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Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea

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Abstract

Numerically small but statistically significant genetic differentiation has been found in many marine fish species despite very large census population sizes and absence of obvious barriers to migrating individuals. Analyses of morphological traits have previously identified local spawning groups of herring (*Clupea harengus* L.) in the environmentally heterogeneous Baltic Sea, whereas allozyme markers have not revealed differentiation. We analysed variation at nine microsatellite loci in 24 samples of spring-spawning herring collected at 11 spawning locations throughout the Baltic Sea. Significant temporal differentiation was observed at two locations, which we ascribe to sympatrically spawning but genetically divergent 'spawning waves'. Significant differentiation was also present on a geographical scale, though pairwise F_{ST} values were generally low, not exceeding 0.027. Partial Mantel tests showed no isolation by geographical distance, but significant associations were observed between genetic differentiation and environmental parameters (salinity and surface temperature) ($0.001 < P \leq 0.099$), though these outcomes were driven mainly by populations in the southwestern Baltic Sea, which also exhibits the steepest environmental gradients. Application of a novel method for detecting barriers to gene flow by combining geographical coordinates and genetic differentiation allowed us to identify two zones of lowered gene flow. These zones were concordant with the separation of the Baltic Sea into major basins, with environmental gradients and with differences in migration behaviour. We suggest that similar use of landscape genetics approaches may increase the understanding of the biological significance of genetic differentiation in other marine fishes.

Keywords: gene flow barriers, isolation by distance, landscape genetics, local adaptation, member-vagrant hypothesis, microsatellite DNA

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Introduction

Although the genetic structure among populations represents the outcome of the interaction among gene flow, genetic drift, selection and mutation, the relative importance of each of these factors may often be difficult to disentangle (Balloux & Lugon-Moulin 2002). For many species, their geographical distribution exceeds the distances that can be covered by any single dispersing individual, a characteristic that often leads to a pattern of isolation by distance (Wright 1943), i.e. a correlation between genetic and geographical distance between populations. However, in some organisms, empirical findings of genetic differentiation may be difficult to reconcile with knowledge on the biology of the species.

For most marine organisms, including marine fishes, the mechanisms affecting genetic structure still remain largely unknown. Comparative studies of different species distributed over the same geographical regions have revealed strong genetic differentiation for some species, but weak genetic differentiation for others, a result that has often been attributed to different dispersal abilities (e.g. Bernardi *et al*. 2003). Most highly abundant and widely dispersed

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marine fishes including heavily exploited species like Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*) exhibit weak genetic differentiation, with typically very low F_{ST} values (< 0.01; Ward *et al*. 1994). Yet, several studies have revealed that these numerically low levels of genetic differentiation often are statistically significant (e.g. cod: Ruzzante *et al*. 1998; Knutsen *et al*. 2003; herring: McPherson *et al*. 2001a). Moreover, a study of cod populations based on historical samples (DNA extracted from otoliths) revealed that the patterns of genetic differentiation are temporally stable on the scale of several decades (Ruzzante *et al*. 2001).

What are the factors shaping the weak, but statistically significant population structure observed in many marine species? Highly abundant and widely distributed marine fishes share three common features that would a priori be expected to preclude genetic differentiation: (i) very large spawning populations, experiencing little or no detectable genetic drift; (ii) absence of obvious migration barriers in the marine environment; and (iii) the presence of highly dispersive and/or migratory life stages, which should lead to high rates of gene flow among populations (Ward *et al*. 1994). The assumption that drift is negligible due to large census spawning population sizes translating into large genetically effective population sizes has recently been challenged by studies suggesting that effective population sizes in marine fishes may be several orders of magnitude lower than census population sizes (e.g. Hauser *et al*. 2002; Turner *et al*. 2002). Moreover, natal homing and/or gyre-like oceanographic features that will keep eggs and larvae of spawning fish in retention areas close to their spawning sites have been suggested as mechanisms leading to partial reproductive isolation among populations (the member-vagrant hypothesis; Iles & Sinclair 1982), and the importance of ocean currents in shaping the genetic structure of cod populations has recently been demonstrated (Knutsen *et al*. 2004). The possibility of natural selection (and possible local adaptation) has recently been addressed more rigorously (reviewed by Guinand *et al*. 2004). In addition, Nielsen *et al*. (2003, 2004) demonstrated low genetic differentiation among populations of cod and turbot (*Scophthalmus maximus*) within the North and Baltic seas, respectively, but a steep increase of genetic differentiation in the transition zone between the two areas. This transition zone is characterized by a sharp increase in salinity from the Baltic Sea to the North Sea, and the results for both species suggest the presence of hybrid zones maintained by natural selection.

One way to obtain further insight into the factors shaping the genetic structure of populations is to combine genetic data with information on landscape characteristics, referred to as *landscape genetics* (Manel *et al*. 2003), partly analogous to the well established discipline of landscape ecology, which aims to study how landscape structure affects the distribution and abundance of species (Turner 1989). Landscape genetics approaches have previously yielded new insights into dispersal patterns and population contingency in terrestrial organisms (e.g. Piertney *et al*. 1998) and freshwater fishes (e.g. Angers *et al*. 1999; Durand *et al*. 1999).

Landscape features can be subdivided into two categories, i.e. *quantitative* factors, such as temperature and humidity, and *qualitative* factors, such as geomorphological barriers to migration (e.g. mountain ranges). In marine environments, landscapes are characterized by quantitative factors such as, e.g. salinity and temperature, and qualitative factors, represented by gyres and other oceanographic attributes. Even though oceanographic features and environmental variables have previously been invoked to explain observed patterns of differentiation in marine fishes (e.g. Ruzzante *et al*. 1998; Nielsen *et al*. 2003, 2004), specific statistical analyses have rarely been conducted to examine their relationship rigorously.

Here, we use landscape genetics approaches to investigate the genetic structure of spring-spawning herring in the Baltic Sea, one of the largest brackish water bodies in the world with a heterogeneous ecosystem divided into several subunits consisting of deep basins partially separated by sills. Surface temperature decreases towards the northwest, and inflow of saline water from the North Sea and freshwater from large rivers creates a strong salinity gradient (Ojaveer & Elken 1997; see also Fig. 1). Thus, the Baltic Sea offers a heterogenous landscape where the genetic structure of populations could be affected by both qualitative and quantitative features (Gabrielsen *et al*. 2002). Atlantic herring is found throughout the Baltic Sea where it exhibits considerable morphological (e.g. vertebrae counts) and ecological (e.g. spawning time differences and migration patterns) variation (e.g. Aro 1989; Parmanne *et al*. 1994). Herring in the Baltic Sea would therefore seem a well-suited system for testing hypotheses about correlations between genetic distance and geographical and environmental variables. In the present study we investigate whether geographical distance (isolation by distance), quantitative landscape features (salinity and temperature at spawning time) or qualitative landscape features (structuring of the Baltic Sea into basins and gyres) individually or in combination can explain observed genetic differentiation in herring.

Materials and methods

Samples studied

The sampling design applied in the present study was based on the previously recognized local spawning groups of spring-spawning herring described by Ojaveer & Elken (1997), and it included all the main subunits of the Baltic Sea. Most of the samples consisted of spawning adults

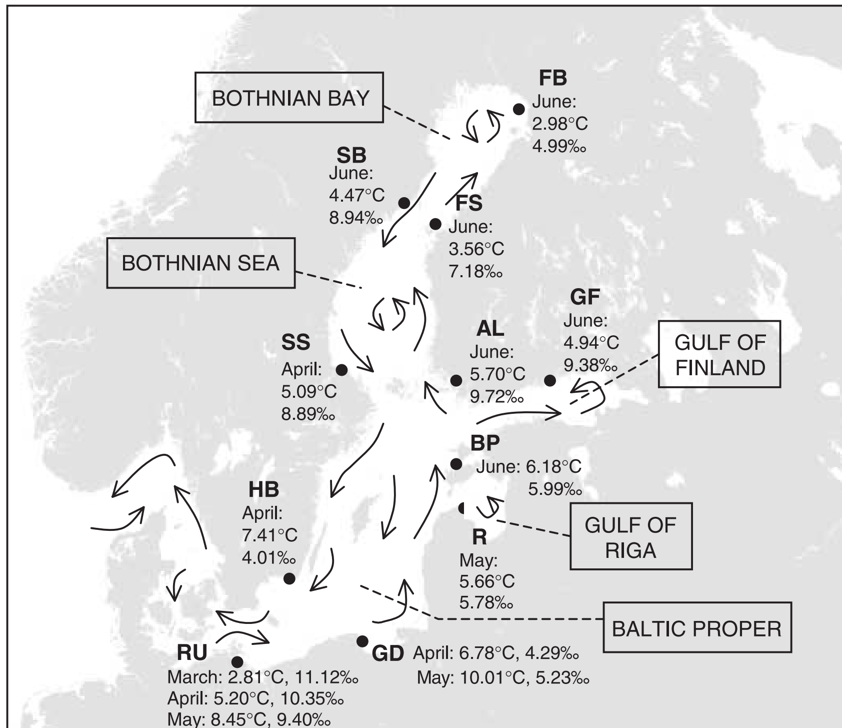

Fig. 1 Sampling locations. Names of sampling locations and exact coordinates are shown in Table 1. Important oceanic features are indicated in the figure, including gyres and mean temperature and salinity at the sampled localities during the month of peak spawning activity. Names of the main basins are given in text boxes.

(except that of the Baltic Proper collected in 2002, BP02, which consisted of nonspawning adults). The samples are listed in Table 1 and their locations are illustrated in Fig. 1. Important oceanographic features including gyres, mean salinity and temperature at the sampled localities during spawning time are also indicated in Fig. 1. It should be noted that even though temperature *decreases* from south to north, the temperatures during spawning time tend to *increase* with latitude, due to a generally later spawning season in northern populations (June) as compared to southern populations (March–May; see Table 1). At five spawning locations [Bothnian Bay, Swedish side (SB), the Åland Archipelago (AL), the Gulf of Riga (R), the Gdańsk Bay (GD) and Rügen (RU)], samples were collected in 2002 and 2003 to assess the temporal stability of the genetic population structure among spawning groups. In addition, three temporal within-season samples were taken during the spawning seasons of 2002 and 2003 (from March to May) at the spawning locations GD and RU. This was done to assess whether or not allele frequency distributions at these locations differed between early- and late-spawning herring (testing for the presence of 'spawning waves'). These latter results are reported in detail in Jørgensen *et al*. (2005).

Microsatellite analyses

DNA was extracted from gill tissue stored in 96% ethanol using either the Chelex–proteinase K protocol by Estoup *et al*. (1996), the Hot Sodium Hydroxide and Tris (HotSHOT) protocol by Truett *et al*. (2000) or the DNeasy Tissue Kit 250 (QIAGEN). Variation was analysed at nine microsatellite loci: Cha1017, Cha1020, Cha1027, Cha1202 (McPherson *et al*. 2001b), Cpa101, Cpa107, Cpa111, Cpa112 and Cpa114 (Olsen *et al*. 2002). The loci were polymerase chain reaction (PCR) amplified individually using standard reagents and their annealing temperatures ranged from 54 to 60 °C for the different loci (exact protocols are available from the author for correspondence upon request). The amplified microsatellite loci were analysed on a BaseStation 51™ DNA fragment analyser (MJ Research) and gels were semi-automatically typed using the software CARTOGRAPHER 1.2.6 (MJ Geneworks, Inc.). A number of measures were taken to eliminate genotyping errors (Bonin *et al*. 2004). To improve typing consistency, two standard individuals were included in each gel run. Between 10% and 20% of the individuals from each sample, depending on the marker, were analysed twice to assure consistency of results. Furthermore, several gels were typed by two persons independently or twice by one person (first and second typing separated by weeks to months) to ensure consistency and agreement with the protocol. The software MICRO-CHECKER 2.2.0 (van Oosterhout *et al*. 2004) was used to test for technical artefacts such as null alleles, stuttering and large allele dropout.

Statistical analyses

Tests for conformity to Hardy–Weinberg expectations were conducted using the procedure by Guo & Thompson

Table 1 Samples of spring-spawning herring from the Baltic Sea used in the present study. Some of the original samples were pooled into the 15 samples indicated in the left-most column

	Sample	Sampling time	Sampling location	Sample size
1	Bothnian Bay; Finnish side (FB02)	June 2002	65.20′N, 24.36′E	66
			65.20′N, 25.18′E	34
2	Bothnian Bay; Swedish side (SB02-03)	June 2002	63.38′N, 20.25′E	116
		June 2003	63.38′N, 20.15′E	115
3	Bothnian Sea; Finnish side (FS02)	June 2002	63.07′N, 21.23′E	100
4	Bothnian Sea; Swedish side (SS02)	April 2002	60.47′N, 17.48′E	100
5	Gulf of Finland (GF02)	June 2002	60.22′N, 26.11′E	50
			60.16′N, 25.39′E	50
6	Åland Archipelago (AL02)	June 2002	60.20′N, 22.10′E	115
7	Åland Archipelago (AL03)	June 2003	60.20′N, 22.10′E	109
8	Baltic Proper (BP02)	June 2002	ICES sq. 47H3	100
	Gulf of Riga (R02-03)	May 2002	ICES sq. 44H2	76
		May 2003		109
10	Hanö Bay (HB02)	April 2002	55.57′N, 15.18′E	100
11	Rügen (RU0302)	March 2002	54.14′N, 13.27′E	100
12	Rügen (RU0402)	April 2002	54.11′N, 13.26′E	100
13	Rügen (RU0502)	May 2002	54.14′N, 13.26′E	100
14	Rügen (RU03)	March 2003	54.14′N, 13.27′E	100
		April 2003	54.11′N, 13.26′E	100
		May 2003	54.11′N, 13.26′E	100
15	Gdańsk Bay (GD02-03)	April 2002	54.28′N, 18.35′E	100
		May 2002	54.27′N, 18.34′E	100
		May 2002	54.27′N, 18.34′E	100
		April 2003	54.26′N, 18.38′E	100
		April 2003	54.28′N, 18.35′E	100
		May 2003	54.28′N, 18.35′E	100

(1992) implemented in GENEPOP 3.3 (Raymond & Rousset 1995). Furthermore, randomization tests (4320 randomizations) for significance of F_{IS} (reflecting heterozygote deficiency/excess) were conducted using FSTAT 2.9.3 (Goudet 1995). For estimating the proportion of genetic differentiation distributed among spawning groups and among temporal samples within spawning groups, we used a hierarchical analysis of molecular variance (AMOVA) implemented in the ARLEQUIN 2.000 software (Schneider *et al*. 2000). The AMOVA included the temporal (2002 and 2003) samples from SB, AL and R. In addition, it included three samples from 2002 and three samples from 2003 from RU and GD, respectively. Furthermore, pairwise genetic differentiation between samples was analysed by Weir & Cockerham's (1984) θ, an unbiased estimator of F_{ST}, and by exact pairwise tests (Goudet *et al*. 1996) using FSTAT 2.9.3 (Goudet 1995). Multidimensional scaling analysis, based on pairwise θ values, was used to visualize the genetic relationships among samples. This analysis was conducted using VISTA 5.6.3 (Young 1996).

To test for correlation between geographical, genetic and environmental distances among samples, we performed partial Mantel tests (Legendre & Legendre 1998) using the program IBD 1.5 (Bohonak 2002). Each test was based on 10 000 randomizations. Pairwise θ values were used to represent genetic distances. The geographical distance input matrix contained the shortest waterway distances between sampling locations, estimated using the GIS program ARCMAP 8.3 (supplied by ESRI®). Genetic and geographical distances were log transformed. Environmental distances were estimated for salinity and surface temperature (upper 10 m) as average differences in minimum, maximum and mean values. Salinity and temperature data were extracted from the International Council for the Exploration of the Sea (ICES) database for the month of peak spawning activity. This period was chosen as we were interested in analysing environmental parameters associated with the spawning, egg and first larval stage, spanning *c*. 1 month. The data represent locations situated as close to the spawning grounds as possible. Additional information on the environmental data is presented in Appendix II.

The program BARRIER 2.2 (Manni *et al*. 2004) was used to highlight geographical areas with pronounced genetic discontinuity between samples, i.e. barriers. Geographical coordinates were supplied for each sample and connected by Delauney triangulation such that each connection has an associated distance (in this case the genetic distance θ described above). Using Monmonier's maximum distance algorithm, barriers were then identified. In short, this

algorithm chooses the edge with the largest associated distance in the triangulation network as its starting edge and then extends the barrier across the directly adjacent edge associated with the largest genetic distance. Additional sections are added to the barrier by such steps until the barrier reaches the outer edge of the network or meets another barrier (see Manni *et al*. (2004) for further details). To ensure that the barriers were not identified due to strong differentiation at only one or few loci, we conducted the analyses both using θ values for each of the nine loci separately and using θ values based on all nine microsatellite loci. In the former case, BARRIER determines 'consensus barriers', i.e. it defines barriers based on a majority rule and it is indicated how many loci support a given section of a barrier. The number of barriers to be identified is set by the user, and based on the number of loci supporting each barrier we found that only three barriers were strongly supported by the data.

Results

In 19 of the 24 initial samples, complete genotypes were scored in > 90% of the individuals. In the remaining five samples (SB02, AL02, R02, RU0402, RU0503), the number of complete genotypes was 77.6 to 89%. The percentage of individuals with missing data was between 0 and 7.9 per sample and microsatellite marker (Appendix I). There was no detectable difference in data quality among samples and the sample sizes were sufficiently large that the missing data were not expected to significantly influence the final results regarding genetic structuring among spawning groups of herring.

Ten out of 432 randomization tests for heterozygote deficiency/excess resulted in significant outcomes (Appendix I). The seven cases of significant positive F_{IS} values that could potentially reflect null alleles were not limited to particular loci or populations (Appendix I), and MICRO-CHECKER (van Oosterhout *et al*. 2004) did not detect problems that could reflect stuttering and large allele dropout (results not shown). Hardy–Weinberg expectations were met in all but 11 of 192 tests after pooling samples that were not significantly genetically differentiated and after Dunn–Šidák adjusting (Sokal & Rohlf 1995) the significance level with $k = 9$ (the number of microsatellite loci).

Temporal stability and genetic structure

The hierarchical AMOVA provided estimates of genetic variance among sampling locations (F_{CT}) and among temporal samples within location (F_{SC}). The variance among locations contributed significantly to the overall genetic variance ($F_{CT} = 0.42\%$; $P < 0.001$) as did variance among temporal samples ($F_{SC} = 0.23\%$; $P < 0.001$). The overall significant differentiation between temporal samples was caused mainly by the significant differentiation between the two Åland (AL) samples ($P = 0.002$) and between RU0302 and the other two Rügen (RU) 2002 samples ($P = 0.022$ and 0.048). The significant differentiation led us to keep AL samples and RU 2002 samples separate during the remaining analyses. Temporal samples were pooled when differentiation between them was nonsignificant. This resulted in a total of 15 samples that were used for all following analyses (see Table 1 for an overview of samples).

Theta estimates between population pairs that included either RU or Gdańsk (GD) samples were mostly significant, whereas many nonsignificant outcomes (after Dunn–Šidák correction) were observed among the remaining samples (Table 2). However, the GD sample differed less from locations farther north in the Baltic compared to RU samples, and GD showed particularly low differentiation to the neighbouring Hanö Bay (HB) and Gulf of Riga (R) samples. Differentiation between RU and GD samples was significant, albeit low. RU0302, the earliest sample (March) of spawning herring at Rügen in 2002, was the sample showing the strongest differentiation from other samples with pairwise θ values ranging from 0.0145 to 0.0271 (Table 2). The possible effect of the large GD sample size was tested by restricting sample size to $n = 100$ (randomly chosen individuals) for GD and estimating pairwise θ values. The results changed very little (results not shown) and the original data were therefore used in the following tests.

Isolation by distance

Partial Mantel tests showed that the correlation between geographical and genetic distances among the spawning locations was nonsignificant, suggesting no isolation by distance among the spawning groups (Table 3). However, correlations between genetic and environmental distances (differences in salinity and temperature between sampling locations) were significant in all tests, whether or not they were controlled for geographical distance (Table 3). The significances were mainly driven by the Rügen (RU) samples. When the most divergent sample (RU0302) was omitted, the correlation between genetic distance and salinity remained significant, whereas the correlation between genetic distance and temperature was not significant. When all RU samples were omitted, all correlations became nonsignificant (data not shown).

Areas of lowered gene flow

The multidimensional scaling plot based on pairwise θ values (Fig. 2) shows the relatively large differentiation among temporal samples from Rügen (RU), which were separated along dimension 2 (explaining 11.8% of variance). Along dimension 1 (explaining 63% of variance), there was a gradient of differentiation from RU to GD and to the

Table 2 Pairwise θ (Weir & Cockerham 1984) estimates calculated using FSTAT (Goudet 1995). Numbers in bold italics indicate statistically significant genetic differentiation ($r < 0.05$) between sample pairs using the test by Goudet *et al*. (1996). Significance levels were adjusted using Dunn-Šidák correction ($k = 120$). Asterisks indicate tests that were significant before Dunn-Šidák adjustment

	SB02-03	FS02	SS02	GF02	AL02	AL03	BP02	R02-03	HB02	RU0302	RU0402	RU0502	RU03	GD02-03
FB02	0.0008	0.0044	0.0017	0.0012	0.0012	0.0001	***0.0033***	0.0011	0.0007*	***0.0193***	***0.0112***	***0.0102***	***0.0095***	***0.0022***
SB02-03	—	0.0024	0.0011	–0.0002	0.0006*	0.0010	0.0014*	0.0011	0.0011*	***0.0227***	***0.0158***	***0.0090***	***0.0114***	***0.0026***
FS02		—	0.0054	0.0054*	0.0024	0.0062*	0.0038*	0.0051	0.0058*	***0.0271***	***0.0246***	***0.0153***	***0.0166***	***0.0083***
SS02			—	0.0002	0.0014	0.0010*	***0.0052***	0.0042*	0.0021*	***0.0259***	***0.0168***	***0.0099***	***0.0143***	***0.0046***
GF02				—	0.0014*	0.0000	0.0029*	0.0012	0.0018*	***0.0252***	***0.0168***	***0.0101***	***0.0125***	0.003*
AL02					—	0.0033*	0.0018*	0.0024*	0.0031*	***0.0235***	***0.0175***	***0.0102***	***0.0127***	***0.0046***
AL03						—	0.0027	0.0018	0.0003	***0.0218***	***0.0133***	***0.0090***	***0.0115***	***0.0022***
BP02							—	0.0005	***0.0033***	***0.0181***	***0.0152***	***0.0088***	***0.0101***	***0.0033***
R02-03									0.0011*	***0.0145***	***0.0108***	***0.0082***	***0.0074***	0.0009
HB02									—	***0.0175***	***0.0078***	***0.0063***	***0.0069***	0.0007*
RU0302										—	0.0064*	***0.0113***	***0.0060***	***0.0113***
RU0402											—	0.0049*	***0.0040***	***0.0062***
RU0502												—	***0.0036***	***0.0032***
RU03													—	**0.0032**

northeastern samples, whereas GD and samples further to the north in the Baltic Sea were separated mainly along dimension 2. The latter group of samples showed some subdivision with the Åland (AL), Bothnian Bay, Swedish side (SB), Bothnian Sea, Swedish side (SS), Gulf of Finland (GF) and Bothnian Sea, Finnish side (FS) samples appearing relatively distinct from the Gulf of Riga (R), Baltic Proper (BP), Gdańsk Bay (GD), Hanö Bay (HB) and Rügen (RU) samples. This group of samples, along with the Bothnian Bay, Finnish side (FB) sample, is from the northernmost part of the Baltic Sea (see Fig. 1). Inspection of scatter plots involving all other dimensions from 1 to 4 did not reveal further obvious patterns of differentiation (data not shown).

Based on pairwise θ values, the BARRIER analyses identified three areas of relatively sharp change in genetic composition. The first barrier, supported by five loci, separated RU0302 (Rügen March 2002) from all other samples (i.e. distance matrices for five of the nine loci lead to this barrier in the analysis of 'consensus' barriers). The second barrier, supported by five to nine loci over different sections of the barrier, separated the other RU samples from all other geographical samples. The third barrier separated the southern samples associated with the Baltic Proper (GD, HB, R, BP) from locations further north and west in basins with their own circulation systems and environmental regimes with respect to salinity and temperature and separated from the Baltic Proper (GF, AL, SS, FS, SB, FB) by sills. This barrier was supported by four to six loci. Allowing BARRIER to identify more genetic breaks resulted in barriers supported by only one to three loci, and we did not consider these results further. The geographical positions of the three identified barriers are shown in Fig. 3, but since the two barriers involving RU samples virtually coincide geographically, only two barriers are shown (i) separating RU samples from all other samples and (ii) separating Baltic Proper samples from northern samples.

Finally, to conduct an a posteriori analysis of the genetic structure of the groups of samples, we conducted a hierarchical AMOVA estimating the distribution of genetic variance within and among the groups of populations identified by BARRIER. Differentiation among populations within groups, F_{SC} was 0.19% ($P < 0.001$), whereas differentiation among groups, F_{CT} was 0.53% ($P < 0.001$). Thus, the zones of lowered gene flow identified by BARRIER did correspond to assemblages of populations exhibiting higher genetic differentiation among groups than within groups, though at the same time populations within groups did not constitute panmictic units.

Discussion

Based on our results we clearly reject the hypothesis that herring in the Baltic Sea constitutes one panmictic unit.

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Table 3 The standardized test statistic, *r*, for Mantel and partial Mantel tests for correlation between genetic, geographical, and indicator (salinity or temperature) distances between spring-spawning groups of herring in the Baltic Sea. The genetic distance was estimated as Weir & Cockerham's (1984) θ. Both genetic and geographical distances were log transformed before Mantel tests. Significant correlations are shown in bold italics

	Salinity						Temperature					
	Max		Mean		Min		Max		Mean		Min	
Distances	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Genetic and indicator	0.682	***< 0.001***	0.370	***0.007***	0.584	***< 0.001***	0.274	***0.031***	0.246	***0.027***	0.141	0.094
controlling for geographical	0.709	***< 0.001***	0.392	***0.010***	0.616	***< 0.001***	0.274	***0.031***	0.247	***0.028***	0.142	0.099
Genetic and geographical			–0.002	0.441					–0.002	0.448		
controlling for indicator	–0.265	0.980	–0.140	0.833	–0.241	0.968	–0.001	0.447	–0.011	0.477	–0.014	0.481

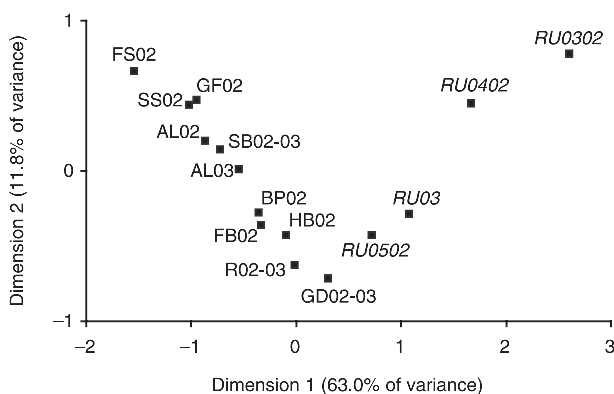

Fig. 2 Multidimensional scaling (MDS) plot based on the matrix of pairwise θ (Weir and Cockerham) values between samples of Baltic Sea herring. Temporal samples from the Rügen (RU) location are denoted by labels in italics.

Previous studies employing allozyme and mitochondrial DNA markers have found significant differentiation among herring populations inhabiting relatively closed marine areas such as fjords (e.g. Jörstad *et al*. 1991; Turan *et al*. 1998), whereas no significant genetic differentiation was found between herring populations from open sea areas, including the Baltic Sea (e.g. Ryman *et al*. 1984; Turan *et al*. 1998). More recently, studies employing microsatellite DNA markers have revealed statistically significant albeit low differentiation among spawning groups from open marine areas (e.g. Shaw *et al*. 1999; McPherson *et al*. 2004), and our results are in accordance with these studies. However, beyond the general finding that genetic differentiation is present among Baltic Sea herring spawning groups, interpretation of the results is complicated. First, temporal genetic differentiation was observed at some locations. Second, genetic differentiation was not correlated with geographical distance between populations. On the other hand, genetic differentiation was significantly correlated with differences in salinity and water temperature, but this correlation primarily reflected the genetic divergence of Rügen (RU) samples in the southwestern Baltic Sea, where salinity and temperature gradients were steepest. Third, we identified two geographical zones that appeared to act as barriers to gene flow, and these zones corresponded to the transition zone in the southwestern Baltic and the separation of the central and northern parts of the Baltic Sea into separate basins. In the following, we first discuss the temporal genetic differentiation observed at some locations. Next, we discuss how Baltic herring populations are structured geographically and by environmental variables. Finally, we consider if the results support previous models suggested to account for the genetic structure of herring and other marine fishes and highlight the implications for management of this commercially important species.

Temporal genetic differentiation

Even though temporal stability of genetic composition of populations was suggested for most sampled locations, temporal genetic differentiation was observed at the locations Rügen (RU) and Åland Archipelago (AL). We attribute this finding to sampling of 'spawning waves', i.e. genetically differentiated spawning groups that spawn at the same locations but at different times, though with some overlap.

The presence of spawning waves in Atlantic herring has originally been suggested on the basis of differences in morphology, growth rate and parasite infestations (e.g. Lambert 1987; Podolska & Horbowy 2002). More recently, McPherson *et al*. (2003) and Jørgensen *et al*. (2005) found corresponding patterns of genetic differentiation among successive samples of spawning adults using microsatellite markers. In the case of the Baltic Sea, Jørgensen *et al*. (2005) found a clear pattern of genetic differentiation between herring spawning at the RU location in March 2002 (RU0302) and May 2002 (RU0502), with the April sample (RU0402) showing intermediate genetic relationships

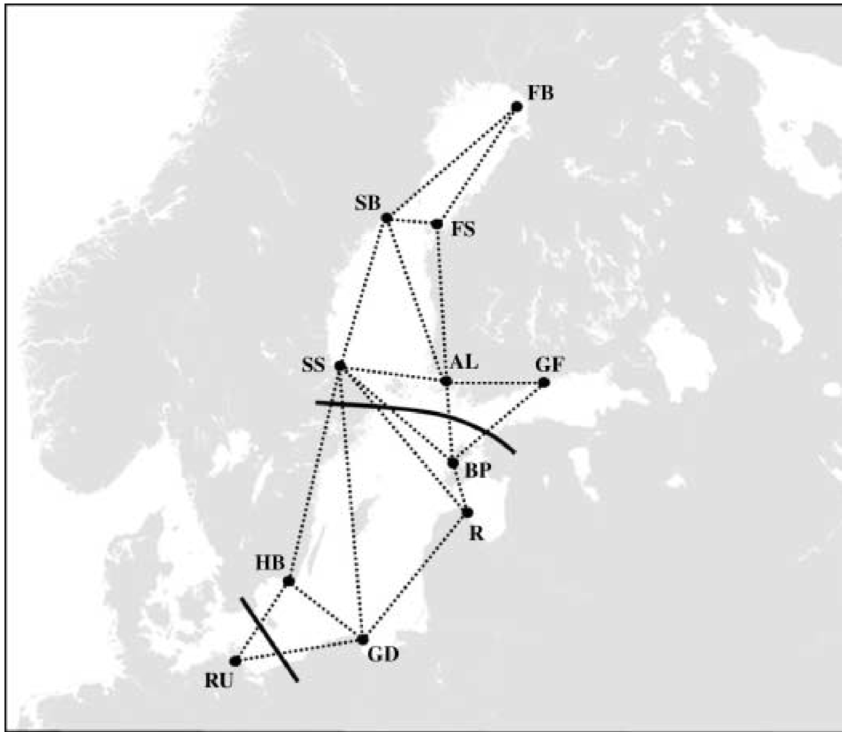

Fig. 3 Areas of limited gene flow as identified by BARRIER using the Monmonier algorithm (Manni *et al*. 2004). Dotted lines: Delaunay triangulation. Full lines: Main barriers to gene flow.

to the March and May samples. However, these relationships could not be recovered in year 2003 samples, possibly because the spawning group represented by the RU0302 sample in 2002 had already spawned at the time of sampling in 2003. The genetic differences observed between the 2002 and 2003 samples of spawning adults in the Åland Archipelago may also result from the sampling of different spawning waves in the two years. Up to four spawning waves per season have been identified at the AL spawning location based on observed egg deposits (Rajasilta *et al*. 1993). The presence of spawning waves significantly complicates interpretation of genetic differentiation in herring. It is noteworthy that the sample showing the highest differentiation to other samples in the study was RU0302, assumed to represent a genetically divergent spawning component at the Rügen location. Clearly, such patterns of within-season temporal differentiation must be taken into account when making inferences about the stability or lack thereof of the genetic structure of herring in the Baltic Sea and possibly elsewhere (e.g. McPherson *et al*. 2003). For instance, estimating effective population size using the temporal method (Waples 1989) could lead to erroneously low estimates if two different spawning waves were analysed in different years.

Geographical differentiation

We found no evidence of isolation by geographical distance, a result in accordance with findings by McPherson *et al*. (2004). We found a significant isolation by salinity and surface temperature measured at the spawning locations during the month of peak spawning and this suggests that environmental divergence has a larger impact on genetic divergence than mere geographical distance. In this context, it should be noted that some controversy exists as to the application of partial Mantel correlation analysis in ecological studies (Raufaste & Rousset 2001) but this issue must as yet be considered unresolved (Castellano & Balletto 2002). We therefore decided to apply the method, keeping in mind that results should be interpreted with caution. Whereas our results initially could be interpreted as structure reflecting differing selective pressures associated with salinity and temperature, the significant correlation between genetic distance and environmental variables depended entirely on the Rügen (RU) samples, and the correlations became nonsignificant when RU samples were omitted from the analyses. Within the geographical area studied, the steepest temperature and salinity gradients are found in the western Baltic Sea, which includes the RU sample localities. Exactly this area has been found to be the centre of hybrid zones between North Sea and Baltic Sea populations of both Atlantic cod and turbot (Nielsen *et al*. 2003, 2004). It is possible that Atlantic herring exhibits a hybrid zone across the same geographical region and that Rügen herring is situated near the centre of this zone, even though this must be confirmed by further analysis of samples from the North Sea and the Kattegat. In any case, the results suggest that environmental gradients have a strong structuring effect on herring populations in the western

Baltic region, and it is possible that populations are adapted to local conditions.

Populations east and north of Rügen also showed no isolation by distance, and neither did genetic distance correlate with temperature and salinity. We argue, however, that the lack of isolation by distance does not mean that geographically explicit population structure was absent. First, the MDS (multidimensional scaling) plot (Fig. 2) did suggest a grouping of samples according to geographical position, with Bothnian and Gulf of Finland populations (SS, FS, GF, AL and SB) forming one group, and Baltic Proper-associated populations (BP, HB, R and GD) forming another group, with the northernmost population FB being the only deviation from this trend. This pattern was further confirmed when geographical coordinates and genetic differentiation were considered together using the Monmonier algorithm employed in the BARRIER analysis. Two main areas of lowered gene flow were identified, leaving three groups of spawning locations: Rügen, the southern spawning locations associated with the Baltic Proper, and northern and western spawning locations in basins separated from the Baltic Proper by sills (see Fig. 3). It is important that samples analysed in BARRIER are not irregularly spaced because barriers would then tend to fall between the most distantly located samples, and the pattern identified by BARRIER could not be distinguished from isolation by distance. Such problems were not detected in this study where barriers were identified between relatively closely located samples (Fig. 3). Hence, we conclude that geographical structure is present among Baltic Sea herring, but it is characterized by areas of low gene flow rather than isolation by distance.

One way to evaluate the biological significance of the BARRIER analyses would be to construct barriers based on environmental 'distances' rather than on genetic distances and assess the concordance between environmental and genetic barriers. Basing the BARRIER analyses on salinity and temperature 'distances' among sampling locations, the most robust barrier is placed just east of HB (results not shown). The contrast between this environmental barrier and the genetic barrier between RU and HB (just west of HB) suggests that the different migration patterns (RU herring migrate into Danish waters to feed while HB herring stay in the Bornholm Basin (Aro 1989) and a high degree of homing of the two spawning groups lead to some degree of reproductive isolation between RU and HB herring. This barrier therefore seems to reflect behavioural differences in the adult stage rather than environmental differences on the spawning grounds. A second robust barrier is placed south of AL, coinciding with the area of lowered gene flow between southern and northwestern groups of samples. Considering only salinity, there is an additional barrier between FS and AL. The coinciding environmental and genetic barriers between northwestern and southern samples also mark an area of change between different hydrological regimes. Topographical and/or hydrographical constraints facilitate individual circulation systems in the basins of the Baltic Sea (Ojaveer & Elken 1997) and north of the identified barrier one large gyre covers the Bothnian Sea and another one the Bothnian Bay (Kullenberg 1981). Such gyres have the potential to facilitate larval retention relatively close to spawning grounds (Iles & Sinclair 1982). This indicates that genetic differentiation may arise, at least partly, by the combined adaptation of eggs and early larval stages to local environmental conditions on the spawning and nursery grounds and retention due to hydrological features. Furthermore, spawning groups of the southern Baltic use overlapping feeding grounds, whereas spawning groups from the northern areas of the Baltic Sea, i.e. the Gulf of Finland, the Bothnian Sea and the Bothnian Bay only migrate within their natal basin (Aro 1989).

Several other abiotic (e.g. O_2 content) and biotic factors (e.g. density and species composition of macroalgae as substrate on the spawning grounds and prey organisms on the nursery grounds) are of importance to the herring (Blaxter & Hunter 1982; Klinkhardt 1996) and could thus be viewed as important components of the marine landscape. A depletion of these factors may lead to an increased mixing of spawning groups competing for resources but it was beyond the scope of this study to include them here.

To sum up, herring populations in the Baltic Sea are not structured according to an isolation-by-distance model. However, geographical structure is clearly present and is characterized by zones of low gene flow as identified by BARRIER, and these zones agree very well with areas of change in salinity and temperature during the month of spawning, with migratory patterns for adults and with hydrographic features. Hence, the results suggest that it is *possible* for local adaptations to occur. Popiel (1984), Parmanne (1990) and others have previously found spawning groups of herring in Scandinavian waters to be characterized by differences in growth rate, morphometric and meristic traits as well as in otolith microstructure. It should, however, also be noted that salinity and temperature during early ontogeny influence at least two of the meristic traits typically used to discriminate herring populations, i.e. the number of vertebrae and pectoral fin rays (Parmanne 1990; Hognestad 1995; Hulme 1995). Thus, whether the morphometric and meristic differences observed are purely environmental in origin or are partially inherited, can best be ascertained by, e.g. common garden experiments (Conover 1998).

Models of genetic structure and implications for management

A number of different models have been suggested to account for population structure in herring, the most

prominent of which are those by Iles & Sinclair (1982) and McQuinn (1997). Iles & Sinclair's (1982) member-vagrant hypothesis (in the following abbreviated MV) assumes that herring populations exist as temporally stable units maintained by both philopatry and oceanic features such as gyres facilitating retention of larvae close to the spawning grounds. This model stresses reproductive isolation between populations, and emigrants from a population are essentially considered lost. McQuinn (1997) on the other hand suggested the adopted migrant hypothesis (AM) that is based on a metapopulation structure involving extinction and recolonization dynamics. The model assumes high levels of gene flow among populations leading to no genetic differentiation because there is no survival disadvantage to hybrids. A central feature of the AM model is 'adoption' of immigrants from other populations into the new population and it assumes homing, though not necessarily *natal* homing, as homing can occur by socially transmitted 'spatial learning'.

The major differences between the predictions of the models are (i) temporal stability (MV) vs. temporal instability (AM), (ii) low levels of gene flow (MV) vs. high levels of gene flow (AM) and (iii) correspondence of genetic structure with environmental and oceanographic features (MV) vs. isolation by distance (AM). Although our results indicate short-term temporal stability of genetic composition of populations, except in cases where spawning waves are invoked, we are unable to evaluate the long-term temporal stability of genetic structure from the present data set. This issue can be addressed by analysis of historical samples (Nielsen *et al*. 1997, 1999). Our results show low genetic structuring, which could suggest high levels of gene flow, especially among spawning groups with overlapping feeding areas, thereby supporting the AM hypothesis. However, the lack of isolation by distance and the finding of two zones of limited gene flow coinciding with oceanographic and environmental features support the MV model. Therefore, our data support a suggestion for reconciliation between the two models (McPherson *et al*. 2004), as they are not necessarily mutually exclusive. Metapopulation dynamics in the Baltic Sea could occur among the spawning groups within the areas separated by zones of low gene flow, whereas these major areas themselves, i.e. the Baltic Proper and the northern populations, respectively, could reflect stable units maintained by oceanographic features.

Our findings also have important implications from a management perspective. The possibility that some of the genetic diversity among spawning groups also reflects underlying adaptive genetic variation suggests that management units should as a minimum reflect the zones of low gene flow identified in this study. The three areas separated by genetic breaks identified here correspond well with the three main groups of Baltic herring identified based on morphological and biological data (ICES 2002): western Baltic herring (RU), open sea herring of the Baltic Proper and Gulf herring. There are a few exceptions, though, since we found that AL and GF herring were separated from the Baltic Proper spawning groups, whereas the R herring were not. These inconsistencies emphasize that it is important to consider genetic data when defining management units for exploited fish, both at regional and local spatial scales.

In total, our results illustrate the usefulness of combining data from molecular markers with information on features of the marine landscape to identify genetic structuring. We envisage that similar use of landscape genetics approaches could greatly improve our understanding of the genetic structure of other marine organisms, and analyses of correspondence between genetic structure and oceanographic features, environmental variables and differences in migratory behaviour may lead to more precise and testable hypotheses about factors limiting gene flow in the marine environment.

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This paper represents a part Hanne Jørgensen's PhD thesis. Hanne Jørgensen's, Dorte Bekkevold's and Michael M. Hansen's current research interests focus on combining genetic, phenotypic and environmental information to examine population structure and local adaptation in marine and freshwater fishes. Daniel Ruzzante's interests focus on the mechanisms responsible for the maintenance and generation of genetic diversity in exploited and nonexploited species. Volker Loeschcke is working on physiological and evolutionary adaptation to environmental gradients with particular emphasis on stressful conditions, combining approaches from the molecular to the population level.

Appendix I

Summary of the genetic data for the 24 initial samples of herring spawning groups analysed in the present study. H_E is expected heterozygosity and H_O is observed heterozygosity. Asterisks denote significant departures from Hardy–Weinberg equilibrium (after Dunn-Šidák adjustment; $k = 9$), using the test procedure by Guo & Thompson (1992). Significant cases of F_{IS} based on radomization tests are shown in italics, and percentage missing indicates the percentage of individuals whose genotype could not be scored

	Cha1017	Cha1020	Cha1202	Cha1027	Cpa101	Cpa107	Cpa111	Cpa112	Cpa114
FB02									
No. of alleles	8	15	13	20	20	4	7	15	18
H_E	0.778	0.891	0.757	0.934	0.914	0.549	0.410	0.806	0.913
H_O	0.580	0.830	0.730	0.900	0.920	0.620	0.400	0.879	0.778
F_{IS}	*0.255*	0.068	0.036	0.036	−0.007	−0.13	0.023	−0.091	*0.149*
% missing	0	0	0	0	0	0	0	1.0	1.0
SB02									
No. of alleles	10	20	12	21	21	4	5	13	20
H_E	0.804	0.909	0.789	0.936	0.915	0.571	0.376	0.764	0.916
H_O	0.703	0.903	0.781	0.939	0.901	0.693	0.395	0.759	0.938
F_{IS}	0.026	0.007	0.011	−0.003	0.015	*−0.215*	−0.049	0.007	0.002
% missing	0.9	2.6	1.7	1.7	4.3	1.7	1.7	0	2.6
SB03									
No. of alleles	12	18	10	21	20	4	5	13	17
H_E	0.811	0.908	0.757	0.929	0.910	0.626	0.411	0.741	0.913
H_O	0.779	0.929	0.771	0.860	0.938	0.576	0.421	0.821	0.911
F_{IS}	0.04	−0.014	0.019	0.075	−0.031	−0.087	−0.023	−0.109	0.002
% missing	1.7	0.9	0	0.9	1.7	0	0.9	2.6	2.6
FS02									
No. of alleles	9	16	11	22	21	4	5	13	19
H_E	0.815	0.902	0.747	0.921	0.913	0.598	0.396	0.809	0.923
H_O	0.780	0.838	0.747	0.800	0.909	0.630	0.400	0.818	0.899
F_{IS}	0.044	0.071	0	*0.132*	0.004	−0.053	−0.01	−0.012	0.026
% missing	0	1.0	1.0	5.0	1.0	0	0	1.0	1.0
SS02									
No. of alleles	10	17	11	21	22	5	6	17	20
H_E	0.795	0.911	0.771	0.919	0.918	0.588	0.364	0.729	0.920
H_O	0.729	0.890	0.798	0.920	0.900	0.650	0.390	0.697	0.890
F_{IS}	0.083	0.023	−0.035	0.001	0.02	−0.107	−0.071	0.044	0.033
% missing	4.0	2.0	1.0	1.0	0	0	0	1.0	0
GF02									
No. of alleles	9	17	12	22	17	4	5	15	17
H_E	0.789	0.912	0.783	0.936	0.906	0.564	0.397	0.687	0.916
H_O	0.750	0.910	0.850	0.940	0.918	0.490	0.410	0.640	0.889
F_{IS}	0.05	0.002	−0.087	−0.004	−0.014	0.132	−0.033	0.069	0.03
% missing	0	0	0	0	2.0	0	0	0	1.0
AL02									
No. of alleles	10	19	10	20	19	4	7	14	16
H_E	0.778	0.911	0.732	0.923	0.914	0.625	0.422	0.753	0.913
H_O	0.777	0.911	0.685	0.929	0.930	0.685	0.438	0.759	0.929
F_{IS}	0.001	0	0.065	−0.006	−0.017	−0.097	−0.036	−0.008	−0.017
% missing	2.6	2.6	3.5	1.7	0.9	3.5	2.6	2.6	2.6
AL03									
No. of alleles	9	21	12	22	18	5	6	14	18
H_E	0.807	0.916	0.810	0.928	0.906	0.547	0.391	0.755	0.919
H_O	0.771	0.889	0.780	0.908	0.861	0.537	0.385	0.697	0.887
F_{IS}	0.046	0.029	0.038	0.022	0.05	0.019	0.015	0.077	0.035
% missing	0	0.9	0	0	0.9	0.9	0	0	2.8

Appendix I *Continued*

	Cha1017	Cha1020	Cha1202	Cha1027	Cpa101	Cpa107	Cpa111	Cpa112	Cpa114
BP02									
No. of alleles	11	17	12	19	20	7	6	11	17
H_E	0.807	0.908	0.728	0.920	0.920	0.643	0.406	0.727	0.916
H_O	0.808	0.899	0.660	0.869	0.850*	0.550*	0.283*	0.700	0.930
F_{IS}	–0.001	0.01	0.094	0.056	0.077	0.145	*0.304*	0.038	–0.016
% missing	1.0	1.0	0	1.0	0	0	1.0	0	0
R02									
No. of alleles	8	15	10	20	18	4	4	14	17
H_E	0.794	0.898	0.698	0.933	0.904	0.543	0.399	0.726	0.916
H_O	0.792	0.863	0.689	0.956	0.843	0.627	0.413	0.699	0.904
F_{IS}	0.003	0.039	0.013	–0.028	0.068	–0.156	–0.035	0.038	0.013
% missing	5.3	3.9	2.6	3.9	7.9	1.3	1.3	3.9	3.9
R03									
No. of alleles	11	18	12	19	22	5	5	13	17
H_E	0.799	0.914	0.776	0.914	0.917	0.566	0.390	0.717	0.914
H_O	0.752	0.898	0.761	0.907	0.926	0.459*	0.358	0.741	0.906
F_{IS}	0.058	0.017	0.019	0.007	–0.01	0.19	0.082	–0.033	0.01
% missing	0	0.9	0	0.9	0.9	0	0	0.9	2.8
HB02									
No. of alleles	11	18	11	24	19	4	5	14	19
H_E	0.838	0.904	0.777	0.937	0.916	0.545	0.441	0.760	0.924
H_O	0.691*	0.888	0.745	0.878	0.936	0.515	0.434	0.701	0.929
F_{IS}	*0.177*	0.018	0.042	0.063	–0.023	0.056	0.015	0.077	–0.005
% missing	2.0	1.0	1.0	1.0	5.0	0	0	2.0	1.0
GD02-1									
No. of alleles	10	17	10	22	19	6	5	16	19
H_E	0.817	0.911	0.744	0.934	0.908	0.522	0.415	0.809	0.914
H_O	0.828	0.850	0.735	0.920	0.889	0.510	0.400	0.860	0.890
F_{IS}	–0.013	0.067	0.013	0.015	0.021	0.023	0.036	–0.063	0.027
% missing	1.0	0	2.0	0	1.0	0	0	0	0
GD02-2									
No. of alleles	9	14	12	19	18	4	6	16	19
H_E	0.812	0.886	0.762	0.932	0.904	0.569	0.448	0.790	0.918
H_O	0.740	0.880	0.747	0.930	0.920	0.540	0.460	0.747	0.850
F_{IS}	0.089	0.007	0.019	0.002	–0.018	0.051	–0.027	0.054	0.074
% missing	0	0	0	0	0	0	0	1.0	0
GD02-3									
No. of alleles	10	17	11	20	20	4	6	16	19
H_E	0.796	0.913	0.808	0.908	0.902	0.539	0.366	0.777	0.918
H_O	0.717	0.939	0.790	0.828	0.898	0.520	0.380	0.768	0.848
F_{IS}	0.099	–0.029	0.022	*0.088*	0.005	0.036	–0.037	0.012	0.076
% missing	1.0	1.0	0	1.0	2.0	0	0	1.0	1.0
GD03-1									
No. of alleles	10	16	11	18	19	4	4	14	20
H_E	0.827	0.900	0.760	0.927	0.908	0.483	0.356	0.749	0.918
H_O	0.770	0.909	0.730	0.920	0.900	0.560	0.384	0.790	0.900
F_{IS}	0.069	–0.01	0.039	0.008	0.009	–0.161	–0.078	–0.054	0.02
% missing	0	1.0	0	0	0	0	1.0	0	0
GD03-2									
No. of alleles	13	18	11	21	21	5	6	16	20
H_{EXP}	0.788	0.910	0.806	0.931	0.920	0.568	0.421	0.764	0.919
H_{OBS}	0.750	0.939	0.750	0.870	0.895	0.520	0.420	0.730	0.929
F_{IS}	0.049	–0.033	0.07	0.066	–0.028	0.084	0.001	0.045	–0.011
% missing	0	1.0	0	0	0	0	0	0	1.0

Appendix I *Continued*

	Cha1017	Cha1020	Cha1202	Cha1027	Cpa101	Cpa107	Cpa111	Cpa112	Cpa114
GD03-3									
No. of alleles	10	16	13	21	19	5	5	15	16
H_E	0.803	0.901	0.760	0.920	0.910	0.553	0.440	0.757	0.911
H_O	0.740	0.859	0.770	0.870	0.899	0.580*	0.440	0.770	0.900
F_{IS}	0.079	0.047	–0.014	0.055	0.012	–0.049	0.001	–0.017	0.012
% missing	0	1.0	0	0	1.0	0	0	0	0
RU0302									
No. of alleles	11	18	9	17	19	2	5	14	16
H_E	0.809	0.868	0.574	0.890	0.884	0.405	0.269	0.780	0.908
H_O	0.860	0.869	0.630	0.870	0.827	0.480	0.270	0.840*	0.939
F_{IS}	–0.063	–0.001	–0.099	0.022	0.065	–0.186	–0.004	–0.077	–0.034
% missing	0	1.0	0	0	2.0	0	0	0	2.0
RU0402									
No. of alleles	9	15	10	17	19	3	5	12	19
H_E	0.812	0.894	0.704	0.903	0.900	0.373	0.429	0.789	0.922
H_O	0.788	0.870*	0.616	0.860	0.969	0.455	0.480	0.726	0.796*
F_{IS}	0.03	0.027	0.125	0.048	–0.077	–0.221	–0.12	0.08	*0.137*
% missing	1.0	0	1.0	0	3.0	1.0	0	5.0	7.0
RU0502									
No. of alleles	11	16	10	19	17	4	4	15	21
H_E	0.823	0.900	0.760	0.918	0.880	0.577	0.452	0.766	0.924
H_O	0.750	0.860	0.760	0.950	0.830	0.450*	0.420	0.707	0.950
F_{IS}	0.089	0.045	0	–0.035	0.058	*0.221*	0.071	0.077	–0.028
% missing	0	0	0	0	0	0	0	1.0	0
RU0303									
No. of alleles	8	17	11	18	19	4	6	16	16
H_E	0.816	0.872	0.715	0.926	0.908	0.511	0.439	0.799	0.909
H_O	0.755	0.796	0.765	0.920	0.910	0.505	0.515	0.847	0.919
F_{IS}	0.075	0.088	–0.07	0.006	–0.003	0.011	–0.173	–0.06	–0.011
% missing	2.0	2.0	2.0	0	0	1.0	1.0	2.0	1.0
RU0403									
No. of alleles	9	16	10	18	19	5	5	18	16
H_E	0.816	0.892	0.718	0.900	0.911	0.478	0.393	0.799	0.909
H_O	0.765	0.879	0.758	0.898	0.899	0.588	0.450	0.889	0.860
F_{IS}	0.063	0.015	–0.055	0.003	0.013	–0.23	–0.145	–0.114	0.054
% missing	2.0	1.0	1.0	2.0	1.0	3.0	0	1.0	0
RU0503									
No. of alleles	9	17	9	18	20	4	6	16	18
H_E	0.801	0.891	0.721	0.920	0.897	0.473	0.501	0.798	0.861
H_O	0.737	0.916	0.730	0.889	0.857	0.677*	0.580	0.847	0.847
F_{IS}	0.08	–0.029	–0.013	0.034	0.044	*–0.434*	–0.159	–0.062	0.017
% missing	1.0	5.0	0	1.0	2.0	1.0	0	2.0	2.0

Appendix II

Environmental data extracted from the ICES data base and used in Mantel tests for correlation between geographical, genetic and environmental distances

		Salinity, mean ± SE				Temperature, mean ± SE				
Sample	Location*	*n*	Max	Mean	Min	*n*	Max	Mean	Min	Years†
FB02	65.15′N, 23.30′E	8	3.17 ± 0.06	2.98 ± 0.07	2.76 ± 0.11	8	6.37 ± 0.77	4.99 ± 0.76	3.70 ± 0.80	1994–1999
SB02-03	63.15′N, 20.30′E	8	4.47 ± 0.13	4.47 ± 0.13	4.46 ± 0.13	8	9.03 ± 0.57	8.94 ± 0.59	8.85 ± 0.62	1993–2000
FS02	63.45′N, 21.30′E	8	3.79 ± 0.09	3.56 ± 0.03	3.40 ± 0.07	8	8.13 ± 0.65	7.18 ± 0.62	6.18 ± 0.69	1993–2000
SS02	61.15′N, 17.30′E	8	5.10 ± 0.05	5.09 ± 0.04	5.08 ± 0.04	8	9.09 ± 0.83	8.89 ± 0.85	8.70 ± 0.91	1993–2000
GF02	ICES sq. 49H6	2	5.04 ± 0.17	4.94 ± 0.21	4.79 ± 0.28	2	9.99 ± 2.02	9.38 ± 1.60	8.20 ± 0.77	1996–1998
AL02 + AL03	59.45′N, 22.30′E	3	5.89 ± 0.25	5.70 ± 0.20	5.59 ± 0.17	3	10.39 ± 1.32	9.72 ± 1.04	8.91 ± 0.73	1994–1996
BP02	59.15′N, 22.30′E	9	6.45 ± 0.08	6.18 ± 0.08	5.85 ± 0.14	9	7.28 ± 0.81	5.99 ± 0.58	4.74 ± 0.58	1992–2000
R02-03	ICES sq. 44H2	3	5.83 ± 0.16	5.66 ± 0.07	5.56 ± 0.04	3	6.69 ± 0.58	5.78 ± 0.81	5.17 ± 0.85	1995–2000
HB02	ICES sq. 40G5	4	7.54 ± 0.09	7.41 ± 0.11	7.31 ± 0.12	4	4.84 ± 0.33	4.01 ± 0.52	3.49 ± 0.55	1994–1997
RU0302	ICES sq. 37G2	7	13.67 ± 0.92	11.12 ± 0.69	8.83 ± 0.26	7	3.26 ± 0.44	2.81 ± 0.49	2.40 ± 0.53	1992–1998
RU0402	ICES sq. 37G2	4	10.57 ± 1.02	10.35 ± 1.18	10.23 ± 1.07	4	5.77 ± 0.54	5.20 ± 0.40	4.79 ± 0.34	1992–1998
RU0502	ICES sq. 37G2	7	10.48 ± 0.59	9.40 ± 0.27	8.52 ± 0.10	7	9.64 ± 0.43	8.45 ± 0.28	7.40 ± 0.22	1992–1998
RU03	ICES sq. 37G2	‡								
GD02-03§	ICES sq. 37G8	A: 4	A: 4.88 ± 0.75	A: 4.29 ± 0.63	A: 3.76 ± 0.65	A: 4	A: 7.35 ± 0.53	A: 6.78 ± 0.88	A: 6.34 ± 0.94	A: 1991–2000
		M: 3	M: 5.85 ± 1.08	M: 5.23 ± 0.89	M: 3.65 ± 0.64	M: 4	M: 10.65 ± 0.80	M: 10.01 ± 1.21	M: 9.54 ± 1.29	M: 1991–1999

*The location at which the salinity and surface temperature data (upper 10 metres) were collected. †Range of years from which the environmental data were extracted (there were some missing values in the data sets). ‡For RU03, the data used were averages of the data used for March, April and May in 2002. §GD data were averaged over April (A) and May (M) data, since the GD sample consists of six temporal samples taken in April and May.