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Two adjacent inversions maintain genomic differentiation between migratory and stationary ecotypes of Atlantic cod

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Abstract

Atlantic cod is composed of multiple migratory and stationary populations widely distributed in the North Atlantic Ocean. The Northeast Arctic cod (NEAC) population in the Barents Sea undertakes annual spawning migrations to the northern Norwegian coast. Although spawning occurs sympatrically with the stationary Norwegian coastal cod (NCC), phenotypic and genetic differences between NEAC and NCC are maintained. In this study we resolve the underlying mechanisms by demonstrating extended linkage disequilibrium (LD) and population divergence in a 17.5 Mb region on linkage group 1 (LG1) based on genotypes of 494 SNPs from 192 parents of farmed families of NEAC, NCC or NEAC x NCC crosses. Linkage analyses revealed two adjacent inversions within the 17.5 Mb region that repress meiotic recombination in NEAC x NCC crosses. We identified a NEAC specific haplotype consisting of 186 SNPs that was fixed in NEAC sampled from the Barents Sea, but segregated under Hardy-Weinberg equilibrium in eight northern NCC stocks. Comparative genomic analyses determine the NEAC configuration of the inversions to be the derived state and date it to ~1.6-2.0 Mya. The haplotype block includes 765 genes, including candidates regulating heme synthesis, skeletal muscle organization and buoyancy conferring adaptation to long-distance migrations and vertical movements down to 500 m. Our results suggest that the migratory ecotype experience strong directional selection for the two adjacent inversions on LG1. Despite interbreeding between NEAC and NCC the inversions are maintaining genetic differentiation, and we hypothesize the co-occurrence of multiple adaptive alleles forming a 'supergene' in the NEAC population.

Introduction

Atlantic cod are widely distributed on the continental shelves and banks on both sides of the North Atlantic Ocean and represent the main demersal fish resource in these regions. The success of this highly exploited fish seems to be related to the different life history strategies of the multiple migratory and stationary populations, but careful management is required as several stocks have been dramatically reduced as a result of overfishing, climate change and pollution (Myers *et al*. 1997; Christensen *et al*. 2003; Robichaud & Rose 2004; MacKenzie *et al*. 2004). Cod fishery dates back to the tenth century A.D. when Vikings used dried Skrei (Old Norse *skríða* means wandering) as a source of nutrition and currency along the European trade routes. Today Skrei are synonymous with the large Northeast Arctic cod (NEAC) population, which feeds in the Barents Sea and near Svalbard, but the adults undertake annual long-distance migrations to and from the spawning banks along the coast of North Norway, mainly offshore the Lofoten Archipelago (Bergstad *et al*. 1987; Sundby & Nakken 2008; Ottersen *et al*. 2014). During foraging and spawning migrations NEAC perform vertical movements down to depths of about 500 m with frequent descending and ascending swimming spanning up to 250 m (Godø & Michalsen 2000; Stensholt 2001). In contrast, the stationary Norwegian coastal cod (NCC) live in shallow coastal waters and fjords throughout the year and generally migrate only short distances at depths down to about 100 m (Hobson *et al*. 2007; Michalsen *et al*. 2014). The vertical divergence between NEAC and NCC is apparent at the 0-group stage when juveniles settle in deep and shallow water, respectively, in northern Norwegian fjords (Løken *et al*. 1994; Fevolden *et al*. 2012). In Iceland, similar ecotypes are represented by the frontal (migratory) and coastal (non-migratory) populations, which exploit different habitats at depths of 200-600 m and less than 200 m, respectively (Pálsson *et al*. 2003; Pampoulie *et al*. 2008; Grabowski *et al*. 2011).

Almost half a century ago, Møller (1966, 1968, 1969) studied the genetic diversity in Atlantic cod along the Norwegian coast and concluded that NEAC and NCC form two genetically separated populations or non-interbreeding sibling species. Although they occur sympatrically on local spawning grounds, differences in phenotypic traits, such as otolith morphology and vertebrae number, seem to be maintained between the populations, but might be influenced by environmental factors (Rollefsen 1933, 1954; Jakobsen 1987; Løken & Pedersen 1996; Nordeide 1998; Nordeide *et al*. 2011). Nuclear DNA analysis has identified divergent allele frequencies within pantophysin (*Pan* I), hemoglobin and rhodopsin, which are of potential relevance for adaptation to different ecosystems (Møller 1966, 1968; Fevolden & Pogson, 1997; Pogson 2001; Andersen *et al*., 2015; Pampoulie *et al*. 2015). The genetic divergence of NEAC and NCC was recently found to be uniquely associated with a large genomic region on linkage group 1 (LG1) with absence of gene flow between the two populations (Hemmer-Hansen *et al*. 2013; Karlsen *et al*. 2013; Therkildsen *et al*. 2013).

The extreme difference between NEAC and NCC at the *Pan* I locus was suggested to be caused by differences in breeding structure, as selection alone would be insufficient to cause the observed levels of genetic differentiation (Fevolden & Sarvas 2001; Sarvas & Fevolden 2005; Westgaard & Fevolden 2007). Accordingly, interbreeding between the populations has been proposed to be hindered by differences in courtship or spawning behavior, or by differences in spawning depths (Hutchings *et al*. 1999; Jeffrey *et al*. 1999; Nordeide & Folstad, 2000; Grabowski *et al*. 2011). In contrast, the mitochondrial genome revealed no reproductive isolation between NEAC and NCC (Karlsen *et al*. 2014), supporting the alternative hypothesis that local selection forces at some loci are strong enough to inhibit, or even override, the levelling effect of the gene flow (Mork & Sundnes 1985). We have finally resolved this controversial issue by demonstrating that the strong genetic divergence between

the two populations is not the result of reproductive barriers nor selection *per se*, but caused by two large inversions on LG1 that repress recombination within heterozygotes preventing introgression between co-segregating haplotypes.

Materials and methods

Fish material and DNA extraction

Wild cod were collected from 14 locations ranging from the Irish Sea in the south to the Barents Sea in the north (see Figure 2). On average, 48 samples were collected from each location. To get a representational sampling of NEAC we collected cod from two locations in the Barents Sea. Farmed cod were sampled from 88 families of the National cod breeding program maintained by Nofima in Tromsø, Norway, and from 8 families of the CODBIOBANK at the Institute of Marine Research in Bergen, Norway.

One hundred and four cod from the National cod breeding program were selected for sequencing. Out of these, 50 fish were of NEAC origin, 11 fish were of NCC origin and 43 fish were offspring of NEAC x NCC crosses. All sequenced fish from the National breeding program belonged to year classes 2005 (P) and 2006 (F1) and represented the second generation of cod produced in captivity. The original broodstock in the base population were sampled from different geographical areas along the Norwegian coast and were assigned to the NCC and NEAC populations based on sampling locations and the *Pan* I^A and I^B alleles (Fevolden & Pogson 1997; Bangera *et al*. 2011). The Greenland cod (*Gadus macrocephalus ogac*) used to date the inversion was sampled at the Uummannaq Island, Northwest Greenland.

DNA was extracted using either a DNeasy kit from Qiagen (Hilden, Germany) according to manufacturer's instructions or a high salt precipitation method

(http://www.liv.ac.uk/~kempsj/IsolationofDNA.pdf). DNA quality was assessed by electrophoresis on 1% agarose gel to estimate the proportion of high molecular weight (HMW) DNA, and low quality samples with negligible levels of MMW DNA were excluded from analysis. DNA concentration was assessed fluorometrically using Qubit technologies (Thermo Fisher Scientific, Carlsbad, USA).

Genotyping

Farmed (n=2951) and wild fish (n=959) were genotyped for 10,913 SNPs using an Illumina custom Infinium II SNP-array (Kent *et al*., in prep) according to manufacturer's instructions (Illumina, San Diego, USA). Poorly performing samples displaying call rates below 0.9 were excluded from analysis. Genotype data was pre-processed by removing low MAF (<0.05) SNPs, and Mendelian errors were set to missing and imputed along with any other failed genotypes using BEAGLE v4 (Browning & Browning, 2007). Wild populations were phased using SHAPEIT v2 (https://mathgen.stats.ox.ac.uk/shapeit) and the family material were accurately phased using linkage information. Phased data for 192 parents were used to estimate linkage disequilibrium (LD) between SNPs using Haploview 4.2 (Barrett *et al*. 2005). All NEAC samples from the Barents Sea were homozygous for a haplotype consisting of 186 SNPs from the SNP-array (see Supplementary Table S1), and the wild fish were assigned to NEAC, NCC or a cross using this NEAC haplotype.

Linkage mapping and inversion detection

The construction of linkage maps for cod using 12K SNP-array is described in detail elsewhere (Grove *et al*., in prep), but begins with performing two-point linkage in CRIMAP (Green *et al*. 1990) to sort SNPs into linkage groups. In the present study we used the 494 SNPs mapped to LG1 to construct separate linkage maps for pure NEAC, pure NCC, and

NEAC x NCC crosses. The sorting of family material for these analyses was determined by haplotyping parents using the 186 SNP-set described above.

SNPs on the cod 12K SNP-array were carefully chosen to tag as many contigs as possible (Kent *et al*. in prep) and are well distributed along the linkage groups, thereby forming a good foundation for building a chromosome sequence for LG1. Scaffolds from two draft assemblies, containing at least one SNP from the linkage map, were selected and used for the construction of chromosome files. Erroneous scaffolds containing SNPs from more than one LG were broken between conflicting SNP positions. Overlapping scaffolds were identified by comparing SNPs mapping to both assemblies and were merged using coordinates from alignment with LASTZ (Harris 2007), resulting in a total of 40 scaffolds that were used to build the final chromosome sequence. Subsequently linkage maps were then updated to take into account the more precise SNP order given by individual scaffolds. Finally, all scaffolds were oriented, ordered and concatenated into a new chromosome sequence based on information from the linkage map. The size of the final chromosome sequence for LG1 was 29,521,491 bp.

Sequencing and variant detection

Genomic DNA from the 104 breeding program fish was prepared for sequencing using the Truseq Library prep kit from Illumina (Illumina, San Diego, USA). Paired-end sequencing (2 x 100nts) of three indexed samples per lane was carried out using an Illumina HiSeq 2000 instrument, generating a total of 13.7 billion reads, with an average of 132 million reads per individual. This represented approximately 10x coverage of the genome for each sample. Reads were processed using default parameters in Trimmomatic version 0.32 (Bolger *et al*. 2014) before being aligned to the unmasked reference genome based on the NCC map described above using Bowtie2 version 2.2.3 (Langmead & Salzberg 2012). Within sample variant detection was performed using GATK HaplotypeCaller version 2.8-1-g932cd3a

(McKenna *et al*. 2010). SnpEff version 4.0e (Cingolani *et al*. 2012) was used to annotate and predict allelic variants. Individual variant calls with a quality score of <20 were excluded from further analysis, as were INDELs and genotypes with read depths below 6 or above 27. Variants not detected in >70% of the samples were removed across all samples.

Genomic DNA from a single Greenland cod was prepared for sequencing using a Nextera XT library preparation kit generating a library with an average size of 650bp. Sequencing was performed using a MiSeq platform with V3 kit chemistry to generate 2 x 301 nt paired-end reads. A total of 18.7 M reads generated 11.2 Gb sequence data. Reads were mapped and variants detected as described above.

Pairwise LD (measured as r2) for the whole linkage group was calculated based on 48 sequenced NEAC using Plink v1.9 (https://www.cog-genomics.org/plink2) with MAF > 0.1 and HWE > 0.001.

Gene annotation

An automated pipeline for protein coding gene annotation was used to build gene models from multiple data sources including (i) approximately 3 million transcriptome reads (http://www.ncbi.nlm.nih.gov/sra?term=SRP013269) obtained from liver, egg, brain, head, kidney, hindgut, gonad, and spleen, generated using GS-FLX 454 Titanium platform (Roche, Switzerland), (ii) ESTs from NCBI (n=257218), (iii) predicted RNAs (n=1541, http://www.codgenome.no/data/ATLCOD1_ANN/), and (iv) roughly 35 million short read mRNA sequences from whole NEAC fish at 12 and 35 days post hatching (Johnsen & Andersen 2012). To enable model building, short reads were mapped to the reference genome sequence using STAR (v2.3.1z12), while long 454 transcriptome reads were mapped using GMAP (version 2014-07-28) with "--no-chimeras" parameter in addition to default parameters. Cufflinks (v2.2.1) with "--multi-read-correct" parameter in addition to the default

parameter assembled the aligned RNA-Seq reads and transcriptome reads into transcripts. Transcript models from RNAseq and 454 transcriptome were merged using Cuffmerge.

Open reading frame (ORF) prediction was carried out using TransDecoder (http://transdecoder.github.io/) (Haas *et al*. 2013) using the pfamA and pfamB databases for homology searches (--search_pfam) and a minimum length of 30 amino acids for ORFs without pfam support (-m 30). In addition to the pfam homology evidence we also performed BLASTP (evalue<1e-10) for all predicted proteins against zebrafish (*Danio rerio*) (v9.75) and three-spined stickleback (*Gasterosteus aculeatus*) (BROADS1.75) annotations downloaded from Ensembl. Only gene models with support from at least one type of homology search (pfam or BLASTP) were kept.

In total we mapped 35 million mRNA-seq reads and 3.3 million 454 transcriptome sequences to the whole genome and used this to annotate LG1. A total of 2323 transcripts were left after merging transcript models using cuffmerge. Functional annotations of the transcripts were done using blastx against the SwissProt database. Results from TransDecoder and homology support filtering of putative protein coding loci are shown in Supplementary Table S2.

Origin and dating of inversions

To determine whether NEAC or NCC represents the ancestral state of the inversions we aligned LG1 sequences representing possible arrangements of the inversions with Northern pike (*Esox lucius*) and stickleback using LASTZ in gap-free mode requiring >= 75% identity and match-count filtering of 100 (Harris 2007).

Hierarchical clustering of the wild stocks was estimated based on genotypes from the SNP-array using the R package SNPrelate (Zheng *et al*. 2012). Four linkage groups (LG1, LG2, LG7 and LG12) were excluded from this analysis because of the presence of extended LD blocks (own data; Bradbury *et al*. 2010; Hemmer-Hansen *et al*. 2013). Reads generated from whole genome sequencing of a single Greenland cod were compared with NEAC and NCC

variant calls to identify a set of fixed sequence differences (FSD; single nucleotides fixed within populations) along LG1. FSD counts were then used to calculate pairwise differences among Greenland cod, NEAC and NCC. Under the assumption of a constant clock we then estimated the NEAC-NCC divergence age relative to their divergence from Greenland cod by calculating the ratio between NEAC-NCC FSD-distance and the mean FSD-distance between Greenland cod-NEAC and Greenland cod-NCC (i.e. $FSD_{NEAC\text{-}NCC}/FSD_{mean(Greenland\ cod\text{-}NEAC,\ Greenland\ cod\text{-}NCC)}$).

Protein modeling

Homology modeling was performed with the MODELLER software (Sali & Blundell, 1993) to build the three-dimensional structure of the NEAC and NCC variants of Ca6 based on the crystal structure of human Ca6 as template (PDB code 3FE4, Pilka *et al*. 2012). The sequences were aligned using ClustalW, and identities between targets and template of 58% (NEAC) and 56% (NCC) allowed using the standard MODELLER protocol implemented in DiscoveryStudio v4.5 (Biovia). We ascertained that no other protein with a known related structure displayed a greater sequence similarity. The best of 50 models according to the PDF (Probability Density Function) score included in MODELLER was selected. The structures were inspected with PROCHECK (Laskowski *et al*. 1993) for inappropriate stereochemistry. Ramachandran maps of NEAC and NCC models revealed that they contained 91.7% of non-Gly-non-Pro residues in most favored, 7.8% in additional allowed, 0.5% in generously allowed and 0.0% in disallowed regions. These models were further validated for their structure quality by Verify 3D available at http://services.mbi.ucla.edu/ and 95% of the residues of the modeled proteins showed satisfactory 3D-1D score (>0.2). DiscoveryStudio v4.5 (Biovia) software was used to visualize the generated models.

Results

Linkage map and LD calculations

A genetic map describing 23 linkage groups in Atlantic cod (Grove *et al*., in prep.) was constructed by genotyping a large family material of 2739 individuals using a 12K SNP-array (Kent *et al*. in prep). The map constructed for LG1 contained 494 SNPs (Figure 1a; Suppl. Table S1) and was used to integrate, order, and orientate scaffolds from two draft cod assemblies into a cohesive chromosome sequence comprising 29.52 Mb. Accurately phased genotypes from 192 parents were used to estimate LD between SNPs, and revealed a distinct block of extended LD from 10-27 Mb (Figure 1c), embracing the *Pan* I locus located at 17.5 Mb. The parents were of known origin and classed as pure NEAC, pure NCC, or NEAC x NCC crosses. Analyses of pure NEAC cod identified a single haplotype of 186 non-consecutive SNPs that were homozygous in all individuals (Supplementary Table S1). All NEAC x NCC crosses had one copy of this haplotype, while the NEAC haplotype was completely absent in pure NCC samples.

Because this distinct haplotype in NEAC indicated substantial differentiation between NEAC and NCC cod, we constructed linkage maps separately for pure NEAC, pure NCC, and NEAC x NCC crosses (Supplementary Table S1). Pure NEAC and NCC showed typical recombination rates between SNPs along the length of LG1, but comparing the linkage maps disclosed a different SNP order within the block with extended LD. NEAC x NCC crosses displayed almost complete repression of recombination within this block, but showed elevated recombination outside the block (Figure 1b). NEAC and NCC linkage maps were used to order and orient scaffolds to create specific assemblies of LG1 for these two ecotypes of Atlantic cod. Alignment of these sequences revealed the presence of two adjacent inversions of 9.55 Mb and 7.82 Mb (Figure 1a). Additional evidence for two inversions, in contrast to a

274 single inversion, was found in the LD pattern of 48 whole genome sequenced NEAC samples.
275 High LD was found between polymorphisms at 18 Mb and 28 Mb in the NCC version of the
276 assembly. In contrast the proposed NEAC orientation of the inversions rearrange these two
277 regions to be located close together (Supplemental Figure S1).
278
279 *Geographical distribution of NEAC haplotype*
280 To validate if the NEAC haplotype precisely identified the differing genotypes of migratory
281 and stationary cod ecotypes we analyzed 48 cod captured in the Barents Sea and representing
282 pure NEAC based on *Pan* I genotyping. All samples were homozygous for the 186 SNPs
283 within the haplotype block, which endorses its utility as a tool to classify cod as NEAC, NCC
284 or crosses. To explore the distribution of the NEAC haplotype we tested individuals from 14
285 different localities across the Northeast Atlantic Ocean. In sharp contrast to the fixation in two
286 locations in the Barents Sea, frequencies of the NEAC haplotype were low or non-existent in
287 more southern stocks and in the White Sea, while intermediate frequencies were found among
288 samples collected along the Norwegian coast north from Bergen (Borgund, Verrabotn,
289 Porsanger and Balsfjord) (Figure 2a). The NEAC haplotype was in HWE in all the stocks
290 examined. These results contrasts with the cluster analysis performed on all SNPs, excluding
291 the LG1 inversions and other genomic regions with suspected inversions due to large LD
292 blocks on LG2, LG7 and LG12 (Figure 2b). In this analysis NEAC from the Barents Sea are
293 clustering together with all the other samples.
294
295 *Origin and age of inversions*
296 To determine whether NEAC or NCC represent the ancestral state we aligned LG1 sequences
297 representing possible arrangements of the inversions with Northern pike and stickleback to
298 identify conserved synteny blocks spanning breakage points defining the inversions. This

analysis revealed a large block in pike spanning the break points flanking inversion 1, and a smaller block in stickleback bridging the two inversions (Figure 3b, Supplementary Figure S2). Taken together these results suggest that NCC represents the ancestral state of the inverted structure.

The relative SNP density between NEAC and NCC across LG1 was calculated using whole-genome resequencing data from samples classified on the basis of the NEAC-haplotype. Analysis of homozygous NEAC (n=50), homozygous NCC (n=11) or NEAC x NCC crosses (n=43) revealed 540,685 SNPs with an average sequencing coverage of 17x. Relative heterozygosity expressed as number of SNPs per 100Kb in NEAC divided by the number in NCC revealed a dramatically reduced SNP density in NEAC samples within the LD block (Figure 3). In contrast, the diversity outside the block was comparable for NEAC and NCC samples and to the rest of the genome.

The NEAC-NCC divergence relative to their divergence from Greenland cod were estimated to 0.57 and 0.13 within and outside the LG1 inversions, respectively. Assuming a divergence age of 3.5 million years between Greenland cod and Atlantic cod (Carr *et al*. 1999; Coulson *et al*. 2006), the inversion is estimated to be ~2 million years old (3.5*0.57=1.99). Although SNP data revealed no apparent genetic population structure between NEAC and NCC outside the inversion (Figure 2a), we find 1553 FSD counts on LG1 outside the inversion. These divergent FSD sites are likely caused by a sample bias within NEAC and NCC fish since they represent a narrow genetic pool of interrelated individuals from a breeding program rather than being a true random sample from both populations. Taking this background bias in FSD into account the normalized Greenland cod – Atlantic cod divergence within the inversion would be ~1.6 million years (3.5*(0.57-0.13)=1.57).

Candidate genes for adaptation to migratory behavior

We annotated the LG1 sequence to search for genes involved in the adaptive divergence between migratory (NEAC) and stationary cod (NCC). The annotation resulted in the prediction of 1262 gene models for the whole chromosome, whereof 763 genes were located within the 17.37 Mb region containing the two inversions (357 and 406 genes, respectively). Variant detection within the same region revealed 19,206 SNPs that were fixed or very close to fixation for alternative alleles in NEAC and NCC and heterozygous in NEAC x NCC crosses, and included 849 plausible functional variants in 321 genes presenting good hits in the SwissProt database (Supplementary Table S4). The corresponding protein variants containing several amino acid substitutions included key enzymes in swim bladder function and heme synthesis, and important factors involved in muscle organization and behavior (Figure 3). Carbonic anhydrase catalyzes the reversible conversion of carbon dioxide and water to bicarbonate and protons of importance for blood acidification and gas secretion into the swimbladder. The predicted NEAC and NCC variants of the secretory carbonic anhydrase (Ca6) differ at five positions, and the replacement of the highly conserved Gln196 with the novel His residue was shown by 3D modelling to reduce the interactions at the dimeric surface in the NCC variant (Figure 4, Supplementary Table S5). Dimeric assembly of this enzyme confers an advantage for efficient CO_2 hydration in a variable extracellular milieu, such as the strong pH fluctuations in the gas gland (Pelster 2004; Pilka *et al*. 2012), and we therefore predict reduced enzyme activity of the NCC variant. The inversions were found to harbor four additional genes involved in swimbladder function by regulating glucose uptake and production of acid metabolites. Glut1a facilitates glucose transport across cell membrane and is highly expressed in the gas gland cells of Atlantic cod (Hall *et al*. 2014). The NEAC and NCC variants of Glut1a differ at two positions that which, together with SNPs in the untranslated regions, might have functional and regulatory effects. We also noted many SNPs

in the genes encoding the three enzymes enolase 1 (Eno1), muscle-type phosphofructokinase (Pfkm) and glucose-6-phosphate dehydrogenase (G6pd) catalyzing the anaerobic conversion of glucose to the acidic metabolites lactate and CO_2.

The inversions also contained candidate genes associated with the strenuous migrations, such as two *alas* genes, which code for enzymes catalyzing the rate-limiting step in heme synthesis. Two aa substitutions were found in the erythroid-specific Alas2 of crucial importance for hemoglobin production. While no globin genes are located on LG1, the aa changes in the rhesus type B glycoprotein (Rhbg) may explain the reported differences in blood type frequencies between NEAC and NCC (Møller *et al*. 1966). Precise regulation of sarcomeric thin filament length is crucial for optimal force generation during muscle contraction. The muscle protein leiomodin 3 (Lmod3) is essential for the organization of thin filaments in skeletal muscle (Yuen *et al*. 2014; Nworu *et al*. 2015), and the predicted cod Lmod3 differ at four positions in NEAC and NCC. Intriguingly, the inversions contain the metabotropic glutamate receptor *mglur4* and *mglur7* genes, which are broadly expressed in the zebrafish brain, including olfactory bulb and retina (Haug *et al*. 2012). Three aa substitutions are located in a highly flexible region of cod mGlur7 (not shown), which in mice plays a significant role in hippocampus-dependent spatial learning (Goddyn *et al*. 2015)

Discussion

Population differentiation of Atlantic cod has been associated with four discrete islands of genomic divergence located on different chromosomes, and hitchhiking selection has been proposed as the underlying mechanism behind NEAC and NCC divergence (Bradbury *et al*. 2010; Hemmer-Hansen *et al*. 2013; Karlsen *et al*. 2013). However, the fragmented nature of

374 the current cod genome assembly (GadMor_May2010; Star *et al*., 2011) has largely restricted
375 our ability to identify genes associated with selection as well as our ability to reveal
376 alternative mechanisms responsible for the observed patterns. To overcome these constraints
377 we constructed a dense linkage map and integrated it with draft genome assemblies to
378 produce a cohesive chromosome sequence for Atlantic cod LG1. Separate linkage maps were
379 constructed for pure NEAC, pure NCC and NEAC x NCC crosses in order to study
380 differences in recombination patterns and potentially highlight rearrangements distinguishing
381 the two ecotypes. These analyses revealed two adjacent inversions of 9.55 Mb and 7.82 Mb
382 (Figure 1b), which clearly differentiate NEAC from NCC, as well as revealing a mechanism
383 resulting in almost complete suppression of homologous recombination in individuals
384 heterozygous for the inversions (Figure 1a). The presence of two inversions rather than one
385 have been shown to have an effect on the possibility for recombination and gene flow. While
386 recombination in single inversions of >20 Mb have been predicted by models and
387 documented in *Drosophila*, more complex inversion structures prevent double crossovers and
388 inhibit gene flow across the inverted regions (Navarro *et al*. 1997; Munte´ *et al*. 2005; Dyer *et*
389 *al*. 2007; Huynh *et al*. 2011).
390
391 Chromosomal inversions have been associated with adaptive phenotypes in various plants and
392 animals, including migratory species displaying high gene flow between the diverging
393 populations (Rieseberg 2001; Hoffmann *et al*. 2004; Hoffmann & Rieseberg 2008).
394 Polymorphic wing color mimicry in butterflies is maintained by chromosomal rearrangements
395 in the *Papilio* genus and in *Heliconius numata* (Joron *et al*. 2011; Nishikawa *et al*. 2015), and
396 a large inversion polymorphism in white-throated sparrow (*Zonotrichia albicollis*) was
397 recently shown to harbor genes displaying expression patterns correlated with territorial song
398 (Thomas *et al*. 2008; Huynh *et al*. 2011; Zinzow-Kramer *et al*. 2015). The repeated evolution

of distinct marine and freshwater ecotypes of three-spined stickleback involves three chromosomal inversions, and alternative orientations of the voltage-gated potassium channel gene *kcnh4* might generate marine- and freshwater-specific isoforms (Jones *et al*. 2012). In rainbow trout (*Oncorhynchus mykiss*), different life-history strategies of anadromous (steelhead) and resident ecotypes were recently shown to be associated with multiple loci with strong LD suggesting the presence of an inversion suppressing recombination (Pearse *et al*. 2014).

The absence of genetic differentiation between NCC and NEAC populations outside the inversions on LG1 supports previous conclusions of high levels of gene flow between migratory and stationary cod ecotypes in the North Atlantic Ocean (Hemmer-Hansen *et al*. 2013; Karlsen *et al*. 2013). However, the fact that the inversion is homozygous in NEAC, but polymorphic and under HWE in NCC populations, suggests that it is under strong directional selection in the migratory ecotype, while confer no fitness effects in the stationary ecotype. Gene flow between populations with divergent adaptive challenges can result in large fitness costs when recombination disrupts coinheritance of advantageous genetic variants. A genetic architecture that enforces strong LD between co-selected gene variants would therefore be highly favorable under extensive gene flow from divergent populations. Such 'supergenes' have been shown to maintain population specific adaptations in various organisms and is often caused by larger chromosome rearrangements (Joron *et al*. 2011; Thompson & Jiggins, 2014; Twyford & Friedman 2015). We therefore hypothesize that the inversions on LG01 act as a supergene to efficiently maintain co-inheritance of several highly favorable genetic variants, which over time have generated the island of genomic divergence observed between migratory and stationary ecotypes of cod.

Several genes associated with gas secretion into the swimbladder were identified within the LG1 inversions. The swimbladder is a crucial organ by maintaining neutral buoyancy that allows fish to stay at their current depth without expending much energy swimming (Fänge 1953; Pelster 2004). Hence, impairment of the swim bladder function was assumed to significantly threaten the success of the spawning migration in the European eel (*Anguilla anguilla*) (Pelster 2014). In a supergene context, this is intriguing because one of the obvious divergent adaptive challenges between NEAC and NCC populations is adaptation to high hydrostatic pressure at large depths. The foraging and spawning migrations of NEAC involve vertical movements at depths of 200-400 m along stable thermal paths (Stensholt 2001), while stationary NCC fish exploit much shallower habitats (Hobson et al. 2007; Michalsen et al. 2014). This is supported by behavioral differences between juvenile NEAC and NCC settling at different depths, whereas the pelagic eggs have similar buoyancy (Løken *et al*. 1994; Fevolden *et al*. 2012; Jung *et al*. 2012). Frequent descents and ascents lead to negative buoyancy, because gas secretion from the gas gland lags behind gas resorption in the swimbladder (Harden Jones & Scholes 1985; Godø & Michalsen 2000). This effect is amplified at greater depths, and the migratory NEAC should therefore benefit from enhanced gas secretion by increased blood acidification in the gas gland. The important role played by carbonic anhydrase in swimbladder function was demonstrated by inhibiting the enzyme activity in the gas gland that resulted in significantly reduced proton production and gas secretion (Fänge 1953; Skinazi 1953; Pelster 1995; Wurtz *et al*. 1999). While the reduced carbonic anhydrase activity predicted for the NCC variant might not be critical for fish inhabiting shallow coastal water, the ability to maintain buoyancy is probably crucial for NEAC during frequent vertical movements to large depths. The energetic costs associated with the strenuous migrations may be further reduced by increased oxygen delivery and

enhanced muscular capacity involving a suite of adaptive alleles identified within the inversions.

Similar to NEAC and NCC, Icelandic migratory and stationary cod populations inhabiting different depths show genetic differentiation at the same genomic region as found in the Norwegian cod populations (Grabowski *et al*. 2011; Pampoulie *et al*. 2008, 2015). This supports an old origin of the inversion polymorphism on LG1 associated with divergent migratory adaptations. We estimated that the inversion arose ~1.6-2 mill years ago during Pleistocene when glacial barriers and lowered sea level greatly influenced the abundance and distribution of marine species. This epoch probably represented the most important vicariance event in the evolution of Arctic fishes (Mecklenburg *et al*. 2011; Owens 2015). Atlantic cod survived in glacial refugia, but also moved southward to ice-free regions during the glacial periods (Bigg *et al*. 2008; Kettle *et al*. 2011). We propose that beneficial alleles were captured within the two inversions that occurred in an isolated refugial population and later became fixed. During interglacial periods local adapted individuals may have dispersed in the Arctic region and are today represented by the large migratory cod populations exploiting the high seasonal productivity in the most northerly environments on both sides of North Atlantic (Robichaud & Rose 2004).

In conclusion, we reveal a major difference in the genomic architecture of the migratory NEAC and stationary NCC ecotypes by documenting two adjacent inversions spanning 17.5 Mb on LG1 that effectively block recombination in individuals heterozygous for the inversions. Despite clear signs of interbreeding, this lack of recombination has caused a supergene comprising adaptive alleles related to the migratory ecotype to be preserved without dilution from the stationary ecotype.

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Data accessibility

All SNPs are referred to by their ss# or rs# available in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/).

Chromosome sequences: Stored at FTP-server hosted by NMBU

Genotype data from SNP array: Dryad (http://datadryad.org/)

Re-sequencing data, 104 farmed cod and 1 Greenland cod: Dryad (http://datadryad.org/)

Author's Contributions

S.L. and Ø.A. designed the study with input from T.G.K. H.G and M.P.K. T.G.K, S.L., H.G., S.R.S and T.N. analyzed the data. Ø.A., T.G.K. and S.L. examined candidate genes. T.J., H.O. and M.B. provided samples from family material and wild populations. M.B. and A.S. provided sequence data from the National cod breeding program. M.C.D.R and B.R. modelled the protein variants. Ø.A., T.G.K., H.G, S.R.S., M.P.K. and S.L. wrote the manuscript with contributions from all authors.

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Figure legends

Figure 1. (a) Linkage map for LG1 created separately for pure NCC and NEAC x NCC crosses. (b) Whole chromosome alignment between the NCC and NEAC sequence. (c) Pairwise LD calculated in 192 parents from the linkage families. Two large inversions inhibit recombination in NEACxNCC crosses corresponding to a region of extended LD on LG1.

Figure 2. Genomic divergence between NEAC and NCC. (a) Proportion of fish containing two (black), one (mid-grey) or no (light grey) copies of the NEAC-haplotype in different Northeast Atlantic stocks. (b) Hierarchical clustering of SNP variation excluding genomic regions with suspected inversions due to large LD blocks (LG1, LG2, LG7, LG12). NEAC and NCC were represented by red and black tips, respectively. The genetic distance was calculated as identity by state across 7238 SNP loci.

Figure 3. Graphical representation of two adjacent inversions on LG1 present in NEAC and NCC. The upper part show the relative difference in heterozygosity, measured as number of polymorphisms per 100kb in NEAC divided by the corresponding values in NCC. Conserved synteny blocks bridging inversion breakage points 1 and 2 suggest that NCC is holding the ancestral state of the inversions. Putative adaptive genes within the inversions are indicated.

Figure 4. Ribbon plots of the modelled carbonic anhydrase (Ca6) dimer interface in a) NEAC and b) NCC. The monomer subunits and key interacting residues (Supplementary Table S5) are given in different colors. The enlarged sections show the dimeric interactions of Gln(Q) and His(H) at position 196.

Supporting Information

Table S1. Linkage maps generated from 96 families of farmed cod. Maps and map distances were calculated separately for NEAC, NCC and NEACxNCC crosses, and also split between males and females.

Table S2. Predicted function of open reading frames were found with TransDecoder and homology search using BLASTP against zebrafish and stickleback protein databases.

Table S3. Reads from a Greenland cod and a NCC where aligned to the NEAC reference. Fixed sequence differences were counted for LG1, both inside the inversions (top right) and outside (bottom left).

Table S4. Genes with non-synonymous SNPs within the inversions. Number of individuals with reference or alternative alleles from NEAC, NCC and NEACxNCC cross are indicated together with transcript identities.

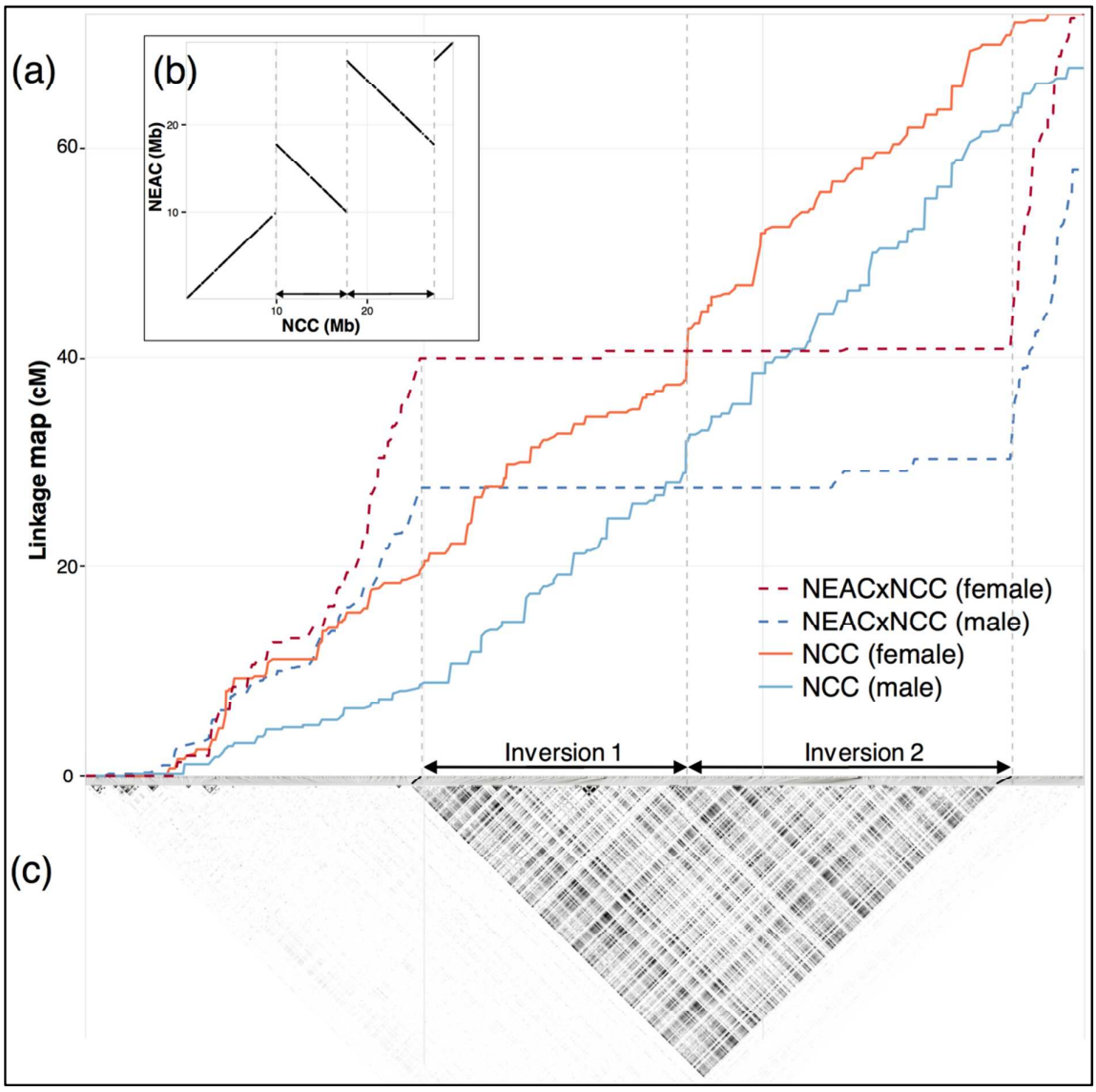
Table S5. Interdimeric contacts in carbonic anhydrase (Ca6) of NEAC (Gln196) and NCC (His196). Protein contacts (within 4.5 Å) in the interfaces between A- and B-monomers of the homology models are reported.

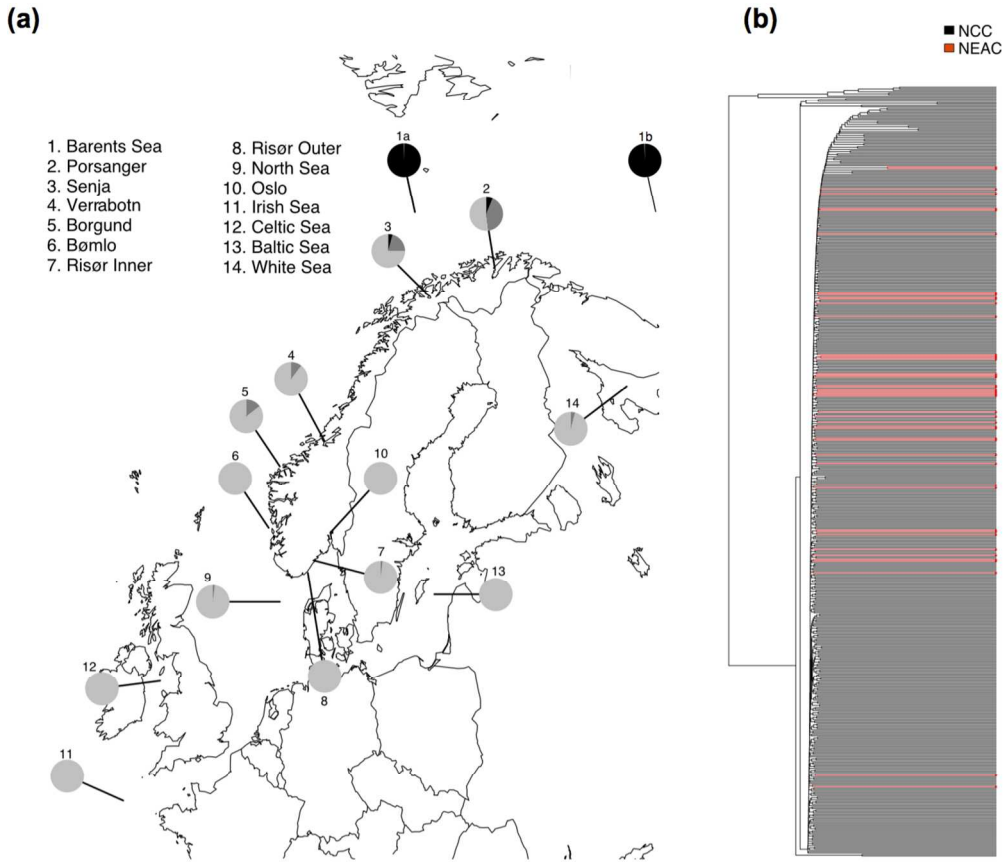
774 **Figure S1.** Figure S1. Pairwise LD for 48 NEAC, measured as r2, between all SNPs
775 (MAF>0.1) detected by re-sequencing on LG1. Left figure is the NCC map while right figure
776 is the NEAC map. Only values above r2=0.7 are shown. Circle indicates a region within the
777 second inversion being in high LD with a region at the end of the chromosome. The NEAC
778 map minimizes the distance between these two regions.

779

780 **Figure S2.** Comparative map using whole chromosome alignment between the NCC version
781 of LG1 and stickleback LGXIII (a) and pike LG12 (b).

Figure 1


(a)
(b)
(c)
NEAC (Mb)
NCC (Mb)
Linkage map (cM)
60
40
20
0
NEACxNCC (female)
NEACxNCC (male)
NCC (female)
NCC (male)
Inversion 1
Inversion 2



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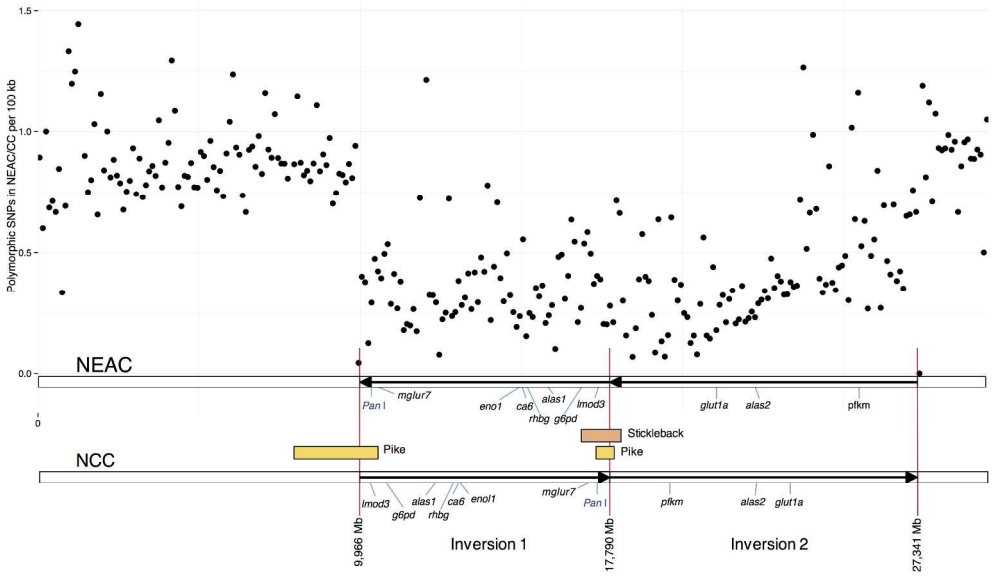

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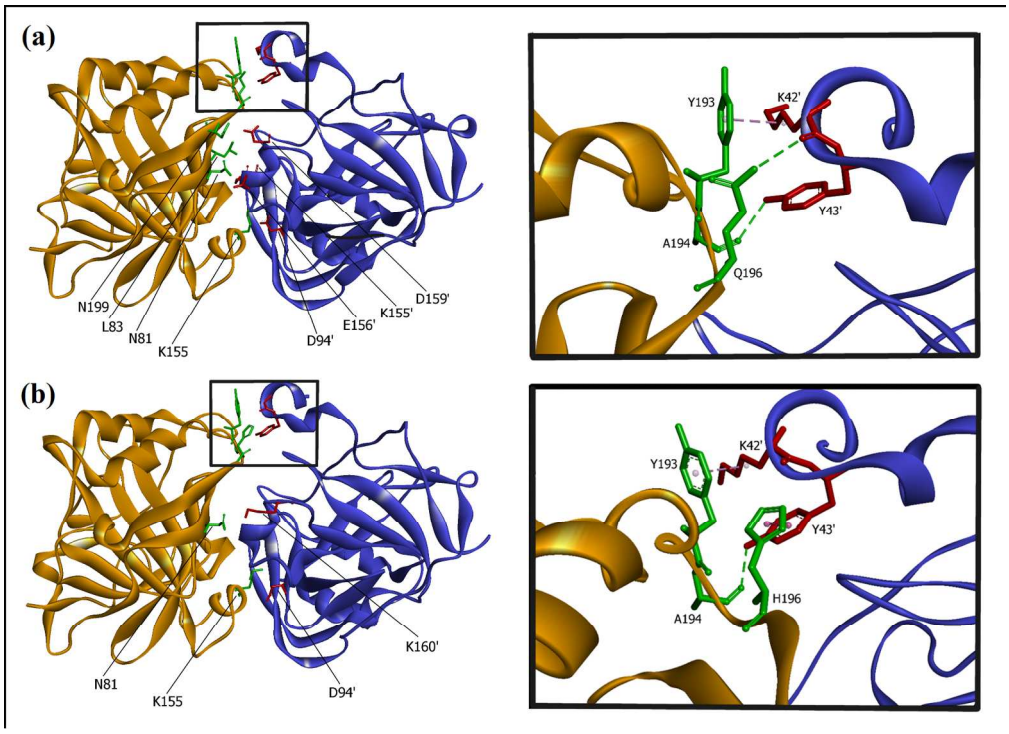

Figure 4. Ribbon plots of the modelled carbonic anhydrase (Ca6) dimer interface in a) NEAC and b) NCC. The monomer subunits and key interacting residues (Supplementary Table S5) are given in different colors. The enlarged sections show the dimeric interactions of Gln(Q) and His(H) at position 196.
484x350mm (96 x 96 DPI)