The Structure of Fish Populations and Traceability of Fish and Fish Products

FishPopTrace

DTU Aqua
Institut for Akvatiske Ressourcer

2007 - 2013

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A genomic island linked to ecotype divergence in Atlantic cod

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34   Running title: Genomic island of divergence in Atlantic cod

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49   **Abstract**

50   The genomic architecture underlying ecological divergence and ecological speciation with

51   gene flow is still largely unknown for most organisms. One central question is whether

52   divergence is genome-wide or localized in "genomic mosaics" during early stages when gene

53   flow is still pronounced. Empirical work has so far been limited, and the relative impacts of

54   gene flow and natural selection on genomic patterns have not been fully explored. Here, we

55   use ecotypes of Atlantic cod to investigate genomic patterns of diversity and population

56   differentiation in a natural system characterized by high gene flow and large effective

57   population sizes, properties which theoretically could restrict divergence in local genomic

58   regions. We identify a genomic region of strong population differentiation, extending over

59   approximately 20 cM, between pairs of migratory and stationary ecotypes examined at two

60   different localities. Furthermore, the region is characterized by markedly reduced levels of

61   genetic diversity in migratory ecotype samples. The results highlight the genomic region, or

62   "genomic island", as potentially associated with ecological divergence and suggest the

63   involvement of a selective sweep. Finally, we also confirm earlier findings of localized

64   genomic differentiation in three other linkage groups associated with divergence among

65   eastern Atlantic populations. Thus, although underlying mechanisms are still unknown, the

66   results suggest that "genomic mosaics" of differentiation may even be found under high levels

67   of gene flow, and that marine fishes may provide insightful model systems for studying and

68   identifying initial targets of selection during ecological divergence.

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73   **Introduction**

74   The genomic architecture underlying adaptation to local environments and ultimately

75   ecological speciation (Schluter 2001; Nosil 2012) is poorly understood for most organisms

76   (Wu 2001; Nosil *et al.* 2009; Feder *et al.* 2012). Recent studies have suggested that, during

77   early stages of ecological divergence where gene flow is still on-going, genetic differentiation

78   may be limited to a few specific genomic locations, or "genomic islands", while the majority

79   of the genome remains homogenized by gene flow (Wu 2001; Turner *et al.* 2005; Via & West

80   2008; Nosil *et al.* 2009; Feder *et al.* 2012a; Feder *et al.* 2012b). Various mechanisms, such as

81   chromosomal inversions (Kirkpatrick & Barton 2006; Feder *et al.* 2011), divergence

82   hitchhiking (Via & West 2008) and processes promoting the genomic co-localization of genes

83   involved in adaptation (Nosil *et al.* 2009; Yeaman & Whitlock 2011), have been proposed as

84   potential mechanisms that would allow differing levels of divergence to evolve within a

85   single genome in the face of gene flow. However, theoretical work has indicated that the

86   conditions, with respect to the relative strengths of selection and gene flow, available for such

87   mechanisms to operate can be relatively restricted (Feder & Nosil 2009; Feder & Nosil 2010;

88   Feder *et al.* 2011; Feder *et al.* 2012b), and that genome-wide divergence should be more

89   common due to effects of reproductive isolation and selection on multiple loci, leading to

90   genome-wide reductions in gene flow (Feder & Nosil 2010). While high gene flow has been

91   predicted to constrain the formation of localized genomic divergence (Feder & Nosil 2009;

92   Feder & Nosil 2010), it has also been suggested that gene flow should promote the clustering

93   of genes involved in local adaptation (Yeaman & Whitlock 2011). Moreover, divergence

94   limited to specific genomic regions should in fact be most readily observable early in the

95   process of divergence, for example between ecotypes (Mallet 2008), rather than at later stages

96   where gene flow is more restricted and genomic divergence pronounced (Via 2009; Weetman

97   *et al.* 2012).

98   Hitherto, the investigation of genomic patterns associated with ecological divergence

99   has been restricted to a few, well known model systems, such as walking stick insects (Nosil

100   *et al.* 2008), *Heliconius* butterflies (Nadeau *et al.* 2012), pea aphids (Via & West 2008),

101   malaria mosquitos (Turner *et al.* 2005; Lawniczak *et al.* 2010), coregonid whitefish

102   (Bernatchez *et al.* 2010), three-spined stickleback (Shapiro *et al.* 2004; Colosimo *et al.* 2005;

103   Roesti *et al.* 2012a) and salmonids (Miller *et al.* 2012). Marine fishes provide excellent

104   models for studying interactions between gene flow and selection in the wild since they are

105   often distributed over diverse ecological habitats, and are typically characterized by high

106   levels of gene flow and large effective population sizes (Nielsen *et al.* 2009a). However,

107   although population genetics of non-model organisms, including most marine fishes, has

108   recently moved from analyses of neutral processes towards targeting adaptation to local

109   environments (Luikart *et al.* 2003; Nielsen *et al.* 2009a; Helyar *et al.* 2011), no studies have

110   yet investigated the genomic architecture associated with ecological divergence in these taxa.

111   Atlantic cod, *Gadus morhua*, has a wide geographical distribution and exploits diverse

112   ecological niches (Mieszkowska *et al.* 2009), ranging from brackish to highly saline

113   environments, and from low temperatures in the Arctic to high and variable temperatures in

114   the southern parts of the distribution (Righton *et al.* 2010). As typical for marine fishes,

115   population structuring is generally shallow (Nielsen *et al.* 2003; O'Leary *et al.* 2007),

116   suggesting high levels of gene flow (Waples 1998) and large effective population sizes

117   (Poulsen *et al.* 2006; Therkildsen *et al.* 2010). Thus, both gene flow and natural selection are

118   predicted to shape genomic patterns of divergence among populations.

119   Ecologically distinct ecotypes, usually characterised as "migratory" and "stationary"

120   behavioural types, have been described for cod in both eastern and western parts of the

121   Atlantic (Palsson & Thorsteinsson 2003; Robichaud & Rose 2004; Grabowski *et al.* 2011;

122   Nordeide *et al.* 2011). In the eastern Atlantic, these ecotypes are well described in both

123   Iceland and Norway. Migratory individuals are also named "frontal cod" in Iceland and

124   "Northeast Arctic cod" in Norway, while stationary individuals are known also as "coastal

125   cod" in Iceland and "Norwegian coastal cod" in Norway. In general, migratory ecotypes

126   exploit deeper and more offshore habitats at some times of the year compared to stationary

127   individuals which frequent coastal water habitats during their entire life (Palsson &

128   Thorsteinsson 2003; Nordeide *et al.* 2011). Migratory individuals from both locations may

129   also undertake pronounced vertical migrations and cross thermal fronts, formed where warm

130   Atlantic and cold Arctic water meet, during the feeding season (Stensholt 2001; Palsson &

131   Thorsteinsson 2003; Pampoulie *et al.* 2008). Furthermore, Norwegian migratory individuals

132   are characterized by long-distance migrations, for example the ~800 km migration from

133   Lofoten on the Norwegian coast to the feeding areas in the Barents Sea (Jørgensen *et al.*

134   2008; Sundby & Nakken 2008). In addition to migratory and feeding characteristics,

135   differences in several other life-history related traits, such as growth rate and age at maturity,

136   and in bioenergetics (Pardoe & Marteinsdottir 2009; Nordeide *et al.* 2011) suggest

137   pronounced ecological differences between the two ecotypes (see Nordeide *et al.* (2011) for a

138   comprehensive review). Thus, it is likely that the two ecotypes represent divergent life-history

139   strategies encompassing several behavioural and physiological characteristics of adaptive

140   importance in both Iceland and Norway. Although the ecotypes are ecologically distinct, there

141   is a potential for hybridization between the two types since spawning areas overlap in some

142   regions (Grabowski *et al.* 2011; Nordeide *et al.* 2011). Individuals displaying an intermediate

143   type of behaviour have been identified through electronic tagging of fish in the wild

144   (Grabowski *et al.* 2011), suggesting that hybridization may occur in nature, but the degree of

145   interbreeding and level of gene flow between ecotypes is presently unknown. Traditionally,

146   morphological characters, such as ear bone structures (otoliths), and single gene markers,

147   such as the membrane protein gene pantophysin (*Pan* I), have been used to designate

148   individuals as either migratory or stationary (Berg & Albert 2003; Pampoulie *et al.* 2008;

149   Wennevik *et al.* 2008). Recently, population genetic work has provided some molecular

150   evidence for adaptive divergence between the ecotypes from Norway (Moen *et al.* 2008;

151   Nielsen *et al.* 2009b), and the finding of consistent migratory profiles over consecutive years

152   for individual fish has suggested a genetic basis for ecotypic divergence in Iceland

153   (Thorsteinsson *et al.* 2012). Yet, the evolutionary relationship between ecotypes is still largely

154   unknown (Nordeide *et al.* 2011) as is the underlying genomic architecture associated with the

155   observed ecotypic differentiation. Furthermore, despite the ecological similarities described

156   above, the evolutionary relationship between Norwegian and Icelandic populations in these

157   parallel systems has not previously been explored.

158   Here we investigate genomic signatures associated with ecological divergence in a

159   high gene flow scenario. We use the migratory and stationary ecotypes in Atlantic cod as a

160   model system, and examine single nucleotide polymorphisms (SNPs) in population samples

161   of both ecotypes from the two partially isolated systems in Iceland and Norway, along with

162   reference samples from the major population complexes in the species. Information from the

163   Atlantic cod linkage map and the Atlantic cod genome assembly is used to investigate

164   genomic patterns associated with ecotypic divergence.

165

166   **Materials and Methods**

167 *Sampling*

168 Tissue samples of 31-40 adult individuals were collected from each of seven spawning

169 locations and one feeding ground (Fig. 1 and Table 1). Samples representing stationary

170 ecotypes, named "coastal cod" or "stationary cod" in Iceland and "Norwegian coastal cod" in

171 Norway, and migratory ecotypes, named "frontal cod" or "migratory cod" in Iceland and

172 "Northeast Arctic cod" in Norway, were collected from spawning grounds from Iceland and

173 Norway, and individuals were assigned to ecotype based on sampling location and depth

174 (Iceland) and ear bone (otolith) morphology (Norway, see also Wennevik *et al.* (2008)). In

175 Iceland, samples were collected in inshore waters (depth: 58 m), known to be mainly

176 inhabited by the stationary ecotype, and from a deeper offshore location (depth: 135 m),

177 where the migratory ecotype has been suggested to predominate (Pampoulie *et al.* 2006;

178 Pampoulie *et al.* 2008). In Norway, stationary and migratory ecotypes were collected on

179 spawning grounds near the island of Lofoten on the northern Norwegian coast. Due to

180 overlapping spawning areas between the two ecotypes (Grabowski *et al.* 2011; Nordeide *et al.*

181 2011) there is a risk of including hybrids and/or misclassified individuals in samples collected

182 from spawning areas. Thus, we included a sample from the extreme northern feeding grounds

183 in the Barents Sea (Fig. 1 and Table 1), which are used only by the migratory ecotype

184 (Nordeide *et al.* 2011) and therefore represents a pure "migratory" ecotype sample. In order to

185 relate findings from the stationary/migratory comparison to neighbouring areas, we also

186 included one sample from the highly divergent Baltic Sea (Nielsen *et al.* 2001) and a sample

187 from the North Sea, representing populations near the southernmost part of the distribution in

188 the eastern Atlantic. Finally, one western Atlantic sample was included as an out-group. Thus,

189 with the reference populations, the sampling scheme targeted the major population complexes

190 in the species (O'Leary *et al.* 2007; Bigg *et al.* 2008). The reference populations in the North

191   Sea and the Baltic Sea are not known to undertake long-distance migrations. However, to

192   allow a direct comparison between the two ecotypes, we refer only to the "stationary" ecotype

193   where it can potentially interbreed with the "migratory" ecotype.

194   In order to assess temporal stability of genomic patterns, we also analysed temporally

195   replicated samples collected from migratory and stationary populations from Norwegian

196   spawning grounds (Lofoten) and from reference populations in the North Sea and Baltic Sea

197   (Table 1).

198

199   *Genotyping and initial data filtering*

200   DNA was recovered from samples using the Omega EZNA Tissue DNA kit (Omega Bio-Tek,

201   USA) and subsequently normalised to 50 ng ul^{-1}. Samples were genotyped for 1536 single

202   nucleotide polymorphisms, most of which were originally developed from EST sequences

203   from western Atlantic cod populations ((Hubert *et al.* 2010), see also Table S1), using

204   Illumina's GoldenGate SAM assay on the Bead Array Reader platform. Data were checked

205   against internal sample independent quality controls, clustered and the resulting genotypes

206   then edited manually using the proprietary GenomeStudio software. A replicate individual

207   was included on all plates to ensure genotype reproducibility. Loci with low signal and/or

208   poor clustering were excluded from the analyses.

209

210   *Linking to the genome assembly*

211   We used the published linkage map consisting of 1310 SNPs (Borza *et al.* 2010) to infer

212   linkage group and position within linkage group for individual SNPs. In addition, a number of

213   SNPs were anchored to the linkage map by mapping the 120 bp flanking sequence of each

214   SNP, available in public data bases, onto the ATLCOD1A genome assembly (Star *et al.* 2011)

215   using BLASTN with an e-value threshold of 10^{-10}. While these SNPs could be assigned to

216   linkage groups, their position within linkage groups is unknown. We highlight loci in linkage

217   groups previously found to be targets of selection in Atlantic cod (i.e. loci in linkage groups 2,

218   7 and 12, see Bradbury *et al.* (2010)) along with loci in linkage group 1, which was found to

219   be highly differentiated between ecotypes in this study (see results). The ATLCOD1A

220   genome assembly was also used to estimate the distance (in base pairs) between adjacent

221   SNPs located within the same scaffolds.

222

223   *Population genetic analyses*

224   For each analysis, loci fixed in all population samples and loci with more than 15% missing

225   genotypes in any sample were removed. Conformance to Hardy-Weinberg equilibrium was

226   tested for each locus in each sample with the package GENETICS v. 1.3.4 for R (R

227   development core team 2011). In order to exclude loci with consistent HWE departures across

228   samples, we excluded loci deviating at the 5% level of significance in more than half of the

229   eight samples. This filtering should assure that loci deviating due to systematic technical or

230   biological reasons were excluded from the analyses. When examining departures from Hardy-

231   Weinberg equilibrium across loci within each sample, we corrected results for multiple testing

232   by using a false discovery rate (FDR) threshold of 5%. FDR correction was done with the

233   package STATS for R, following (Benjamini & Hochberg 1995).

234   Individual locus pairwise FST coefficients, following (Weir & Cockerham 1984),

235   were estimated with the R package GENELAND (Guillot *et al.* 2005), and mean and 95%

236   confidence intervals were estimated from 1000 data sets generated by bootstrapping over loci.

237   Population structuring over all loci was examined through correspondence analysis in

238   the package ADEGENET for R (Jombart 2008), using six axes to describe the relationship

239   among the seven eastern Atlantic population samples. In addition to the full data set, overall

240   pairwise FST was estimated and correspondence analysis conducted on a data set where

241   highly divergent outlier loci identified through Bayesian regression (see below) had been

242   excluded. Loci in the reduced dataset were presumed to be primarily affected by neutral

243   evolutionary forces, such as gene flow and genetic drift. We also investigated the effects of

244   removing loci with global minor allele frequencies below 10% in both the full and the

245   reduced data set, since correspondence analyses gives higher weight to rare alleles (Jombart *et*

246   *al.* 2009), potentially biasing these analyses.

247   Observed levels of heterozygosity within samples were estimated for each locus with

248   the R package GENETICS v. 1.3.4, and the R package ZOO was used to calculate moving

249   averages of single locus estimates with a window size of 10 SNPs along each individual

250   linkage group.

251   A statistical test for FST outliers was conducted by the Bayesian regression method

252   implemented in BAYESCAN 2.1 (Foll & Gaggiotti 2008). The method uses reversible-jump

253   Markov chain Monte Carlo sampling to estimate posterior odds for a model with selection

254   against a model without selection for individual loci. Prior odds for a model without selection

255   were set to 10:1 and 20 pilot runs of each 5000 samplings were used to adjust acceptance

256   rates and to obtain a prior estimate of mean and variance of parameter distributions. Pilot runs

257   were followed by an additional burn in of 50000 and 5000 samplings with a thinning interval

258   of 10 for the estimation of posterior distributions. The false discovery rate was controlled at

259   5% with the R function plot_bayescan distributed with the package (available from

260   http://cmpg.unibe.ch/software/bayescan/). Outliers were identified in a dataset excluding the

261   highly divergent western Atlantic sample in order to reduce bias due to hierarchical levels of

262   population structuring (Excoffier *et al.* 2009) and to allow a more detailed investigation of

263   patterns among eastern Atlantic samples. Loci with minor allele frequencies below 2% across

264   all samples were excluded since loci with low information content may bias computations

265   (Beaumont & Balding 2004). The additional filtering step reduced the number of loci to 975

266   in this analysis. Since loci with low levels of variation may bias outlier tests due to a

267   depression of global FST (Roesti *et al.* 2012b), we estimated global FST for different minor

268   allele frequency thresholds in the eastern Atlantic data set to examine if the chosen threshold

269   had an effect on global FST. In addition, we conducted the outlier test for a dataset where loci

270   with a minor allele frequency below 10% had been excluded in order to examine if outliers

271   were confirmed at a more stringent threshold.

272

273   **Results**

274   *Data filtering and control*

275   Following genotyping and initial data filtering, 295 individuals and 1282 loci were exported

276   for statistical analyses (Table S1). Data quality among retained loci was generally high, with

277   95% of loci having an average GenCall (GC) score above 0.61 for called genotypes. Initial

278   blast results identified three pairs of identical loci mapping to the same scaffold and position

279   within scaffold (Table S1). One locus from each pair was removed from further analyses. Ten

280   loci were removed from all analyses due to departures from Hardy-Weinberg equilibrium in

281   more than half of the eight samples. After this filtering, only a few loci (between 0 and 11, see

282   Table S1) deviated significantly in each sample, suggesting conformance to Hardy-Weinberg

283   expectations within each of the sampled populations. Following the removal of loci fixed in

284   all population samples and loci with more than 15% missing genotypes in any sample, 1199

285   loci remained for further analyses when all eight population samples were used. For analyses

286   focusing on the seven eastern Atlantic samples, similar data filtering resulted in a dataset

287   consisting of 1164 loci. The lower number resulted from a higher number of monomorphic

288   loci among these samples. In addition, observed levels of heterozygosity (Ho) were similar in

289   the eastern Atlantic and Baltic Sea (range of average Ho: 0.23-0.26), but lower than in the

290   western Atlantic (average Ho: 0.34, Table S1), indicating effects from ascertainment bias (see

291   also Discussion).

292

293   *Genomic distribution of SNPs*

294   The majority of analysed loci, 983 of 1199, were already placed on the linkage map (Table

295   S1). In addition, we were able to assign linkage groups to another 161 SNPs, although with

296   unknown position within linkage groups, through blasting against the ATLCOD1A genome

297   assembly (Table S1). Among the remaining 55 loci, 32 SNPs did not map to a scaffold while

298   23 SNPs were found in scaffolds that did not contain mapped SNPs. Thus, these loci could

299   not be assigned to any linkage group. While most loci mapped to a scaffold, 227 SNPs

300   mapped to scaffolds containing just the one SNP. The remaining loci were distributed on 236

301   scaffolds, with the majority of scaffolds containing only few SNPs (Fig. S1). This distribution

302   illustrates the relatively fragmented nature of the current genome assembly. The distribution

303   of distances between adjacent SNPs within scaffolds was also skewed towards lower values

304   (Fig. S2). Thus, the distance to the previous SNP within the same scaffold was below 50,000

305   bp for most loci and only few pairwise distances were above 1Mb.

306

307   *Population genetics*

308   Correspondence analysis showed marked differences between the two ecotypes with

309   migratory and stationary samples forming completely separate clusters, each containing both

310   Icelandic and Norwegian samples, when all markers were included in the analysis (Fig. 2a).

311   In contrast, these samples grouped according to geographic origin when a reduced "neutral"

312   data set (i.e. where 87 significant and highly divergent outlier loci had been removed, see also

313   below) was analysed (Fig. 2b). The North Sea and Baltic Sea samples, representing

314   geographically isolated samples, were also genetically isolated in both data sets (Fig. 2).

315   These results were confirmed when loci with a minor allele frequency below 10% were

316   removed (Figure S3), illustrating that these global patterns were robust to the inclusion of rare

317   alleles. The patterns were supported by estimates of pairwise FST (Table S2). With the

318   reduced (neutral) data set, confidence intervals overlapped with zero when comparing

319   ecotypes from spawning grounds within localities. In contrast, although pairwise FST

320   estimates were low, confidence intervals did not overlap with zero when similar ecotypes

321   were compared across the two localities (Table S2).

322   Levels of population differentiation, assessed through individual locus pairwise FST,

323   varied along the linkage groups (Fig. 3; see also Fig. S4 for all comparisons). The pairwise

324   comparisons of migratory and stationary ecotypes collected in both Norway and Iceland (Fig.

325   3a-c) showed markedly increased levels of differentiation for loci in linkage groups 1, 2 and 7

326   in addition to a few loci that were not mapped to a linkage group. In contrast, the pairwise

327   comparisons between similar ecotypes across geographic locations (Fig. 3d and 3e) showed

328   that differentiation was very shallow across all linkage groups. The pairwise comparison

329   between the southernmost eastern Atlantic location from the North Sea and the Norwegian

330   stationary ecotype collected in the northern Atlantic (Fig. 3f) revealed elevated levels of

331   structure for loci in linkage groups 2, 7 and 12, while most remaining loci were weakly

332   differentiated, thus confirming earlier findings of high differentiation in these linkage groups

333   (Bradbury *et al.* 2010). The comparison between the North Sea and the Baltic Sea samples

334   (Fig. 3g), representing reproductively isolated populations (Nielsen *et al.* 2003, see also

335   Discussion), showed elevated differentiation for loci across most linkage groups, as did the

336   comparison between the North Sea and the western Atlantic sample (Fig. 3h).

337   Observed levels of heterozygosity also varied among linkage groups (Fig. 4).

338   Remarkably different patterns in the distribution of heterozygosity were observed among the

339   populations, with dramatic reductions in linkage group 1 in the migratory ecotype samples

340   (Fig. 4a-c). In addition, reduced levels of heterozygosity were observed in linkage group 7 for

341   the migratory ecotype samples (Fig. 4a-c), the North Sea population sample (Fig. 4d) and the

342   western Atlantic sample (Fig. 4h), while the stationary ecotype samples showed increased

343   levels of heterozygosity for the same genomic region (Fig. 4e and 4f).

344   Eighty-seven high FST outlier loci were identified through Bayesian regression on a

345   data set excluding the highly divergent western Atlantic sample and loci with a minor allele

346   frequency below 2%. These outlier loci were primarily located in linkage groups 1, 2, 7 and

347   12 (71 of 87 outliers; Table S3). Global FST changed only slightly (from 0.056 to 0.065)

348   between minor allele frequency thresholds of 0% and 20% (Fig. S5). Changes in global FST

349   were larger for thresholds above 20%, but these analyses only included few loci since most of

350   the loci were removed from analysis at these very high thresholds. In addition, an outlier test

351   including only loci with minor allele frequencies above 10% identified almost the same set of

352   outliers as the test applied on loci with minor allele frequencies above 2% (only four outlier

353   loci were not identified with a threshold of 10%, see Table S3). Thus, results from the outlier

354   test appear very robust to the effects of loci with low information content (see also discussion

355   in Roesti *et al.* (2012b)).

356   Patterns of single locus population differentiation and genetic diversity were

357   confirmed when temporal replicates of the samples from the North Sea, the Baltic sea and

358   both migratory and stationary ecotypes from Norwegian spawning grounds were analysed

359   (Fig. S6 and Fig. S7). Differentiation was increased in linkage groups 1, 2 and 7 in the

360   comparison between the two ecotypes, while differentiation was increased in linkage groups

361   2, 7 and 12 in the comparison between the North Sea and the stationary samples.

362   Differentiation was low across the remaining linkage groups in these comparisons, while

363   differentiation was high across all linkage groups in comparisons involving the Baltic Sea

364   sample (Fig. S6). Genetic diversity was drastically reduced in linkage group 1 in the

365   migratory sample. In addition linkage group 7 showed decreased diversity in the migratory

366   and North Sea samples, while it showed increased diversity in the stationary sample. Finally,

367   loci in linkage group 12 showed decreased diversity in the North Sea sample (Fig. S7). These

368   results indicate temporal stability of observed patterns.

369   A detailed investigation of the loci in linkage group 1 revealed that loci displaying

370   elevated levels of population differentiation between migratory and stationary ecotypes were

371   located between 14.3 and 37.2 cM (Fig. 5 and Table S4). This pattern was evident for both

372   Norwegian and Icelandic comparisons. The previously intensely studied locus in the gene

373   pantophysin (*Pan* I) is located at position 25.1 cM in this linkage group ((Borza *et al.* 2010)

374   and Table S1).

375

376   **Discussion**

377   In addition to identifying a region of high differentiation between ecotypes in linkage group 1,

378   we confirmed earlier findings suggesting selection in linkage groups 2, 7 and 12 in Atlantic

379   cod (Bradbury *et al.* 2010). However, these signals were not specifically associated with the

380   migratory ecotype as was the case for the highly differentiated region in linkage group 1. The

381   region of elevated differentiation between ecotypes extends over 20 cM in a genome subject

382   to high levels of gene flow (see below). Thus, our results suggest that extensive divergence of

383   local genomic regions may be possible even in situations with extensive gene flow (Yeaman

384   & Whitlock 2011; Weetman *et al.* 2012). In addition, genomic studies of high gene flow

385   scenarios, like ecotypes in marine organisms, may indeed provide valuable model systems for

386   elucidating evolutionary processes at the genomic level associated with ecological divergence

387   (Via 2009; Via 2012).

388

389   *Origin of migratory ecotype*

390   Despite decades of research on the ecotypes in both Norway and Iceland (Palsson &

391   Thorsteinsson 2003; Nordeide *et al.* 2011), no study has so far directly compared populations

392   from the two regions through the use of a large number of genetic markers. Genetic

393   differentiation between Norway and Iceland (across ecotypes) revealed with neutral genetic

394   markers (Fig 2b and Table S2) suggest reproductive isolation between these locations. Yet,

395   results illustrate marked similarities in genomic signatures associated with ecotypic

396   divergence. Thus, although the description of the ecotypes (or behaviour types) in Icelandic

397   waters has so far only been based on information from data storage tags (Palsson &

398   Thorsteinsson 2003; Pampoulie *et al.* 2008; Grabowski *et al.* 2011), our study confirms the

399   presence of two divergent groups in coastal and deep off-shore locations, respectively.

400   The region of increased differentiation between ecotypes is also characterized by

401   dramatically reduced levels of diversity in samples representing the migratory ecotype, a

402   classical signal of a selective sweep (Storz 2005). This suggests that initially these

403   populations may have experienced a selective sweep involving the specific region on linkage

404   group 1.

405   Extremely shallow population differentiation across most of the genome (Fig. 3a-c) as

406   well as the close relationship among populations within geographic locations (across

407   ecotypes), as estimated with neutral genetic markers (Fig. 2b), suggest two possible scenarios

408   for the origin of migratory ecotype populations. In one scenario, the migratory ecotype arose

409   twice through convergent evolution in two parallel systems (Iceland and Norway) following

410   colonization after the last glacial maximum (LGM) around 21,000 years ago. Similarities

411   within geographic regions (Fig. 2b) could then reflect shared ancestry and recent divergence

412   (Pogson *et al.* 2001) rather than effects from gene flow between ecotypes. However, highly

413   divergent allele lineages for one gene in the region affected by the selective sweep,

414   pantophysin (Pogson & Mesa 2004), suggest that the split of the two ecotypes is ancient

415   compared to the LGM. If the pantophysin gene is representative for the region, these data

416   suggest that recent convergent adaptation is not likely. In contrast, a more parsimonious

417   scenario is that the two ecotypes were already present when deglaciated regions around

418   Iceland and Norway were colonized following the LGM (Kettle *et al.* 2011) and that the

419   geographically based structure at neutral markers is caused by on-going gene flow between

420   ecotypes within localities. This scenario is also consistent with the hypothesized, though still

421   highly speculative, existence of both coastal and off-shore refugia for Atlantic cod during the

422   LGM (Pampoulie *et al.* 2008; Kettle *et al.* 2011). Modelling work has suggested that periods

423   of allopatry, for instance in isolated glacial refugia, could favour the establishment of local

424   genomic differentiation under some models of adaptive divergence (Feder *et al.* 2011). With

425   the current data set it is not possible to determine if secondary contact between ecotypes

426   occurred before or after colonization. However, the combination of highly divergent allele

427   lineages within and extremely shallow differentiation outside the region on linkage group 1 is

428   difficult to explain without a significant role for gene flow. Indeed, if the split is very old and

429   gene flow is not occurring between ecotypes, we would expect to see similar patterns of

430   structuring for neutral markers as those observed for the loci within this specific genomic

431   region since neutral markers would then reveal common ancestry of ecotypes across

432   locations. In addition, on-going gene flow is also indirectly supported by observations of

433   individuals expressing an intermediate type of behaviour in nature (Grabowski *et al.* 2011),

434   which could suggest on-going hybridization between the ecotypes.

435   Neutral genetic differentiation between Norway and Iceland (for both ecotypes) also

436   suggests at least partial isolation of the two geographical systems (Waples & Gaggiotti 2006),

437   and that gene flow mostly occurs between ecotypes within the two regions. This gene flow

438   would then be counteracted by on-going selection in the two parallel systems in the specific

439   genomic region in linkage group 1.

440

441   *Underlying mechanism for genomic differentiation*

442   A number of mechanisms could be responsible for generating and maintaining strong

443   differentiation between ecotypes in the specific region in linkage group 1. If, as suggested

444   above, natural selection is involved, both exogenous (e.g. adaptation to local environmental

445   conditions) and endogenous (i.e. intrinsic incompatibilities) factors could be important and it

446   may be very difficult to disentangle such effects (Bierne *et al.* 2011). While an intrinsic

447   incompatibility unrelated to known ecological and environmental differences cannot be ruled

448   out, the data are also consistent with the alternative interpretation that the migratory ecotype

449   was affected by a selective sweep linked to the unique life-history characteristics known for

450   these populations. It is plausible that the life-history strategy of the migratory ecotype is

451   linked to utilizing high productivity frontal niches in the Arctic for feeding (Stensholt 2001;

452   Grabowski *et al.* 2011), and that the well-described migratory and behavioural characteristics

453   reflect this adaptation. Alternative and more specialized adaptations to different temperature

454   conditions (Righton *et al.* 2010; Grabowski *et al.* 2011) are also likely linked to these

455   differences in life-history strategies between ecotypes.

456   Many studies have discussed selection on the pantophysin gene (e.g. (Pogson 2001;

457   Karlsson & Mork 2003; Case *et al.* 2005; Skarstein *et al.* 2007)), while some authors have

458   noted that observed patterns of linkage disequilibrium within the gene could indicate that

459   selection is instead targeting a linked gene (Fevolden & Pogson 1997). The latter hypothesis

460   is supported by the present study, which suggests that pantophysin may be linked to a large

461   genomic region, potentially harbouring hundreds of genes, rather than the actual target of

462   selection.

463   Although the link between ecotypes and genomic patterns are consistent with patterns

464   resulting from natural selection (through exogenous or endogenous factors) in local

465   populations, alternative explanations could, in principle, also explain our findings. For

466   instance, it has been suggested that transient phases during the fixation process of a globally

467   favourable mutation could generate signals similar to selective sweeps in local populations

468   (Bierne 2010). However, in a scenario of a globally favourable mutation, sweep signals of

469   different magnitudes should be observed in all populations and should be unrelated to specific

470   ecological characteristics (see also (Roesti *et al.* 2012a)). Thus, expected patterns under a

471   globally favourable mutation model are difficult to reconcile with observed patterns, where

472   sweep signals are specifically observed in populations characterized by the migratory life-

473   history strategy. Similarly, structural chromosomal features, such as chromosome

474   centromeres, could potentially explain localized genomic increases in population

475   differentiation due to reduced recombination rates in these regions (Lawniczak *et al.* 2010;

476   Roesti *et al.* 2012a). However, while recombination rate variation would be expected to result

477   in increased levels of differentiation in some parts of the genome, it cannot explain the

478   extreme reduction in diversity observed only in the migratory population samples. Thus, the

479   most plausible explanation remains a balance between local selection and gene flow. Finally,

480   ascertainment bias could have affected some of the analyses conducted in this study since

481   markers were primarily developed from western Atlantic cod populations. Previous studies

482   have not found markedly different levels of diversity in eastern and western Atlantic cod

483   populations (O'Leary *et al.* 2007; Bigg *et al.* 2008), and the lower levels of variation observed

484   in the eastern Atlantic in this study could therefore suggest an effect from ascertainment bias.

485   However, we still do not expect these effects to severely bias the major conclusions drawn

486   from analyses focusing on eastern Atlantic populations, since levels of variation are similar in

487   the eastern Atlantic samples (Table S1) and since all samples in the eastern Atlantic

488   (migratory and stationary populations, in particular) are weakly differentiated from each other

489   and show common divergence from the western Atlantic (Table S2, see also e.g. Rosenblum

490   & Novembre (2007)). Thus, ascertainment bias would be expected to affect eastern Atlantic

491   samples to the same degree.

492   While data suggest increased differentiation over one large genomic region, the

493   relatively modest genome coverage in this study and the fragmented nature of the current cod

494   genome assembly (see Fig. S1 and Fig. S2) does not allow a formal assessment of whether the

495   signals reflect few or several targets of selection (see discussion in Via (2012)). It is possible

496   that future studies applying higher genome coverage may identify more complex patterns of

497   differentiation between cod ecotypes, such as observed in malaria mosquitos (Lawniczak *et*

498   *al.* 2010; Neafsey *et al.* 2010). Similarly, the data do not allow for an assessment of whether

499   divergence hitchhiking, chromosomal rearrangement, such as inversions, or another

500   mechanism is most likely responsible for the observed patterns. It is likely, however, that

501   dense sequencing of the region could elucidate the underlying processes responsible.

502

503　*Genomic mosaic of differentiation in Atlantic cod*

504　In contrast to patterns observed in linkage group 1, regions of increased differentiation in

505　linkage groups 2, 7 and 12 are not associated with the migratory ecotype samples. These

506　patterns have previously been attributed to coevolution of several genes in response to

507　common environmental conditions (temperature; (Bradbury *et al.* 2010)), but they have not

508　been related to the extremely low levels of differentiation across other parts of the genome, as

509　observed here.

510　　　　　　Collectively our results suggest that, on a genome-wide scale, relatively few and

511　potentially large regions, or "genomic islands", could be affected by selection in populations

512　still influenced by gene flow. These patterns are consistent with a "genomic mosaic of

513　divergence" (Wu 2001; Via & West 2008), originally proposed to underlie early stages of

514　ecological divergence in malaria mosquitos and pea aphids (Turner *et al.* 2005; Via & West

515　2008; Via 2009; White *et al.* 2010; Via 2012). Since these original studies, theoretical and

516　conceptual work has considered whether divergence should be localized or genome-wide

517　during different stages of the "divergence-with-gene-flow continuum" (Feder *et al.* 2012a;

518　Feder *et al.* 2012b; Via 2012). Although the number of empirical studies is increasing,

519　relatively few model systems have so far been studied. While some studies have identified

520　genome-wide patterns of divergence, for instance in walking stick insects (Nosil *et al.* 2008)

521　and three-spined stickleback (Roesti *et al.* 2012a), others have suggested localized

522　divergence, for example in pea aphids (Via & West 2008; Via *et al.* 2012) and *Heliconius*

523　butterflies (Nadeau *et al.* 2012). Interestingly, results from the original model case

524　introducing the "genomic island" metaphor (Turner *et al.* 2005) have been reinterpreted with

525　the availability of genome-wide data to actually reflect pervasive divergence throughout the

526   genome (Lawniczak *et al.* 2010; Neafsey *et al.* 2010), and even studies on the same species

527   under different settings have arrived at different conclusions (Hohenlohe *et al.* 2012; Roesti *et*

528   *al.* 2012a). Thus, so far empirical work has not identified a universal remnant genomic

529   signature following ecological divergence, and it seems likely that different processes operate

530   on different stages of the continuum from panmixia to complete reproductive isolation (Feder

531   *et al.* 2012a).

532   In Atlantic cod, patterns of genomic differentiation associated with clearly

533   differentiated populations from the Baltic Sea and the western Atlantic were different from

534   those observed between weakly differentiated groups. Among highly divergent populations,

535   population differentiation was found across all linkage groups (Fig. 3 and Fig. S4), suggesting

536   reproductive isolation and reduced gene flow (Nielsen *et al.* 2003; Feder *et al.* 2012a).

537   Divergence between the eastern and western Atlantic is believed to be more than 100,000

538   years old, predating the last glacial maximum (Bigg *et al.* 2008). Thus, it may not be

539   surprising that time has allowed genomic differentiation to develop across the Atlantic. In the

540   case of the Baltic Sea, however, Atlantic cod most likely colonized the region following the

541   last glacial retreat from this area around 8,000 years ago (Nielsen *et al.* 2003; Johannesson &

542   Andre 2006). For Atlantic cod and many other marine species, it is therefore plausible that

543   genomic differentiation arose over a relatively short evolutionary time scale following a

544   colonization process involving adaptation, reproductive isolation and increased levels of

545   genetic drift in the Baltic Sea (Johannesson & Andre 2006). Indeed, several life-history

546   characteristics, such as unique sperm activity and egg buoyancy (Nissling & Westin 1997), as

547   well as pronounced genetic differentiation for both neutral and non-neutral genetic markers

548   (Nielsen *et al.* 2003; Nielsen *et al.* 2009b) of Atlantic cod in the Baltic Sea, suggest

549   significant roles for both neutral and non-neutral evolutionary forces in Baltic Sea

550   populations. The scenarios represented by the Atlantic cod system may therefore represent

551   different stages on the continuum from panmixia to complete isolation (Feder *et al.* 2012a;

552   Via 2012). Importantly, even though the initial split between ecotypes was not recent *per se*,

553   the scenario may still represent an early stage of divergence, i.e. a stage where populations

554   remain connected through significant levels of gene flow (Via 2009). In contrast, reductions

555   in gene flow between highly differentiated groups illustrate that genome-wide effects from

556   neutral evolutionary forces will make it difficult to detect genomic regions associated with

557   initial stages of divergence if populations are investigated at later stages (Via 2009; Via

558   2012).

559

560   *Conclusions*

561   The Atlantic cod ecotypes have contributed novel insights on the possible genomic signatures

562   underlying ecological divergence in a high gene flow species. Even though the responsible

563   mechanism and the nature of targets of selection are still unknown, our findings provide

564   additional insights into the long-standing controversy on the interactions between diversifying

565   selection and homogenizing gene flow (Ehrlich & Raven 1969; Mayr 1969; Lenormand 2002;

566   Garant *et al.* 2007). While predictions on the extent and pattern of adaptive divergence can be

567   tested using comparisons of phenotypic traits across populations, analysis at the genomic

568   level allows for unequivocal identification of the integrated effects of selection and gene flow,

569   as well as indicating genes potentially of major effect. Importantly, the frequently

570   documented negative correlations between phenotypic differences and gene flow (Rasanen &

571   Hendry 2008) may be underlain by a much more complex genomic mosaic of response even

572   in high gene flow species (see also Nadeau *et al.* (2012)). Thus, the Atlantic cod ecotypes

573   represent an informative model to study evolution in action (Via 2009), particularly in relation

574   to the dramatic environmental changes predicted for Arctic marine environments under future

575   climate change (Solomon *et al.* 2007).

576

577   **Acknowledgements**

578   We are grateful to Thomas Damm Als for discussions improving the manuscript, and to Sean

579   Rogers and five anonymous reviewers who provided very useful comments on earlier

580   versions of the manuscript. The research leading to these results has received funding from

581   the European Community's Seventh Framework Programme (FP7/2007-2013) under grant

582   agreement n° KBBE-212399 (FishPopTrace).

583

584   **References**

585   Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among

586   populations from genome scans. *Molecular Ecology,* **13**, 969-980.

587   Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a practical and

588   powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-*

589   *Methodological,* **57**, 289-300.

590   Berg E, Albert O (2003) Cod in fjords and coastal waters of North Norway: distribution and

591   variation in length and maturity at age. *ICES Journal of Marine Science,* **60**, 787-797.

592   Bernatchez L, Renaut S, Whiteley AR, et al (2010) On the origin of species: insights from the

593   ecological genomics of lake whitefish. *Philosophical Transactions of the Royal Society of*

594   *London Series B-Biological Sciences,* **365**, 1783-1800.

595   Bierne N (2010) The distinctive footprints of local hitchhiking in a varied environment and

596   global hitchhiking in a subdivided population. *Evolution,* **64**, 3254-3272.

597   Bierne N, Welch J, Loire E, Bonhomme F, David P (2011) The coupling hypothesis: why

598   genome scans may fail to map local adaptation genes. *Molecular Ecology,* **20**, 2044-2072.

599   Bigg GR, Cunningham CW, Ottersen G, Pogson GH, Wadley MR, Williamson P (2008) Ice-

600   age survival of Atlantic cod: agreement between palaeoecology models and genetics.

601   *Proceedings of the Royal Society of London Series B-Biological Sciences,* **275**, 163-172.

602   Borza T, Higgins B, Simpson G, Bowman S (2010) Integrating the markers Pan I and

603   haemoglobin with the genetic linkage map of Atlantic cod (*Gadus morhua*). *BMC Research*

604   *Notes,* **3**, 261.

605   Bradbury IR, Hubert S, Higgins B, et al (2010) Parallel adaptive evolution of Atlantic cod on

606   both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society*

607   *of London Series B-Biological Sciences,* **277**, 3725-3734.

608   Case R, Hutchinson W, Hauser L, Van Oosterhout C, Carvalho G (2005) Macro- and micro-

609   geographic variation in pantophysin (PanI) allele frequencies in NE Atlantic cod *Gadus*

610   *morhua*. *Marine Ecology-Progress Series,* **301**, 267-278.

611   Colosimo P, Hosemann K, Balabhadra S, et al (2005) Widespread parallel evolution in

612   sticklebacks by repeated fixation of ectodysplasin alleles. *Science,* **307**, 1928-1933.

613   Ehrlich PR, Raven PH (1969) Differentiation of populations. *Science,* **165**, 1228-1232.

614   Excoffier L, Hofer T, Foll M (2009) Detecting loci under selection in a hierarchically

615   structured population. *Heredity,* **103**, 285-298.

616   Feder JL, Egan SP, Nosil P (2012a) The genomics of speciation-with-gene-flow. *Trends in*

617   *Genetics,* **28**, 342-350.

618   Feder JL, Gejji R, Powell TH, Nosil P (2011) Adaptive chromosomal divergence driven by

619   mixed geographic mode of evolution. *Evolution,* **65**, 2157-2170.

620   Feder JL, Gejji R, Yeaman S, Nosil P (2012b) Establishment of new mutations under

621   divergence and genome hitchhiking. *Philosophical Transactions of the Royal Society of*

622   *London Series B-Biological Sciences,* **367**, 461-474.

623   Feder JL, Nosil P (2010) The efficacy of divergence hitchhiking in generating genomic

624   islands during ecological speciation. *Evolution*, **64**, 1729-1747.

625   Feder JL, Nosil P (2009) Chromosomal inversions and species differences: when are genes

626   affecting adaptive divergence and reproductive isolation expected to reside within inversions?

627   *Evolution,* **63**, 3061-3075.

628   Fevolden SE, Pogson GH (1997) Genetic divergence at the synaptophysin (Syp I) locus

629   among Norwegian coastal and north-east Arctic populations of Atlantic cod. *Journal of Fish*

630   *Biology,* **51**, 895-908.

631   Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for

632   both dominant and codominant markers: a Bayesian perspective. *Genetics,* **180**, 977-993.

633　Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow

634　on contemporary adaptation. *Functional Ecology,* **21**, 434-443.

635　Grabowski TB, Thorsteinsson V, McAdam BJ, Marteinsdottir G (2011) Evidence of

636　segregated spawning in a single marine fish stock: Sympatric divergence of ecotypes in

637　Icelandic cod? *PLoS ONE,* **6**, e17528.

638　Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape

639　genetics. *Molecular Ecology Notes,* **5**, 712-715.

640　Helyar SJ, Hemmer-Hansen J, Bekkevold D, et al (2011) Application of SNPs for population

641　genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology*

642　*Resources,* **11**, 123-136.

643　Hohenlohe PA, Bassham S, Currey M, Cresko WA (2012) Extensive linkage disequilibrium

644　and parallel adaptive divergence across threespine stickleback genomes. *Philosophical*

645　*Transactions of the Royal Society of London Series B-Biological Sciences,* **367**, 395-408.

646　Hubert S, Higgins B, Borza T, Bowman S (2010) Development of a SNP resource and a

647　genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC Genomics,* **11**, 191.

648　Johannesson K, Andre C (2006) Life on the margin: genetic isolation and diversity loss in a

649　peripheral marine ecosystem, the Baltic Sea. *Molecular Ecology,* **15**, 2013-2029.

650　Jombart T, Pontier D, Dufour AB (2009) Genetic markers in the playground of multivariate

651　analysis. *Heredity,* **102**, 330-341.

652   Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers.

653   *Bioinformatics,* **24**, 1403-1405.

654   Jørgensen C, Dunlop ES, Opdal AF, Fiksen O (2008) The evolution of spawning migrations:

655   State dependence and fishing-induced changes. *Ecology,* **89**, 3436-3448.

656   Karlsson S, Mork J (2003) Selection-induced variation at the pantophysin locus (PanI) in a

657   Norwegian fjord population of cod (*Gadus morhua* L.). *Molecular Ecology,* **12**, 3265-3274.

658   Kettle AJ, Morales-Muniz A, Rosello-Izquierdo E, Heinrich D, Vollestad LA (2011) Refugia

659   of marine fish in the northeast Atlantic during the last glacial maximum: concordant

660   assessment from archaeozoology and palaeotemperature reconstructions. *Climate of the Past,*

661   **7**, 181-201.

662   Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation and speciation.

663   *Genetics,* **173**, 419-434.

664   Lawniczak MK, Emrich SJ, Holloway AK, et al (2010) Widespread divergence between

665   incipient *Anopheles gambiae* species revealed by whole genome sequences. *Science,* **330**,

666   512-514.

667   Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology &*

668   *Evolution,* **17**, 183-189.

669   Luikart G, England P, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of

670   population genomics: From genotyping to genome typing. *Nature Reviews Genetics,* **4**, 981-

671   994.

672   Mallet J (2008) Hybridization, ecological races and the nature of species: empirical evidence

673   for the ease of speciation. *Philosophical Transactions of the Royal Society of London Series*

674   *B-Biological Sciences,* **363**, 2971-2986.

675   Mayr E (1969) *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

676   Mieszkowska N, Genner MJ, Hawkins SJ, Sims DW (2009) Chapter 3. Effects of climate

677   change and commercial fishing on Atlantic cod Gadus morhua. *Advances in Marine Biology,*

678   **56**, 213-273.

679   Miller MR, Brunelli JP, Wheeler PA, et al (2012) A conserved haplotype controls parallel

680   adaptation in geographically distant salmonid populations. *Molecular Ecology,* **21**, 237-249.

681   Moen T, Hayes B, Nilsen F, et al (2008) Identification and characterisation of novel SNP

682   markers in Atlantic cod: evidence for directional selection. *BMC Genetics,* **9**, 18.

683   Nadeau NJ, Whibley A, Jones RT, et al (2012) Genomic islands of divergence in hybridizing

684   *Heliconius* butterflies identified by large-scale targeted sequencing. *Philosophical*

685   *Transactions of the Royal Society of London Series B-Biological Sciences,* **367**, 343-353.

686   Neafsey DE, Lawniczak MK, Park DJ, et al (2010) SNP genotyping defines complex gene-

687   flow boundaries among African malaria vector mosquitoes. *Science,* **330**, 514-517.

688   Nielsen EE, Hansen MM, Schmidt C, Meldrup D, Grønkjær P (2001) Population of origin of

689   Atlantic cod. *Nature,* **413**, 272.

690   Nielsen E, Hansen M, Ruzzante D, Meldrup D, Gronkjaer P (2003) Evidence of a hybrid-zone

691   in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual

692   admixture analysis. *Molecular Ecology,* **12**, 1497-1508.

693   Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009a) Population genomics of

694   marine fishes: identifying adaptive variation in space and time. *Molecular Ecology,* **18**, 3128-

695   3150.

696   Nielsen EE, Hemmer-Hansen J, Poulsen NA, et al (2009b) Genomic signatures of local

697   directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*).

698   *BMC Evolutionary Biology,* **9**, 276.

699   Nissling A, Westin L (1997) Salinity requirements for successful spawning of Baltic and Belt

700   Sea cod and the potential for cod stock interactions in the Baltic Sea. *Marine Ecology-

701   Progress Series,* **152**, 261-271.

702   Nordeide JT, Johansen SD, Jorgensen TE, Karlsen BO, Moum T (2011) Population

703   connectivity among migratory and stationary cod *Gadus morhua* in the Northeast Atlantic-A

704   review of 80 years of study. *Marine Ecology-Progress Series,* **435**, 269-283.

705   Nosil P (2012) *Ecological Speciation*, 1st edn. Oxford University Press, New York.

706   Nosil P, Egan SP, Funk DJ (2008) Heterogeneous genomic differentiation between walking-

707   stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. *Evolution,*

708   **62**, 316-336.

709   Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic

710   divergence. *Molecular Ecology,* **18**, 375-402.

711   O'Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF (2007) Microsatellite variation

712   in cod *Gadus morhua* throughout its geographic range. *Journal of Fish Biology,* **70**, 310-335.

713   Palsson O, Thorsteinsson V (2003) Migration patterns, ambient temperature, and growth of

714   Icelandic cod (*Gadus morhua*): evidence from storage tag data. *Canadian Journal of*

715   *Fisheries and Aquatic Sciences,* **60**, 1409-1423.

716   Pampoulie C, Jakobsdottir KB, Marteinsdottir G, Thorsteinsson V (2008) Are vertical

717   behaviour patterns related to the pantophysin locus in the Atlantic cod (*Gadus morhua* L.)?

718   *Behavior Genetics,* **38**, 76-81.

719   Pampoulie C, Ruzzante DE, Chosson V, et al (2006) The genetic structure of Atlantic cod

720   (*Gadus morhua*) around Iceland: insight from microsatellites, the Pan I locus, and tagging

721   experiments. *Canadian Journal of Fisheries and Aquatic Sciences,* **63**, 2660-2674.

722   Pampoulie C, Stefansson MO, Jorundsdottir TD, Danilowicz BS, Danielsdottir AK (2008)

723   Recolonization history and large-scale dispersal in the open sea: the case study of the North

724   Atlantic cod, *Gadus morhua* L. *Biological Journal of the Linnean Society,* **94**, 315-329.

725   Pardoe H, Marteinsdottir G (2009) Contrasting trends in two condition indices: bathymetric

726   and spatial variation in autumn condition of Icelandic cod *Gadus morhua*. *Journal of Fish*

727   *Biology,* **75**, 282-289.

728   Pogson GH (2001) Nucleotide polymorphism and natural selection at the pantophysin (Pan I)

729   locus in the Atlantic cod, *Gadus morhua* (L.). *Genetics,* **157**, 317-330.

730   Pogson GH, Mesa KA (2004) Positive Darwinian selection at the pantophysin (Pan I) locus in

731   marine gadid fishes. *Molecular Biology and Evolution,* **21**, 65-75.

732 Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic

733 cod, *Gadus morhua*, at large and small geographic scales. *Evolution,* **55**, 131-146.

734 Poulsen N, Nielsen E, Schierup M, Loeschcke V, Gronkjaer P (2006) Long-term stability and

735 effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular*

736 *Ecology,* **15**, 321-331.

737 Rasanen K, Hendry AP (2008) Disentangling interactions between adaptive divergence and

738 gene flow when ecology drives diversification. *Ecology Letters,* **11**, 624-636.

739 Righton DA, Andersen KH, Neat F, et al (2010) Thermal niche of Atlantic cod *Gadus*

740 *morhua*: limits, tolerance and optima. *Marine Ecology-Progress Series,* **420**, 1-U344.

741 Robichaud D, Rose G (2004) Migratory behaviour and range in Atlantic cod: inference from a

742 century of tagging. *Fish and Fisheries,* **5**, 185-214.

743 Roesti M, Hendry AP, Salzburger W, Berner D (2012a) Genome divergence during

744 evolutionary diversification as revealed in replicate lake-stream stickleback population pairs.

745 *Molecular Ecology*, **21**, 2852–2862.

746 Roesti M, Salzburger W, Berner D (2012b) Uninformative polymorphisms bias genome scans

747 for signatures of selection. *BMC Evolutionary Biology,* **12**, 94.

748 Rosenblum EB, Novembre J (2007) Ascertainment bias in spatially structured populations: a

749 case study in the eastern fence lizard. *The Journal of Heredity,* **98**, 331-336.

750 Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution,* **16**, 372-

751 380.

752 Shapiro M, Marks M, Peichel C, et al (2004) Genetic and developmental basis of evolutionary

753 pelvic reduction in threespine sticklebacks. *Nature,* **428**, 717-723.

754 Skarstein TH, Westgaard J, Fevolden S (2007) Comparing microsatellite variation in north-

755 east Atlantic cod (*Gadus morhua* L.) to genetic structuring as revealed by the pantophysin

756 (Pan I) locus. *Journal of Fish Biology,* **70**, 271-290.

757 Solomon S, Qin D, Manning M*, et al*, eds (2007) *Climate Change 2007: The Physical Science*

758 *Basis. Contribution of Working Group I to the Fourth Assessment Report of the*

759 *Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge,

760 United Kingdom and New York, NY, USA.

761 Star B, Nederbragt AJ, Jentoft S, et al (2011) The genome sequence of Atlantic cod reveals a

762 unique immune system. *Nature,* **477**, 207-210.

763 Stensholt B (2001) Cod migration patterns in relation to temperature: analysis of storage tag

764 data. *ICES Journal of Marine Science,* **58**, 770-793.

765 Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population

766 divergence. *Molecular Ecology,* **14**, 671-688.

767 Sundby S, Nakken O (2008) Spatial shifts in spawning habitats of Arcto-Norwegian cod

768 related to multidecadal climate oscillations and climate change. *ICES Journal of Marine*

769 *Science,* **65**, 953-962.

770 Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS (2010) Large effective population size

771 and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St.

772 Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences,* **67**, 1585-1595.

773　Thorsteinsson V, Palsson OK, Tomasson GG, Jonsdottir IG, Pampoulie C (2012) Consistency

774　in the behaviour types of the Atlantic cod: repeatability, timing of migration and geo-location.

775　*Marine Ecology-Progress Series,* **462**, 251-260.

776　Turner TL, Hahn MW, Nuzhdin SV (2005) Genomic islands of speciation in *Anopheles*

777　*gambiae*. *PLoS Biology,* **3**, e285.

778　Via S (2012) Divergence hitchhiking and the spread of genomic isolation during ecological

779　speciation-with-gene-flow. *Philosophical Transactions of the Royal Society of London Series*

780　*B-Biological Sciences,* **367**, 451-460.

781　Via S (2009) Natural selection in action during speciation. *Proceedings of the National*

782　*Academy of Sciences of the United States of America,* **106 (Suppl 1)**, 9939-9946.

783　Via S, Conte G, Mason-Foley C, Mills K (2012) Localizing F(ST) outliers on a QTL map

784　reveals evidence for large genomic regions of reduced gene exchange during speciation-with-

785　gene-flow. *Molecular Ecology, 21*, 5546–5560.

786　Via S, West J (2008) The genetic mosaic suggests a new role for hitchhiking in ecological

787　speciation. *Molecular Ecology,* **17**, 4334-4345.

788　Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some

789　genetic methods for identifying the number of gene pools and their degree of connectivity.

790　*Molecular Ecology,* **15**, 1419-1439.

791　Waples R (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in

792　high gene flow species. *Journal of Heredity,* **89**, 438-450.

793   Weetman D, Wilding CS, Steen K, Pinto J, Donnelly MJ (2012) Gene flow-dependent

794   genomic divergence between *Anopheles gambiae* M and S Forms. *Molecular Biology and*

795   *Evolution,* **29**, 279-291.

796   Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure.

797   *Evolution,* **38**, 1358-1370.

798   Wennevik V, Jorstad KE, Dahle G, Fevolden S (2008) Mixed stock analysis and the power of

799   different classes of molecular markers in discriminating coastal and oceanic Atlantic cod

800   (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. *Hydrobiologia,* **606**,

801   7-25.

802   White BJ, Cheng C, Simard F, Costantini C, Besansky NJ (2010) Genetic association of

803   physically unlinked islands of genomic divergence in incipient species of *Anopheles gambiae*.

804   *Molecular Ecology,* **19**, 925-939.

805   Wu C (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology,*

806   **14**, 851-865.

807   Yeaman S, Whitlock MC (2011) The genetic architecture of adaptation under migration-

808   selection balance. *Evolution,* **65**, 1897-1911.

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810   **Data Accessibility**

811   Novel SNPs analysed in this study are available in GenBank (dbSNP) under accession

812   numbers ss678251294-ss678251301. Individual SNP genotypes have been deposited in the

813   DRYAD data repository (doi:10.5061/dryad.9gf10).

814

815 **Author Contributions Box**

816 JHH and EEN designed the study with input from MIT, RO, DB, SH and GRC. AG, CP and

817 TJ contributed samples. JHH and NOT analysed the data. JHH wrote the paper with

818 contributions from all authors.

819

820 **Figure legends**

821 *Figure 1*

822 Locations of samples included in the present study. See Table 1 for detailed sample

823 information.

824

825 *Figure 2*

826 Population relationships among eastern Atlantic samples based on correspondence analysis

827 with all markers (a, 1164 loci) and with neutral markers only (b, 1077 loci).

828

829 *Figure 3*

830 Estimates of pairwise levels of population differentiation (Weir and Cockerhams θ (Weir &

831 Cockerham 1984)) based on 1199 loci ordered by position within linkage groups between (a)

832 Norway migratory on spawning grounds and Norway stationary, (b) Norway migratory on

833 feeding grounds and Norway stationary, (c) Iceland migratory and Iceland stationary, (d)

834 Norway migratory on spawning grounds and Iceland migratory, (e) Norway stationary and

835 Iceland stationary, (f) Norway stationary and North Sea, (g) North Sea and Baltic Sea and (h)

836 North Sea and western Atlantic. Horizontal dashed and dotted lines represent mean and 95th

837 percentiles generated by bootstrapping over loci. Loci in linkage groups 1, 2, 7 and 12 are

838   coloured red, blue, green and purple, respectively, while additional linkage groups are

839   coloured in alternating shades of grey and loci with unknown linkage group are shown in

840   black. Location within linkage group is unknown for loci to the right of the vertical line.

841

842   *Figure 4*

843   Observed levels of heterozygosity based on 983 loci with known linkage group position,

844   estimated as moving averages within linkage groups, in (a) Norway migratory on spawning

845   grounds, (b) Norway migratory on feeding grounds, (c) Iceland migratory, (d) North Sea, (e)

846   Norway stationary, (f) Iceland stationary, (g) Baltic Sea and (h) western Atlantic. Horizontal

847   dashed line marks the 1st percentile over all linkage groups. Estimates for linkage groups 1, 2,

848   7 and 12 are coloured red, blue, green and purple, respectively, while additional linkage

849   groups are coloured in alternating shades of grey.

850

851   *Figure 5*

852   Pairwise FST, estimated by Weir and Cockerhams θ (Weir & Cockerham 1984), between

853   migratory and stationary ecotypes from Norway (a) and Iceland (b) for 57 loci with known

854   linkage group position in linkage group 1. Loci identified as FST outliers by Bayesian

855   regression are shown in red.

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862 **Tables**

863 *Table 1*

864 Samples of Atlantic cod included in the present study.

Sample	Sample size	Latitude	Longitude	Sampling month/year
Ecotype samples				
Norway migratory (feeding)	35	75.64	16.82	August/2009
Norway migratory (spawning)	35	67.33	11.38	March/2009
Norway stationary	31	68.15	14.48	March/2009
Iceland migratory	39	63.20	-19.30	April/2002
Iceland stationary	38	63.49	-21.05	April/2002
Reference samples				
North Sea	38	56.91	7.83	February/2007
Baltic Sea	40	55.04	15.30	March/2006 and April/2007
Western Atlantic	39	48.01	-63.55	May/2008
Temporal replicates				
Norway migratory	35	68.35	12.14	April/2003
Norway stationary	27	68.12	14.44	March/2003
North Sea	40	58	-3	March/2003

Baltic Sea	40	54.87	15.46	April/1997

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Norway migratory (feeding)
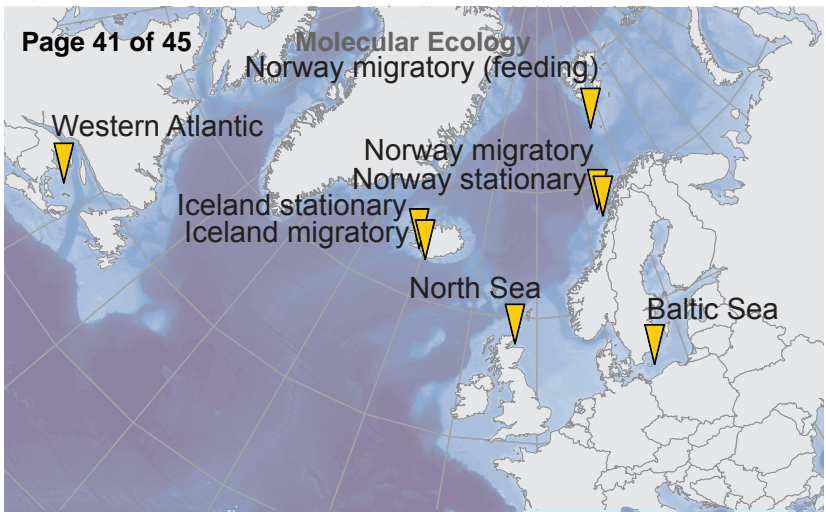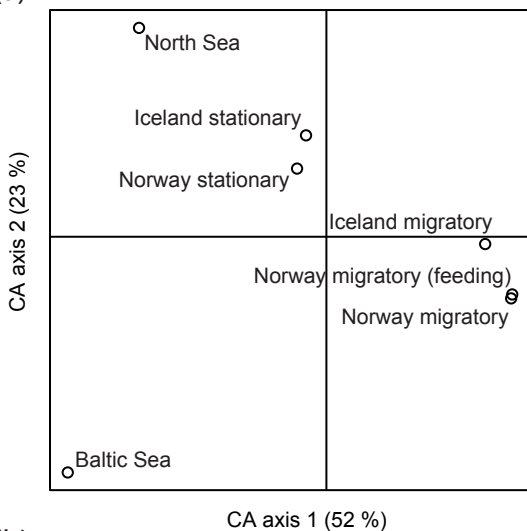
Western Atlantic

Norway migratory

Norway stationary

Iceland stationary

Iceland migratory

North Sea

Baltic Sea

(a)

(b)