisted when controlling (partial Mantel test) for geographic distance, whereas the correlation between genetic and geographic distances was nonsignificant or marginally significant when the environmental components (monthly average, monthly maximum and monthly minimum salinity) were controlled for (Table 3). Tests including maximum salinity, salinity variance, or any of the temperature parameters gave no evidence of covariance with genetic differentiation (Tables 3, 4).

DISCUSSION

Differentiation among Spawning Locations

We identified temporally stable differentiation among spawning locations along an environmental gradient despite the fact that individuals migrate freely across it. Due to annual variation in recruitment success our samples varied in age composition between the two sampling years (Table 1). Unequal sampling of potentially differentiated age classes may lead to erroneous conclusions about spatial versus temporal allele frequency variance (Jorde and Ryman 1995), but the AMOVA indicated that no detectable variability was found among age classes sampled within locations. Estimates of population differentiation are commonly almost an order of magnitude lower in marine fishes compared to estimates in freshwater fishes and terrestrial organisms. Summarizing estimates from a range of marine fishes Ward et al. (1994), for example, reported a median F_{ST} of 2%. In comparison, the overall F_{ST} estimated for our population samples was less than 1%, whereas between-individual sample estimates came up to 2.7% (Table 2). Proper interpretation of such low levels of differentiation requires consideration of the negative relationship between maximal attainable F_{ST} and level of polymorphism of the genetic markers used (very high in the loci employed here; c.f. Appendix 1)(Hedrick 1999). Regardless, it is pertinent to question the biological significance of low F_{ST} values. One way of doing so is to examine whether observed patterns are temporally stable (Waples 1998). We therefore consider the fact that the observed patterns of genetic differentiation were stable across two years of sampling evidence for their biological significance. Moreover, the samples showed a clear grouping along a west-east axis corre-

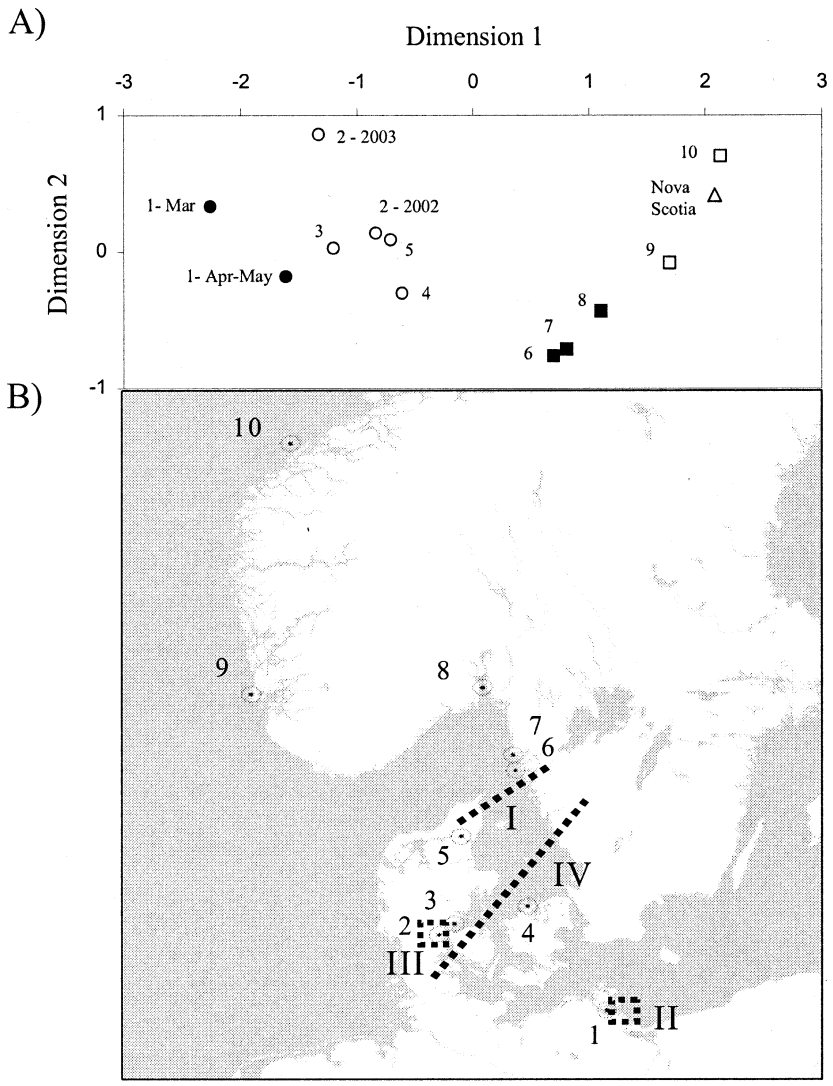

FIG. 2. (A) MDS plot of samples from the Western Baltic (closed circles), Kattegat and inner Danish waters (open circles), Skagerrak (closed squares), North Sea and Norwegian Sea (open squares), and the Northwest Atlantic outgroup (triangle). Locality numbers refer to Table 1. Respectively, 68% and 9% of the variance was explained by the first and second axes (stress = 0.16). (B) Major shifts in gene flow suggested by BARRIER, with the order of importance given in roman numerals. The second and third barriers separate, respectively, the Rügen March sample from all other samples and the Kolding Fjord 2003 sample from all other samples.

sponding with the North Sea–Baltic Sea transition zone (Fig 2A). The sets of genetic markers applied in our study were associated with high power for detection of even very small allele frequency differences among samples (Ryman et al., pers comm). Thus, our study represents the first demonstration of a well-defined spatial relationship for the genetic structure in a clupeoid fish.

The levels of genetic differentiation detected in our study were higher than those reported for *C. harengus* populations across comparable geographical ranges in the northwestern Atlantic, where no relationship between geographic and genetic differentiation is evident (McPherson et al. 2004). A microsatellite study of autumn and winter spawning *C. harengus* populations spanning a >1000 km north-south transect in the western North Sea reported very low, albeit statistically significant differentiation (F_{ST} = 0.1%, $P < 0.001$) and lack of distinct geographical structure in this area (Mariani et al., in press). We found that northwestern Atlantic (Nova Scotia) and northeastern Atlantic (North Sea) spawning components did not differ genetically (Table 2). Lack of differentiation of neutral genetic markers across scales of several thousands of kilometers may reflect recent colonization histories coupled with large populations experiencing low levels of genetic drift (Grant and Bowen 1998) and need not invoke high gene flow per se. The large contrast detected in levels of differentiation among samples within the Atlantic and those between the Atlantic and the Baltic show that levels of gene flow can be expected to be highly variable over the distribution of the species.

Failing to follow a sound sampling scheme is likely to lead to erroneous conclusions about population structure in highly migratory organisms (Carvalho and Hauser 1997). Our samples included high proportions of ripe and running fish and hence satisfy the requirements for sampling of reproductively

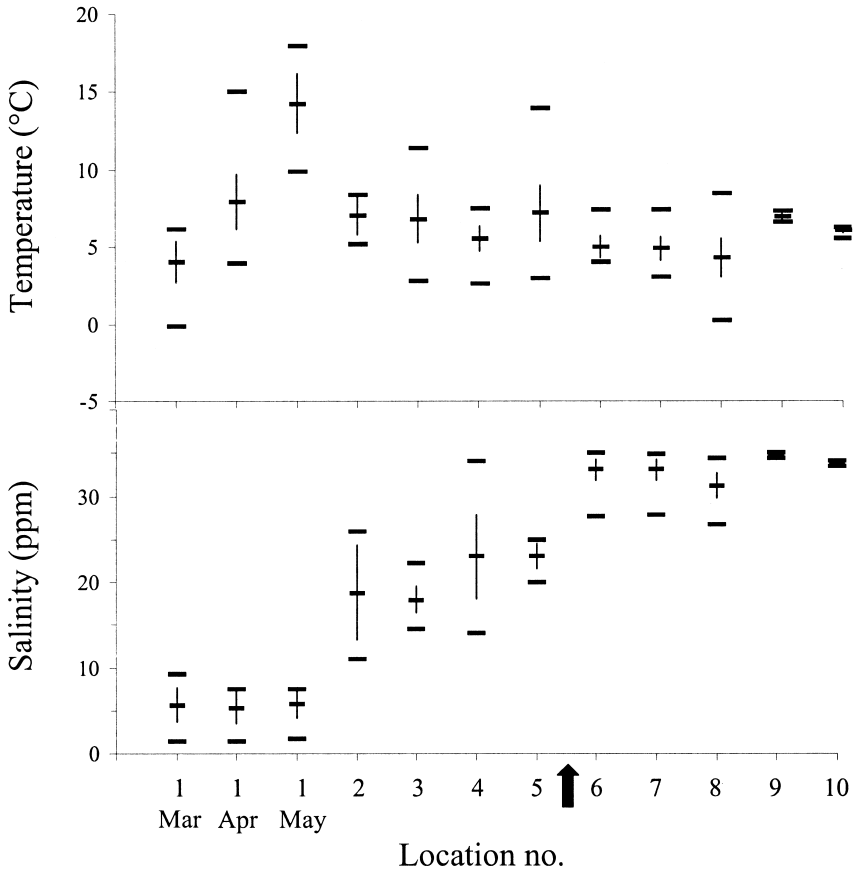

FIG. 3. Temperature (upper diagram) and salinity (lower diagram) on spawning locations in the month of sampling. Values are averages over the years 1997–2003, error bars indicate standard deviations estimated over days across years, and vertical bars indicate local maxima and minima. Location numbers refer to Figure 1. Values for location 1 are presented for each of the three sampling months (see text). The arrow indicates where the computational geometric analysis indicated the largest discontinuity in gene flow among spawning locations.

TABLE 3. Mantel tests (regular and partial) for matrix correlations incorporating pairwise differences in genetic, geographic, and each of four salinity parameters. Significant (at $\alpha = 0.05$) *P*-values following correction for four sequential tests are indicated with asterisks. Test results for genetic versus geographic distances are similar across the four analyses and are only given once.

Salinity parameter	Average salinity			Minimum salinity			Maximum salinity			Salinity variance		
	Z	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2
Genetic vs. geographic distance	1.507	<0.001*	0.145									
Partial, controlling for environmental parameter		0.306	0.005		0.883	0.027		0.009*	0.099		0.002*	0.122
Genetic vs. environmental difference	9.034	<0.001*	0.426	10.401	<0.001*	0.598	7.308	0.014	0.129	1.374	0.033	0.078
Partial, controlling for geographic distance		<0.001*	0.332		<0.001*	0.542		0.039	0.082		0.063	0.052

TABLE 4. Mantel tests (regular and partial) for matrix correlations incorporating pairwise differences in genetic, geographic, and each of four temperature parameters. Significant (at $\alpha = 0.05$) *P*-values following correction for four sequential tests are indicated with asterisks. Results for genetic versus geographic distances are similar across tests and are only given once.

Temperature parameter	Mean temperature			Minimum temperature			Maximum temperature			Temperature variance		
	Z	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2
Genetic vs. geographic distance	1.507	<0.001*	0.145									
Partial, controlling for environmental parameter		<0.001*	0.156		0.002*	0.127		<0.001*	0.149		<0.001*	0.189
Genetic vs. environmental difference	1.138	0.156	0.043	1.477	0.082	0.042	1.862	0.239	0.012	1.613	0.954	0.068
Partial, controlling for geographic distance		0.126	0.056		0.148	0.021		0.203	0.016		0.989	0.116

coherent components. At the one locality where temporal allele frequency stability was rejected (Kolding Fjord 2002 vs. 2003) the sample from 2002 contained only 8% ripe individuals and may not have represented the local spawning component adequately. This sample showed closest similarity with samples collected at locations outside the fjord (Fig. 2A), suggesting that a proportion of the sampled fish originated from neighboring locations. The Limfjord sample also contained few ripe and running fish, but the single temporal sample prevented determination of whether fish were local or comprised of migrants originating elsewhere. The main access to the Limfjord is via the eastern mouth of the fjord, and although Limfjord herring are assumed to perform feeding migrations into the Kattegat, the relative isolation of the fjord nonetheless suggests that individuals present around the time of spawning would be of local origin.

We found some indication for reproductive isolation between short-term temporally differentiated ''spawning waves'' at the Rügen location. The early (March) sample was significantly differentiated from later samples (Table 2), but our samples did not allow tests of annual stability. Within-year samples showed slightly differing age class distributions because older fish tended to spawn earlier than younger fish (Table 1), and tests for differentiation among short-term temporal samples using subsets of individuals belonging to the same age class revealed no evidence for structure (results not shown). Although we could not determine the biological significance of the short-term genetic differentiation at this location, our results emphasize the importance of sampling scheme (i.e., equal cohort representation and assuring that samples represent spawning individuals) in analyses of temporal stability. Failure to recognize local substructure would, for example, be critical to attempts at generating estimates of effective population size and could lead to severe underestimation of this evolutionarily important parameter.

Environmental Correlates of Population Structure

Applying Mantel tests we demonstrated a signal of isolation by distance, but also that both minimum and average salinity exhibited high correlations with genetic differentiation. The latter trends persisted when effects of geographic distance were controlled for using partial Mantel analysis, whereas conversely the trend for isolation by distance disappeared when these environmental parameters were held constant (Table 3). Caution should be taken when relationships between geographic, population genetic, and environmental parameters are examined by means of partial Mantel tests (Raufaste and Rousset 2001). Multicollinearity between independent variables, in our case geographic distance and environmental parameters, is likely to lower power through inflated type-I errors in tests of the partial correlation between the environmental parameter and the dependent variable, in this case genetic differentiation (Castellano and Balletto 2002). Nonetheless, the highly significant correlation between salinity and genetic differentiation when controlling for geographic distance showed that salinity parameters and/or associated factors were correlated with gene flow among spawning locations, and suggested that salinity differences rather than distance per se affected levels of reproductive isolation among spawning components. In the North Sea–Baltic Sea transition zone salinity, or factors correlated with salinity, may act as cues for homing to spawning grounds. Factors associated with salinity parameters and the timing of juvenile and adult migratory behavior may also act to produce a strong signal of reproductive isolation being associated with salinity. We stress that the analysis does not demonstrate local adaptation to a specific salinity regime (see below) but that our approach offers a means of comparing genetic and various environmental parameters to evaluate the relative magnitudes of their covariances.

Our analyses allowed us to compare environmental patterns with the BARRIER computational geometric approach. The BARRIER analysis indicated that the largest change in gene flow (i.e., the largest reproductive isolation) occurred between spawning locations in the Skagerrak and Kattegat (Fig. 2B). Although the statistical significance of this result could not be determined directly, the consensus analysis showed that the barrier was supported by multiple independent loci. The delimitation between these areas corresponds with a major transition in salinity parameters and a general trend for a more stable environment northwest of the barrier, as opposed to more variable local conditions southeast of the suggested barrier (Fig. 3). The second to fourth identified barriers were supported by fewer loci and only partially corresponded with environmental changes. The low salinity in the Baltic relative to the inner Danish waters was, for ex-

ample, only partly associated with a signal of reproductive isolation (compare Fig. 2B with Fig. 3).

The computational geometric approach BARRIER enables visualization of hierarchical patterns of differentiation, irrespective of actual magnitudes of differentiation. Interpretation of the biological significance of barriers should therefore be treated with caution and ideally be combined with other types of evidence for isolating mechanisms, such as behavioral and environmental factors. A caveat when applying the approach to populations following an isolation-by-distance model occurs when the sampled locations are not equally spaced geographically (Manni et al. 2004). Here, the algorithm will return a signal of a reproductive barrier between the geographically, and hence genetically most distant neighboring populations. In such cases, it is not possible to determine whether the constructed barrier reflects a true barrier to gene flow or merely that information for intermediate populations was not included in the analysis. We identified the largest increase in levels of reproductive isolation in regions that were relatively well represented by samples, indicating that the identified barrier is unlikely to reflect mere sample coverage. Moreover, the area in which the highest level of population differentiation was observed encompassed an ecologically meaningful gradient that is likely to impact growth and recruitment.

The largest reproductive barrier was estimated between groups of samples scored using different genotype visualization techniques and inconsistent scoring between approaches could potentially have affected this analysis. Our quality control measures and the high resulting consistency between techniques, together with the combined evidence from the spatially explicit distribution of samples shown in Figure 2A and the fact that the barrier was supported by multiple individual loci strongly suggest that the result is unlikely to have been caused by technical artifacts.

The barriers to gene flow detected between spawning components could presumably result from distinct hydrographic circulation systems that retain larvae and/or adults within local areas, as has been suggested for herring (Iles and Sinclair 1982) and other species including cod, *Gadus morhua* (Ruzzante et al. 1998); sea bass, *Dicentrarchus labrax* (Bahri-Sfar et al. 2000); and marine invertebrates with pelagic larvae (Wares et al. 2001). However, in the herring populations studied here, juveniles and adults are not retained within natal areas. The North Sea hydrographic circulation transports autumn hatched larvae from the east coast of the United Kingdom to juvenile nursery areas in the Skagerrak and the Kattegat (Rosenberg and Palmén 1982, Fig. 1), and tagging studies on spring spawning western Baltic herring from the Rügen area indicate feeding migrations to the same areas (Aro 1989). The proportions of the two major groups in the area of mixing have previously been based on analyses of differences in growth and maturity combined with metrics like vertebrae numbers (ICES 1991). Otolith microstructure analysis has been used to identify the hatching season of mixed individuals and has indicated the existence of several subcomponents among spring spawners feeding in the Skagerrak-Kattegat area (Mosegaard and Madsen 1996; Mosegaard et al. 2001), and a genetic study corroborates this (Bekkevold et al., unpubl. ms.). These observations hence refute that the population structure reported here is caused solely by physical isolation between population components, and instead invoke a role for active homing and possibly locally differentiated selection pressures.

The Potential for Local Adaptation in Herring

A number of studies have sought to use divergence in ecologically relevant habitat factors as a surrogate of divergent selection to examine associations with gene flow (e.g., Smith et al. 1997; Reusch et al. 2001; Ogden and Thorpe 2002). We identified correlations between population differentiation and environmental (salinity) parameters associated with the spawning, egg, and early larval phase that were of greater magnitude than correlations between geographical and genetic differentiation (Table 3). Whereas such observations do not demonstrate that genetic differentiation is maintained by selection, salinity parameters are expected to exert strong selective pressures in marine organisms. Fertilization and larval developmental success are, for example, reduced at both low and high salinity levels in Pacific herring, *C. pallasi*, and this species' optimal salinity range was found to be higher than that of Baltic herring (Griffin et al. 1998). Baltic herring spawn in coastal low salinity habitats where larval retention is high due to limited large-scale hydrographical activity (Lehmann et al. 2002) and therefore developing larvae have high probability of experiencing a predictable environment. Across the North Sea–Baltic Sea transition zone spatially variable but locally predictable environmental conditions suggest that herring from different spawning locations experience stabilizing selection for different salinity tolerance optima. Locally differentiated selective pressures would consequently lead to selection against dispersal between spawning locations of differing salinity conditions, and may provide an adaptive explanation for the homing behavior reported in herring tagging studies (reviewed by McQuinn 1997). Based on estimates of dispersal between spawning locations, levels of gene flow have previously been considered to be substantial and to preclude local adaptation in herring (McQuinn 1997). However, gene flow constrains, but need not preclude, adaptive evolution (e.g., King and Lawson 1995; Calsbeek and Smith 2003; Saint-Laurent et al. 2003; Hendry et al. 2004). Moreover, rates of dispersal between populations cannot be directly translated into realized gene flow, because the reproductive success of dispersers and their offspring is influenced by local selective pressures. The relative fitness returns associated with dispersing versus philopatric behavior are at present unknown in herring, as in most other marine fishes. The present study suggests that salinity conditions on spawning locations affect the fitness associated with different dispersal behaviors. Common garden experiments, such as applied by Billerbeck et al. (2001), could be used to evaluate such effects.

Evidence for a Multispecies North Sea–Baltic Sea Hybrid Zone?

Clines in neutral genetic markers are often associated with ecotone shifts and hybrid zone boundaries (Barton and Hewitt 1985). Our study complements genetic studies in other fishes from the North Sea–Baltic Sea transition zone. Two recent

studies identified genetic clines in the western Baltic in Atlantic cod (Nielsen et al. 2003) and turbot, *Scopthalamus maximus* (Nielsen et al. 2004). In both species, relatively low differentiation was indicated among samples within both the North Sea and the Baltic Sea, and the authors suggested that genetic patterns reflect the presence of a hybrid (interaction) zone between ''pure'' Baltic and North Sea populations. We sampled single spawning components from the Baltic and the North Sea only, and thus were not able to evaluate levels of differentiation within each of these areas. However, two recent microsatellite studies reported, respectively, population structure in Baltic Sea herring (Jørgensen et al. 2005), and limited structure in North Sea autumn and winter spawning components (Mariani et al. in press). Presence of a hybrid zone can be evaluated by examination of linkage disequilibria through the suspected zone of interaction (e.g., Barton 2000). However, the relatively low levels of differentiation detected in most marine fishes including herring using neutral genetic markers requires analysis of large numbers of loci to obtain sufficient statistical power for demonstrating linkage, and was not attempted here. We evaluated spatial differences in genetic variability by estimating allelic richness and found that variability decreased from the North Sea to the Baltic. A similar trend was reported in Nielsen et al.'s studies of cod and turbot and in the aquatic plant *Zostera marina* from the same area (Olsen et al. 2004). Such patterns correspond with founder events following colonization from the North Sea via the Skagerrak when the Baltic Sea was created following glacial retreat about nine thousand years ago. Based on our data it is not possible to determine whether the observed population structure reflects the existence of a hybrid zone where selection acts against hybrids (endogenous selection), or of multiple reproductively isolated spawning components, each exhibiting maximal fitness in native environments (exogenous selection). Whether the former or latter scenario yields better explanatory power has implications to predictions about population fitness curves through the area, and about the temporal stability of the genetic cline (Dasmahapatra et al. 2002). However, such congruity in the geographic positioning of the largest reproductive barrier in herring and the distribution of cline centers reported for both cod and turbot (Nielsen et al. 2003; 2004) (to within a few hundred kilometers) further suggests that spatial genetic variance in these three species is related to selective differences along a common ecological gradient, rather than a mere result of secondary contact between allopatrically differentiated North Sea and Baltic population components, as has been suggested for *Macoma balthica* for example (Luttikhuizen et al. 2003).

Conclusions

We have shown that genetic structure can be maintained in marine fish populations exhibiting substantial mixing during larval and adult life stages. Analyses incorporating genetic, spatial, and environmental parameters indicated that isolating mechanisms are associated with the specific salinity conditions on spawning locations. Our results do not imply a role for linkage between microsatellite DNA loci and traits under selection, but that populations experiencing dissimilar salinity conditions on spawning locations follow different evolutionary trajectories. This shows that the North Sea–Baltic Sea transition zone offers an insightful opportunity for studying local adaptation in ''classical'' marine fishes with continuous distributions, such as herring, cod, and turbot. Little is yet known about selective patterns in species inhabiting the open sea, although it is evident that geographic separation and dispersal potential are poor predictors of the spatial scale of the potential for local adaptation in marine systems. Our approach of combining results from partial Mantel tests with the computational geometric approach offers a promising means of evaluating relationships between barriers to gene flow and environmental variance across marine fishes and ecosystems, as it can be applied both within and across species.

Acknowledgments

The authors are grateful to N. Ryman, L. Laikre, S. Mariani, B. Hutchinson, E. Hatfield, R. Stet, and J. Simmonds for inspiring discussions and comments, to B. Jönsson for assistance in the laboratory, and to the Swedish Meteorological and Hydrological Institute for providing oceanographic data. This work is part of the research project HERGEN (http://www.hull.ac.uk/hergen/) funded by the European Union within the 5th framework program. C. André acknowledges support from the Swedish Research Council, FORMAS.

Literature Cited

Aro, E. 1989. A review of fish migration patterns in the Baltic. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 190:72–96.

Bahri-Sfar, L., C. Lemaire, O. K. Ben Hassine, and F. Bonhomme. 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proc. R. Soc. Lond. Biol. Ser. B 267:929–935.

Barton, N. H. 2002. Estimating multilocus linkage disequilibria. Heredity 84:373–389.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

Billerbeck, J. M., T. E. Lankford, and D. O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates. I. Tradeoffs with swimming performance in *Menidia menidia*. Evolution 55:1863–1872.

Bohonak, A. J. 2002. IBD (Isolation By Distance): a program for analyses of isolation by distance. J. Hered. 93:153–154.

Borsa, P. 2002. Allozyme, mitochondrial-DNA, and morphometric variability indicate cryptic species of anchovy (*Engraulis encrasicolus*). Biol. J. Linn. Soc. 75:261–269.

Calsbeek, R., and T. B. Smith. 2003. Ocean currents mediate evolution in island lizards. Nature 426:552–555.

Carvalho, G. R., and L. Hauser. 1997. Advances in the the molecular analysis of fish population structure. Ital. J. Zool. 65(Suppl.): 21–33.

Castellano, S., and E. Balletto. 2002. Is the partial Mantel test inadequate? Evolution 56:1871–1873.

Dasmahapatra, K. K., M. J. Blum, A. Aiello, S. Hackwell, N. Davies, E. P. Bermingham, and J. Mallet. 2002. Inferences from a rapidly moving hybrid zone. Evolution 56:741–753.

Dragesund, O., A. Johannessen, and O. Ulltang. 1997. Variation in migration and abundance of Norwegian spring spawning herring (*Clupea harengus* L.). Sarsia 82:97–105.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (ver. 2.9.3.2). Available from http://www.unil.ch/izea/software/fstat.html.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories

in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. J. Hered. 89:415–426.
Griffin, F. J., M. C. Pillai, C. A. Vines, J. Kaaria, T. Hibbard-Robbins, R. Yanagimachi, and G. N. Cherr. 1998. Effects of salinity on sperm motility, fertilization, and development in the Pacific herring, *Clupea pallasi*. Biol. Bull. 194:25–35.
Hauser, L., C. Turan, and G. R. Carvalho. 2001. Haplotype frequency distribution and discriminatory power of two mtDNA fragments in a marine pelagic teleost (Atlantic herring, *Clupea harengus*). Heredity 87:621–630.
Hedrick, P. W. 1999. Perspective: highly variable loci and their intepretation in evolution and conservation. Evolution 53: 313–318.
Hendry, A. P., E. B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58: 2319–2331.
Hoarau, G., A. D. Rijnsdorp, H. W. Van der Veer, W. T. Stam, and J. T. Olsen. 2002. Population structure of plaice (*Pleuronectes platessa* L.) in northern Europe: microsatellites revealed large-scale spatial and temporal homogeneity. Mol. Ecol. 11: 1165–1176.
ICES. 1991. Report of the Herring Assessment Working Group for the Area South of 62°N. International Council for the Exploration of the Seas, CM 1991/Assess: 15.
———. 2003. Report of Herring Assessment Working Group for the Area South of 62°N. International Council for the Exploration of the Seas, CM 2003/ACFM: 17.
Iles, T. D., and M. Sinclair. 1982. Atlantic herring: stock discreteness and abundance. Science 215:627–633.
Johannessen, A., and E. Moksness. 1991. Herring larvae (*Clupea harengus*) in the Skagerrak area from December 1987 to April 1988. Fish. Res. 11:155–170.
Johnston, I. A., V. L. A. Vieira, and G. K. Temple. 2001. Functional consequences and population differences in the developmental plasticity of muscle to temperature in Atlantic herring *Clupea harengus*. Mar. Ecol. Prog. Ser. 213:285–300.
Jorde, P. E., and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139:1077–1090.
Jørgensen, H. B. H., M. M. Hansen, D. Bekkevold, D. E. Ruzzante, and V. Loeschcke. 2005. Marine landscapes and population structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol. Ecol. 14:3219–3234.
Jørstad, K. E. 2004. Evidence for two highly differentiated herring groups at Goose Bank in the Barents Sea and the genetic relationship to Pacific herring, *Clupea pallasi*. Environ. Biol. Fishes 69:211–221.
King, R. B., and R. Lawson. 1995. Color-pattern variation in Lake Erie water snakes: the role of gene flow. Evolution 49:885–896.
Knutsen, H., P. E. Jorde, C. Andre, and N. C. Stenseth. 2003. Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol. Ecol. 12:385–394.
Knutsen, H., C. André, P. E. Jorde, M. Skogen, E. Thuroczy, and N. C. Stenseth. 2004. Influx of North Sea cod larvae into the Skagerrak coast. Proc. R. Soc. Lond. Ser. B 271:1337–1344.
Legendre, P., and L. Legendre. 1998. Numerical ecology: developments in environmental modelling, no. 20. Elsevier, Amsterdam.
Lehmann, A., W. Krauss, and H. H. Hinrichsen. 2002. Effects of remote and local atmospheric forcing on circulation and upwelling in the Baltic Sea. Tellus Dyn. Meteorol. Oceanogr. 54A: 299–316.
Luttikhuizen, P. C., J. Drent, and A. J. Baker. 2003. Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol. Ecol. 12:2215–2229.
Manni, F., E. Guérard, and E. Heyer. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by ''Monmonier's algorithm''. Hum. Biol. 76:173–190.
Mariani, S., W. F. Hutchinson, E. M. C. Hatfield, D. E. Ruzzante, E. J. Simmond, T. G. Dahlgren, C. Andre, J. Brigham, E. Torstensen, and G. R. Carvalho. North Sea herring population structure as revealed by microsatellite analysis. Mar. Ecol. Prog. Ser. *In press*.
McQuinn, I. H. 1997. Metapopulations and the Atlantic herring. Rev. Fish Biol. Fish. 7:297–329.
McPherson, A. A., R. L. Stephenson, P. T. O'Reilly, M. W. Jones, and C. T. Taggart. 2001a. Genetic diversity of coastal Northwest Atlantic herring populations: implications for management. J. Fish Biol. 59(Suppl. A):356–370.
McPherson, A. A., P. T. O'Reilly, T. L. McParland, M. W. Jones, and P. Bentzen. 2001b. Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). Mol Ecol. Notes 1:31–32.
McPherson, A. A., P. T. O'Reilly, and C. T. Taggart. 2004. Genetic differentiation, temporal stability, and the absence of isolation by distance among Atlantic herring populations. Trans. Am. Fish. Soc. 133:434–446.
Moksness, E., and P. Fossum. 1991. Distinguishing spring- and autumn spawned herring larvae (*Clupea herengus* L.) by otolith microstructure. ICES J. Mar. Sci. 48:61–66.
Mosegaard, H., and K. P. Madsen. 1996. Discrimination of mixed herring stocks in the North Sea using vertebral counts and otolith microstructure. ICES C.M. 1996/H:17.
Mosegaard, H., L. A. Worsøe, J. Modin, J. Groeger, T. Gröhsler, M. Storr-Paulsen, and M. A. St. John. 2001. EU study report in support of the DG:XIV: a new sampling regime for resource assessment of herring in the Skagerrak, Kattegat, and SW Baltic: final report. 98/026. 2001. DIFRES, EU Study Report, Charlottenlund, Denmark.
Nielsen, E. E., M. M. Hansen, D. E. Ruzzante, D. Meldrup, and P. Grønkjær. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea, revealed by individual admixture analysis. Mol. Ecol. 12:1497–1508.
Nielsen, E. E., P. H. Nielsen, D. Meldrup, and M. M. Hansen. 2004. Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. Mol. Ecol. 13:585–595.
Nissling, A., and L. Westin. 1997. Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. Mar. Ecol Prog. Ser. 152:261–271.
Nissling, A., L. Westin, and O. Hjerne. 2002. Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. ICES J. Mar. Sci. 59:93–108.
Norris, R. D. 2000. Pelagic species diversity, biogeography, and evolution. Paleobiology 26(Suppl. S):236–258.
Ogden, R, and R. S. Thorpe. 2002. Molecular evidence for ecological speciation in tropical habitats. Proc. Natl. Acad. Sci. USA 99:13612–13615.
Olsen, J. B., C. J. Lewis, E. J. Kretschmer, S. L. Wilson, and J. E. Seeb. 2002. Characterization of 14 tetranucleotide microsatellite loci derived from Pacific herring. Mol. Ecol. Notes 2:101–103.
Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham, C. Bostrom, and 12 additional author. 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. Mol. Ecol. 13: 1923–1941.
O'Reilly, P. T., M. F. Canino, K. M. Bailey, P. Bentzen. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. 13:1799–1814.
Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. Ann.
Pogson, G. H., C. T. Taggart, K. A. Mesa, and R. G. Boutilier. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution 55:131–146.
Raufaste, N., and F. Rousset. 2001. Are partial mantel tests adequate? Evolution 55:1703–1705.
Raymond, M., and F. Rousset. 1995. GENEPOP (ver. 1.2): popu-

lation genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.
Rechlin, O. K. W. 2000. Differences in length growth of western Baltic Sea spring spawning herring. Arch. Fish. Mar. Res. 48: 153–160.
Reusch, T. B. H., K. M. Wegner, and M. Kalbe. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. Mol. Ecol. 10:2435–2445.
Rosenberg, R., and L.-E. Palmén. 1982. Composition of herring stocks in the Skagerrak-Kattegat and the relations of these stocks with those of the North Sea and adjacent waters. Fish. Res. 1: 83–104.
Ruzzante, D. E., C. T. Taggart, D. Cook, and S. Goddard. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: Microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. 53:634–645.
Ruzzante, D. E., C. T. Taggart, and D. Cook. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Mol. Ecol. 7:1663–1680.
Ryman, N., U. Lagercrantz, L. Andersson, R. Chakraborty, and R. Rosenberg. 1984. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). Heredity 53:687–704.
Saint-Laurent, R., M. Legault, and L. Bernatchez. 2003. Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). Mol. Ecol. 12:315–330.
Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 2000. Arlequin Ver. 2000. A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Shaw, P. W., C. Turan, J. M. Wright, M. O'Connell, and G. R. Carvalho. 1999. Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity 83: 490–499.
Smith, T. B., R. K. Wayne, D. J. Girman, and M. W. Bruford. 1997. A role for ecotones in generating rainforest biodiversity. Science 276:1855–1857.
Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35:627–632.
Stamatis, C., A. Triantafyllidis, K. A. Moutou, and Z. Mamuris. 2004. Mitochondrial DNA variation in northeast atlantic and mediterranean populations of norway lobster, *Nephrops norvegicus*. Mol. Ecol. 13:1377–1390.
Truett, G. E., P. Heeger, A. A. Mynatt, J. A. Truett, J. A. Walker, and M. L. Warman. 2000. Preparation of PCR-quality mouse genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT). Biotechniques 29:52–54.
Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 10:506–513.
Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high geneflow species. J. Hered. 89: 438–450.
Wares, J. P., S. D. Gaines, and C. W. Cunningham. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. Evolution 55:295–306.
Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.
Wright, S. E. 1943. Isolation by distance. Genetics 28:114–138.
Yamahira, K., and D. O. Conover. 2002. Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83:1252–1262.
Yang, R.-C. 2004. A likelihood-based approach to estimating and testing for isolation by distance. Evolution 58:1829–1845.
Young, F. W. 1996. ViSta: the visual statistics system. Research Memorandum 94-1(b) 2d. ed. L. L. Thurstone Psychometric Laboratory, University of North Carolina. Chapel Hill, NC.
Zardoya, R., R. Castilho, C. Grande, L. Favre-Krey, S. Caetano, S. Marcato, G. Krey, and T. Patarnello. 2004. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. Mol. Ecol. 13:1785–1798.

Corresponding Editor: D. Ayre