Widespread but spotty: Atlantic cod is a complex of five species through ecological speciation

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Abstract

Marine environments are home to a significant portion of fish and other organisms' biodiversity. However, our knowledge and empirical evidence of speciation in the marine realm is quite limited. Here we use genomics and study population differentiation and speciation in the Atlantic cod, a marine teleost of biological and economic importance. We show that the non-inversion part of the genome separates individuals into five clusters. At the four chromosomes carrying large inversions that define ecotypic variation, individuals also cluster into the same five clusters in conjunction with individuals' inversion genotypes. Within each cluster, individuals homozygous or heterozygous for the inversions are divergent and there is an adaptive divergence of

each type of inversion homozygote among clusters. These interchromosomal correlations imply the reproductive isolation of five cryptic species. We show that the inversion haplotypes are ancient trans-species balanced polymorphisms that would evolve towards homozygous lethality under Muller's ratchet and recombination suppression of inversions. However, gene flux from gene conversion rescues homozygous fitness, and recombination and selection lead to the evolution of habitat specialists through ecological speciation. The specialized genotypes actively seek out their preferred habitat resulting in a widespread but patchy distribution that contradicts the traditional geographical speciation model and the stock concept in fisheries biology. Our results highlight the power of genomics in studying selection and speciation while informing conservation and management of essential fisheries resources.

Keywords: Speciation, ecology, selection, adaptation, Atlantic cod

1 Introduction

Geography and geographical barriers to gene flow play an important role in models of population differentiation and geographic speciation where reproductive isolation arises as a by-product of divergence under allopatry [1]. In contrast, ecological speciation models [2, 3] propose that the primary drivers of phenotypic divergence are natural and sexual selection occurring in either allopatric or sympatric populations. In these models, the divergence of reproductive traits occurs as a consequence of the divergence and specialization of ecological traits facilitated by mechanisms such as pleiotropy or genetic linkage. Genomics often shows 'genomic islands of differentiation', clusters of loci that show large differentiation among populations in a sea of less differentiated genetic regions. Some are thought to be 'genomic islands of speciation', divergence under strong selection in the face of gene flow. The islands are also often associated with chromosomal structural variation, such as inversions that suppress recombination. Recombination in meiosis of inversion heterozygotes may lead to aneuploid gametes and lower fitness of heterozygotes (hybrids). Inversion may thus facilitate the evolution of post-mating isolation. Inversions can be ancestral polymorphisms associated with reproductive isolation via incomplete lineage sorting [4] or they can be trans-species balanced polymorphisms [5, 6].

The marine environment has few if any, geographic barriers, but isolation by distance is often thought to be important. Eggs and larvae can be dispersed over large areas by currents and some organisms, such as fish, are highly mobile and can actively search for suitable habitats, even over large distances. However, cryptic species are common in the sea [7, 8] and speciation may involve differences in chemical mate recognition, gametic incompatibility, and specialization in adaptive habitat utilization [9] influenced by sexual selection [3].

Marine fish species of significant commercial value often contribute to important fisheries managed as distinct stocks. The stock structure is a geographical concept derived in one way or another from the geographical (allopatric) speciation model. Fish stocks are considered subpopulations of a particular species with a particular ecology and life-history parameters (growth, reproduction, and mortality). Potential cryptic units are largely ignored, and stocks are managed using a geographical stock concept with regional or nation-state boundaries.

The genome of Atlantic cod (*Gadus morhua*) is liable to recurrent selective sweeps [10] showing that selective processes are of great importance. Atlantic cod and its related Pacific and Arctic gadid relatives [8] exhibit many features related to the above considerations of population differentiation. Four chromosomes carry large inversions related to ecotypic variation [11, 12] of deep-water-migratory and shallow-water-stationary behavioral ecotypes [13] as well as behavioral ecotypes of habitat choice related to salinity, oxygen, and temperature [14]. The chromosomal inversions are 'islands of divergence' [15] that are likely supergenes under balancing selection [8, 16, 17]. Additional complexity is also seen. For example, the presence of a distinct hybrid zone between Baltic cod and Danish Belt Sea cod [18] and with natural selection and adaptation to the specific conditions of the Baltic Sea contribute to reproductive isolation between the two populations [14]. Furthermore, certain localities have unusually high genetic diversity as demonstrated by variations in microsatellites in the North Sea [19] and biocomplexity of SNP variation at Western Greenland [20]. There are reports of parallel clines in the western and eastern Atlantic suggestive of selection [21] and of cryptic speciation [8, 22]. But no clear patterns have emerged for the variation in general.

Here we report findings from our 'two thousand-genomes cod project.' We have sequenced at medium to high coverage whole genomes of Atlantic cod from throughout its distributional range in the North Atlantic Ocean as well as other gadid taxa, the Pacific cod (*G. macrocephalus*) and walleye pollock (*G. chalcogrammus*) of the Pacific Ocean, Greenland cod (*G. ogac*) at western Greenland, and Polar cod (*Arctogadus glacialis*) and Arctic cod (*Boreogadus saida*) of the Arctic ocean. We provide conclusive answers to patterns of variation within Atlantic cod and lay the foundation for further studies of selection, recombination, speciation, and adaptation. We also reveal patterns and processes important for the conservation and management of important fisheries resources.

2 Results

2.1 Population resequencing across the codfish range

We sequenced 1,668 Atlantic cod from across the North Atlantic. We had reasonably large samples from most localities ranging clockwise from Cape Cod to the Faroe Islands (Fig. A1), including the largest sample of 828 individuals from Iceland (marginal sum in Table 2). Average sequencing depth was 10.8

(average of mean coverage) and ranged from 2.3 to 94.2; individuals with less than 2× depth or more than 0.1 missing variant calls were removed. We genotyped 17,886,971 SNPs in total across the genome after filtering with GATK best practices [23], for an estimated pairwise nucleotide diversity $\theta_\pi = 0.0019$ for non-inversion chromosome implying effective population size of 1.3×10^5 (assuming a mutation rate 3.62×10^{-9} per nucleotide per generation [10]). Compare that to effective population size 1.3×10^5 to 4.3×10^5 for a locally circumscribed population of *R* fish on the South/south-east coast of Iceland [10].

2.2 Local structure of genome

Previous work has characterized large inversions on four chromosomes, Chr01, Chr02, Chr07, and Chr12 [11, 12]. To verify the presence of these inversions and detect new inversions, we computed local structure [24]across the genome. Briefly, the method divides each chromosome into segments, performs PCA on each segment, estimates the pairwise distance between all segment-pair PCA results, and performs multidimensional scaling on the resulting pairwise distance matrix [24]. Every chromosome showed a local structure (Fig. B2). The `lostruct` analysis [24] showed that pairwise distances between chromosomal segments clustered in two or more groups. Four chromosomes stood out, Chr01, Chr02, Chr07, and Chr12, showed evidence for large inversions that have already been described [11, 12] and are related to ecotypic variation. Other chromosomes showed many peaks covering smaller chromosomal segments (Fig. B2). These may represent smaller chromosomal structural variation, such as small inversions, or they may represent population structure due to selection or drift. The chromosomal segments outside of the large inversions also showed multiple peaks for multidimensional scaling coordinate 2 (MDS2, Fig. B2) possibly related to adaptive differentiation of the haplotypes of each inversion.

2.3 Non-inversion chromosomes and population structure

In this study, we classified the four chromosomes containing large inversions as 'inversion chromosomes,' while the remaining 19 chromosomes not carrying large inversions (Fig. B2) were referred to as 'non-inversion chromosomes.' To assess population differentiation, we utilized principal component analysis (PCA) and admixture analysis [25]. We specifically distinguished between the two types of chromosomes mentioned earlier. Our previous findings indicate the presence of selective sweeps throughout the Atlantic cod genome [10]. Variation at inversion chromosomes may be maintained at intermediate frequencies due to balancing selection [8, 10, 17], while variation at non-inversion chromosomes may primarily reflect demographic processes. Although the non-inversion chromosomes may be distorted due to linked selection from the recurrent sweeps in the Atlantic cod genome, the decay of linkage disequilibrium is also very high [10].

2.3.1 Principal components analysis of non-inversion chromosomes

Principal components analysis (Fig. 1) showed that individuals clustered into five groups that we refer to as PCA group *R*, *S*, *SS*, *T*, *U*. The *SS* group is associated with shell-sand seabed (hence *SS*) on the Faroe Bank [26], and it is also a predominant group in the Breidafjordur Bay in Iceland close to the fishing ground for the Iceland clam (*Chlamys islandica*). This apparent correlation with a type of seabed may indicate specific habitat requirements or choices. The other groups were named by single letters around them. The groups were defined by clustering individuals on the first four principal components. Thus *U* was separate from the rest on PC2 and *S*, *SS*, and *T* separated on PC3 and PC4. The largest *R* group comprising 76% of individuals was very tight-knit. On PC3 and PC4 (but not on PC1), this group was centered on coordinate 0,0, which has been thought to indicate missingness of data [27]. However, even 90% missingness can allow recovery of population structure [28] and the centering of the largest cluster to the center of the multidimensional space may be but the pull of the large sample size, as our data were already screened for missingness (0.1). The admixture analysis of latent clusters (below) also supported the reality of these groups. Overall the first four PCs explained 76% of the variation (Fig. 1) and PC5 and PC6 added another 9%. The patterns of five groups were also seen for each of the individual non-inversion chromosomes (Fig. C3) so the observed pattern of the combined data (Fig. 1) was not due to averaging out individual differences but a general genomic pattern.

2.3.2 Admixture analysis of non-inversion chromosomes

Admixture analysis [25] also revealed the five groups detected by PCA with additional details (Fig. 2). Importantly the tight knit *R* group splits into six well-defined clusters at $k = 10$ ancestral populations while the other PCA groups remained distinct. All ten replicate runs of admixture showed similar results and pong [29] detected only a single mode for all replicate runs at each k. Individual chromosomes showed much the same pattern (Fig. C4). The differentiation among admixture populations measured by F (Table 1) was 0.07 to 0.12 among the five PCA groups (with the *T* group most divergent) and 0.01 to 0.02 among the population clusters within the *R* group (with pop6 most divergent; Fig. C4).

2.3.3 Spotty but widespread geographical distribution of PCA groups

The geographical distribution of individuals belonging to the five PCA groups was widespread but spotty (Fig. 3). For example the *S* group fish were found in the Baltic (particularly the eastern Baltic), the North, Celtic, and Irish Seas, the White Sea, and the Barents Sea, a spot in south Iceland and at Cape Cod on the east coast of north America. Similarly, the *SS* group was found in the western Baltic Sea, on the Faroe Bank (where it is associated with a seabed

of shell sand) but not on the Faroe Plateau, in one spot on the south coast of Iceland and the Breidafjord Bay on the western part of the island (where the seabed has an abundance of Iceland scallop *Chlamys islandica*) as well as in several other spots in the North Atlantic. A similar pattern holds for the other groups. *R* was found in all localities except the White Sea where only *S* and *SS* were found. The widespread but spotty distribution suggests either habitat fragmentation or very specific habitat requirements or habitat choice, a specialization of narrow niches.

2.3.4 Geographic distribution of clusters within PCA groups

Admixture showed that populations (latent clusters) within the *R* group were widely distributed and spotty but also unevenly distributed (Fig. 2, bottom). Thus, pop6, found in Iceland and several eastern localities (including Borgenfjord but not in Verrabotn (two adjacent infjords in Norway), was the most common type in the western localities. In contrast, pop5, found in the West, was more frequent in the eastern localities (Table 2). Asking if there is evidence for simple isolation by distance (under which frequency of a type decreases monotonically with geographic distance from the source) within the *R* group the answer is no. For example, the relative frequency of pop6 is U-shaped in Newfoundland, Iceland, and Borgenfjord (Fig. 2; Table 2).

Principal component analysis of the PCA groups also showed a complex connection to geography (Fig. 4). Thus within the *R* group, the western cod individuals (Cape Cod, Nova Scotia, and Newfoundland) were somewhat separate. Still, a fair number also overlapped with the Barents Sea and Iceland samples. Verrabotn, an in-fjord way inside the long Trondheim fjord in Norway, overlaps with the Faroe Islands across the Norwegian Sea and with Irish Sea samples but is distinct from Borgenfjord, which is a neighboring in-fjord in the Trondheim fjord. Borgenfjord, in turn, overlaps with some Icelandic samples (Fig. 4, **a**). Similarly, in the *S* group, Iceland samples are surrounded by Cape Cod samples, and Baltic and Barents Sea samples are close together (Fig. 4, **b**). The *SS*, *T*, and *U* groups also show the complexity of the connection between geography and genetic variation of separation and overlap. Overall, there was some effect of geography. However, there are no clear signs of expansion tracts from refugia [30] or isolation by distance in a regular manner. Therefore, it is unclear whether geography is an isolating factor or if the geographic locality is a proxy for some specific environmental conditions important for habitat choice.

2.4 Inversion chromosomes and genotypic variation

Principal component analysis of variation inside of the inversions of the inversion chromosomes (genetic variation that defines ecotypic variation) showed a complex pattern of clusters. Using a novel but simple method, we generated what we call the composite genotypes of the inversion haplotypes or haplotypic genotypes (Appendix D, Figs. D6, D7, D8, D10, and D11) in a robust

way (Fig. D12). The five PCA groups defined by variation at the non-inversion chromosomes (Fig. 1) and the haplotypic genotypes fully explained the clusters observed at the four inversion chromosomes (Figs. 5 and 6). Admixture analysis also showed the complexity seen in the PCA with heterozygotes showing close to a 50:50 population membership (Figs. D5). Differentiation among and within haplotypic genotypes was also evident and heterozygous genotypes were frequently not intermediate between the two respective homozygotes (Fig. 7).

2.4.1 Recombinational breakup of inversions

There was an apparent recombinational breakup of the inversions, a gene-flux [31] possibly from double-crossovers but more likely gene conversion [32]. The genotyping (Appendix D) showed that individuals that were called with a composite homozygous genotype frequently were heterozygous for individual sites. Similarly, individuals that were called composite heterozygous genotype frequently were homozygous for individual sites (Fig. D6). Often adjacent or close sites in individuals that were called heterozygotes were homozygous for both the reference and alternative homozygote (red and blue, (Fig. D6, top left) suggesting independent recombination events. There were 643 diagnostic sites covering 17 Mb of inversion on Chr01 (Fig. D6, top left), and thus, a site covers roughly a 25 kb segment on average. By the size of segments and independence of events, we suggest that these are signs of gene conversion tracts rather than double crossovers. In *Drosophila* the estimated size of gene conversion tracts is tens to a couple of thousand bp [Table S4 32]. If the size distribution of Atlantic cod gene conversion tracts is similar to *Drosophila* our observations taking a peek at 25 kb intervals revealed only the tip of the iceberg. There was a continuous distribution of numeric genotypic score with three modes (Fig. D6, bottom right). Principal component analysis showed that the numeric genotype score of heterozygotes of the four inversion chromosomes covered the genic space between the homozygotes and also extended beyond intermediateness (Fig. 8). There was a general heterozygote excess at the four inversions chromosomes (Table D3, although not significant for Chr12) overriding any potential Wahlund effect of population structure expected from large genotype frequency differences among localities (Appendix G, Figs. G23, G24, G25, G26, and G27). Overall, there were significant genotypic associations among the inversion chromosomes (Table D1). This effect was, however, not found considering a local Icelandic population that had been screened for population structure [10] (Table D2). Therefore, the the overall association was likely due to population structure rather than direct epistatic interactions. The frequency supports this differences of the various genotypes among localities (Appendix G, Figs. G23, G24, G25, G26, and G27). This analysis also shows how heterozygotes can be close to the predominant homozygote but also extend beyond intermediateness for all inversion chromosomes and the five PCA groups.

2.4.2 Trans-species and trans-oceanic supergenes

Maximum likelihood phylogenetic trees of individuals homozygous for alternative inversion haplotypes of the five PCA groups and the Arctic and Pacific gadid taxa revealed a trans-species and trans-oceanic relationships (Fig. 9 and Appendix E and Fig. E13) that was not observed among representative non-inversion chromosomes (Fig. E14). Reference-allele homozygotes were in one clade and alternative-allele homozygotes in another for all four inversion chromosomes. Yet there was also divergence among the PCA groups and gadid taxa within each homozygous genotype representing an inversion haplotype (Fig. 9). There were many other noteworthy patterns such as apparent paraphyly of Pacific cod and Greenland cod, the clustering of PCA group T with Pacific cod and Greenland cod, and the clustering of PCA group U and walleye pollock. The walleye pollock was the least polymorphic, and only a single homozygote for the reference allele was found for Chr07. The walleye pollock thus has a reduced frequency of the reference allele for Chr01, Chr02, Chr07 but not for Chr12 inversion (Fig. 9).

Treemix analysis showed similar trans-species relationships of the inversion chromosomes (Figs. E15, E16, E17, and E18) and not for the sample of non-inversion chromosomes (Figs. E19, E20, and E21). Here the individuals homozygous for alternative inversion haplotypes (Fig. 9) are lumped together to form a population. Treemix also showed evidence for introgression, often between the two homozygotes within a PCA group or species, but also between taxa. For example, there was evidence for migration between U and walleye pollock, between T and Pacific cod/Greenland cod, and polar cod and PCA group S, some of which were also seen for the non-inversion chromosomes.

3 Discussion

We show that the non-inversion part of the Atlantic cod genome, which is not associated with ecotypic differences, is differentiated into (at least) five groups. Population variation and differentiation of the four inversion chromosomes that code for ecotypic variation important for differential niche utilization is accounted for by these five groups account in conjunction with inversion genotypes. The evolution of these groups and genome-wide interchromosomal correlations can only have happened in reproductively isolated units. Thus, the Atlantic cod is a complex of at least five hitherto cryptic species [7, 33, 34]. We further show that the inversion supergenes [8, 16, 17], 'islands of divergence' related to ecotypic variation, are ancient trans-species and trans-ocean balanced polymorphisms that are older than speciation of the Atlantic, Arctic, and Pacific gadid clade.

Previously we showed that recurrent selective sweeps permeate the Atlantic cod genome, a selection that may be chasing ephemeral optima but also driving longer-duration adaptive traits such as those of balanced polymorphisms [10]. The following ecological speciation scenario is fully compatible with our

results. Inversion haplotypes (e.g. A and B) will rapidly diverge due to selective sweeps within each. Deleterious mutations will hitch-hike, drift due to lowered effective population size, fix, and accumulate in both homozygotes (e.g. AA and BB) sinking them to lethality with Muller's ratchet [35–37] leading to increasing overdominance [37] as suggested by a general heterozygote excess. In the absence of recombination this system would evolve to become a balanced lethal system [38]. However, rampant gene-flux [31] likely due to gene conversion [32] rescues homozygous fitness and allows heterozygotes and homozygotes, in turn to explore fitness space providing material for divergent selection leading to divergent haplotypes of ecologically relevant variation. The ecotypic haplotypes branch out, for example, with haplotype structuring of bimodal fitness distribution of homokaryotypes [37] (with free recombination within homokaryotypes), and diverge either allopatrically or sympatrically [39] by natural selection and possibly also by sexual selection [40–42] into specialized niches and habitat specialization by depth, temperature, salinity, type of bottom substrate, and so on. They then breed within their habitat of choice defined by fine-scaled environmental differences [43] so that their ecology defines reproductive isolation, a case of ecological speciation [2, 39, 44]. The specialized types actively seek out the preferred habitat that they are adapted to, resulting in a widespread but spotty geographic distribution.

Speciation-with-gene-flow [45, 46] was proposed to explain population differentiation in Atlantic cod [22, 47]. The model assumes low levels of differentiation in large parts of the genome because of ongoing gene flow, which homogenizes those genomic regions. Under this model, 'islands of genomic divergence,' genomic regions showing high differentiation, are loci connected to isolating traits between the incipient species, speciation genes in effect. Introgression of islands is prevented by selection, but recombination homogenizes the rest of the genome. This model assumes the establishment of interchromosomal linkage disequilibrium, although chromosomes assort independently. Maintaining such a strong association at unlinked loci at different chromosomes implies that most recombinant genotypes are less viable than non-recombinants. Only genotypes with the correct allelic combination survive. Even with a model of only few islands participating the number of gametes that can form viable zygotes will be very small [48]. Although the high-fecundity Atlantic cod has high reproductive excess and should be able to withstand large selection pressures [10, 49] such strong selective forces should lead to the evolution of effective reproductive barriers between the forms. A model of divergence-after-speciation [48] is an alternative to the model of speciation-with-gene-flow. Under this model, there is no differential gene flow among loci. Instead, reproductive isolation is rapid, even instantaneous, and complete, as in the scenario above. The divergence-after-speciation model explains the low level of divergence throughout most parts of the genome as shared ancestral polymorphisms. The more recent the split, the more homogenized the genomes. The elevated differentiation in the 'islands of divergence.' is explained as being

due to heterogeneous natural selection over the genome but not due to heterogeneous migration rates. The genomic islands are loci of ecological adaptations. This model also explains linkage disequilibrium between islands on different chromosomes because ecological adaptation within species involving genes of different chromosomes necessarily results in inter-chromosomal correlations.

The above speciation scenario settles the major features of the results. But there are several other patterns in the data that will be the basis for further analysis. The F_{ST} differentiation of non-inversion chromosomes within the R group is similar to the differentiation of neutral loci in a sensory drive speciation in cichlids [50]. A rhodopsin polymorphism important for Atlantic cod vision at different depths and under turbid conditions [51] is inside the Chr01 inversion and several other opsin genes are located within the inversion as well. Is the differentiation of the R group a case of incipient speciation along similar lines [50]? The widespread, partly overlapping, but spotty distribution of the species we have found, has a bearing on several previous findings. For example, a study of a hybrid zone of Baltic cod [18] possibly or even likely sampled primarily S in the eastern Baltic, SS in the transition zone, along with R in both localities. Unusually high genetic diversity of microsatellites in the North Sea [19] and pronounced biocomplexity of SNP variation at Western Greenland [20] are localities where four of our species co-occur. Parallel clines in the western and eastern Atlantic suggestive of selection [21] may have sampled cryptic units to different extents and suggestions of cryptic speciation [8, 22] accord well with our findings. The claim of 'Two races of cod at Faroe' [52] are understandable as the SS and R species found at the Faroe Islands. Our results may shed light on the enigma of the walleye pollock niche shift [8] which may have all but sorted out certain chromosomes. Paraphyly and trans-ocean relationship of deep lineages within the Pacific cod/Greenland cod duo suggests a more complex situation than the notion that Greenland cod is a recent re-invasion of Pacific cod into the Atlantic at Greenland [53]. The results of widespread and spotty distributions are at variance with an allopatric geographic mode of differentiation and also are a challenge for the geographical stock concept of fisheries management for cod.

4 Conclusion

We conclude that the Atlantic cod is a complex of five cryptic species that have adapted to specific habitats. Trans-species balanced inversion polymorphisms subject to recurrent selective sweeps [10] and recombination are important in this process. One species split into five highlights the importance of genomics for elucidating cryptic biodiversity in the marine environment.

The results are important for conservation and management of important fisheries resources. However, much remains to be done. What is the origination and evolutionary age of the trans-species balanced polymorphisms? Does hybridization and introgression play a role? Can we quantify selection and recombination involved in the processes described here [54, 55]? What specific

genes are involved in the adaptation and speciation, and what is the role of potential structural variation at the non-inversion chromosomes? How old are these speciation events, and what is the role of migration and introgression in these processes? How can we simulate the complex selection and demography involved [56] to test the hypothesis quantitatively? Answering these and other such questions is beyond the scope of this paper. We will address them subsequently.

5 Tables

Table 1 Differentiation of populations revealed by principal component analysis and admixture. Pairwise F_{ST} for non-inversion chromosomes among populations revealed by PCA and `admixture`[25] analysis for $k = 10$ ancestral populations (Fig. 2) and labeled by PCA group (Fig. 1).

	Principal Component Analysis (PCA group)								
	R						*S*	*SS*	*T*
	Admixture population (pop)								
	pop0	pop3	pop4	pop5	pop6	pop9	pop2	pop7	pop8
*R*_pop3	0.007								
*R*_pop4	0.006	0.007							
*R*_pop5	0.008	0.008	0.007						
*R*_pop6	0.019	0.019	0.018	0.017					
*R*_pop9	0.006	0.007	0.006	0.009	0.019				
*S*_pop2	0.096	0.096	0.095	0.092	0.092	0.098			
*SS*_pop7	0.083	0.084	0.081	0.080	0.082	0.085	0.052		
*T*_pop8	0.114	0.115	0.113	0.111	0.110	0.117	0.076	0.067	
*U*_pop1	0.073	0.074	0.072	0.071	0.073	0.075	0.083	0.073	0.085

Table 2 Frequency of types revealed by principal component analysis and admixture in relation to geographic locality.

	Principal Component Analysis (PCA group)										
	R						*S*	*SS*	*T*	*U*	
	Admixture population (pop)										
	0	3	4	5	6	9	2	7	8	1	Sum
Cape cod	0	0	0	0	6	0	24	0	0	0	30
Nova Scotia	4	2	0	7	42	2	0	0	1	0	58
Newfoundland	0	2	2	1	61	4	0	0	1	0	71
Greenland	8	4	8	15	41	4	0	8	8	9	105
Iceland	144	130	139	58	29	253	3	30	41	1	828
Barents Sea	4	2	8	13	11	2	12	40	0	0	92
White Sea	0	0	0	0	0	0	1	4	0	0	5
Norway coastal	0	4	6	12	23	0	0	0	8	10	63
Verrabotn	14	5	17	31	0	1	0	0	27	0	95
Borgenfjord	2	5	4	6	34	1	0	0	5	1	58
North Sea	0	0	1	1	0	1	31	0	31	22	87
Baltic Sea	0	2	3	2	0	0	18	18	0	0	43
Celtic Sea	0	0	1	0	0	0	7	0	0	0	8
Irish Sea	4	3	7	5	0	1	8	6	0	0	34
Faroes	17	8	21	28	0	1	0	16	0	0	91
Sum	197	167	217	179	247	270	104	122	122	43	1668

6 Figures

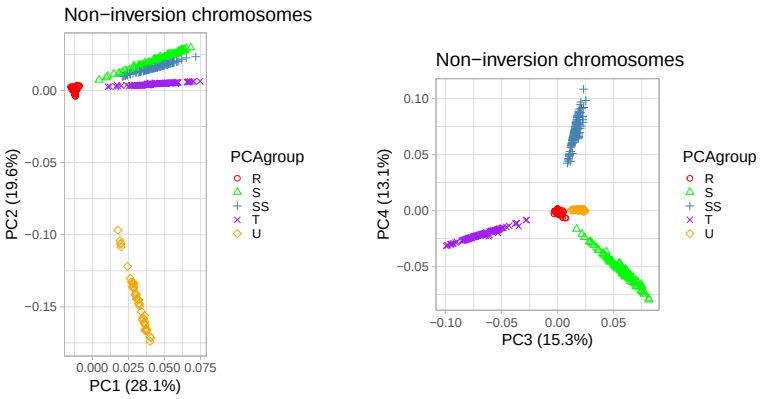

Fig. 1 Principal components analysis (PCA) of non-inversion chromosomes in Atlantic cod. PCA of the 19 non-inversion chromosomes combined (Chr03 to Chr06, Chr08-Chr11, and Chr13-Chr23) shows five groups *R*, *S*, *SS*, *T*, and *U*. PC1 to PC4 explain 76% of the variation. Bi-allelic SNPs with singletons excluded, LD pruned, and thinned to 450,953 SNPs.

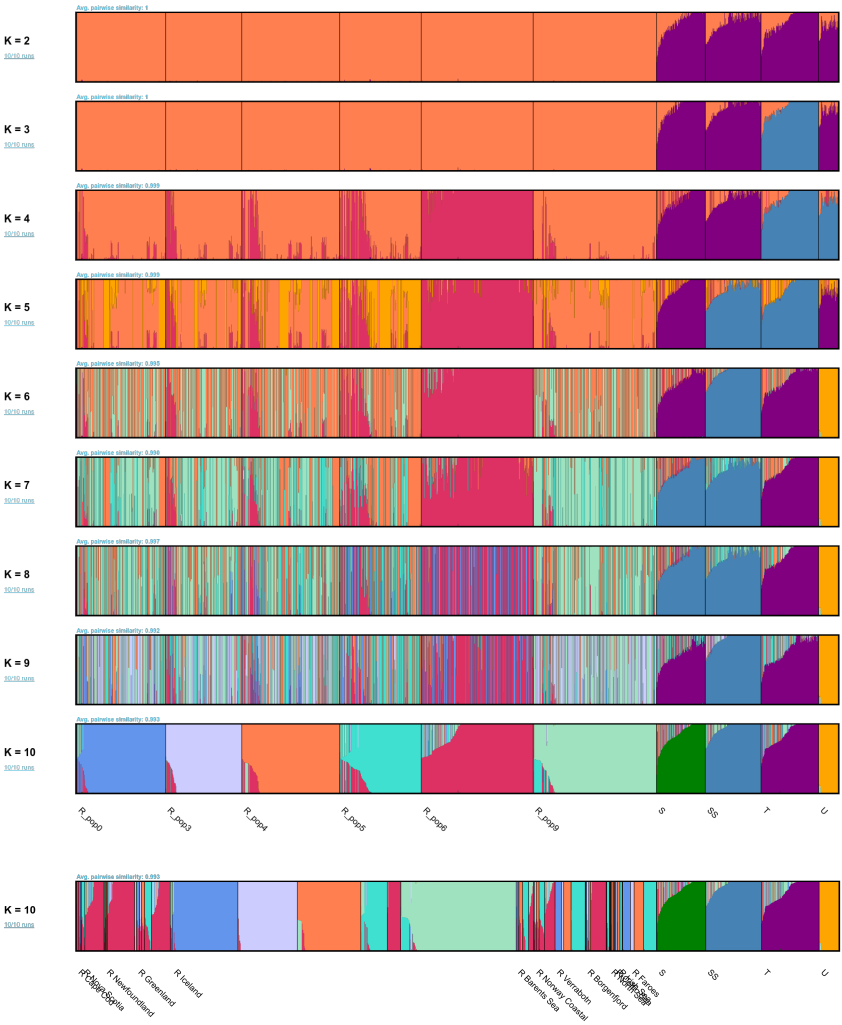

Fig. 2 Admixture analysis of non-inversion chromosomes. Pong [29] analysis and display of 10 `admixture`[25] runs for non-inversion chromosomes. Admixture runs for $k = 2 \ldots 10$ with individuals assigned to populations detected by admixture (pop0 ... pop9) by maximum membership at $k = 10$ and labeled according to the admixture population (top panel). The PCA groups S, SS, T, and U (pop1, pop7, pop8, and pop2 respectively) are separate and different from R. The R group is heterogeneous and admixture detects six clusters of R at $k = 10$. All ten admixture runs agree for every $k = 2 \ldots 10$. Color indicates population revealed by admixture for $k = 10$ and R populations are each labeled by populations revealed by admixture (top) and ordered by geographic locality (ranging from Cape Cod in the west to the Faroe Islands in the east) and admixture population within the locality indicated by color (bottom).

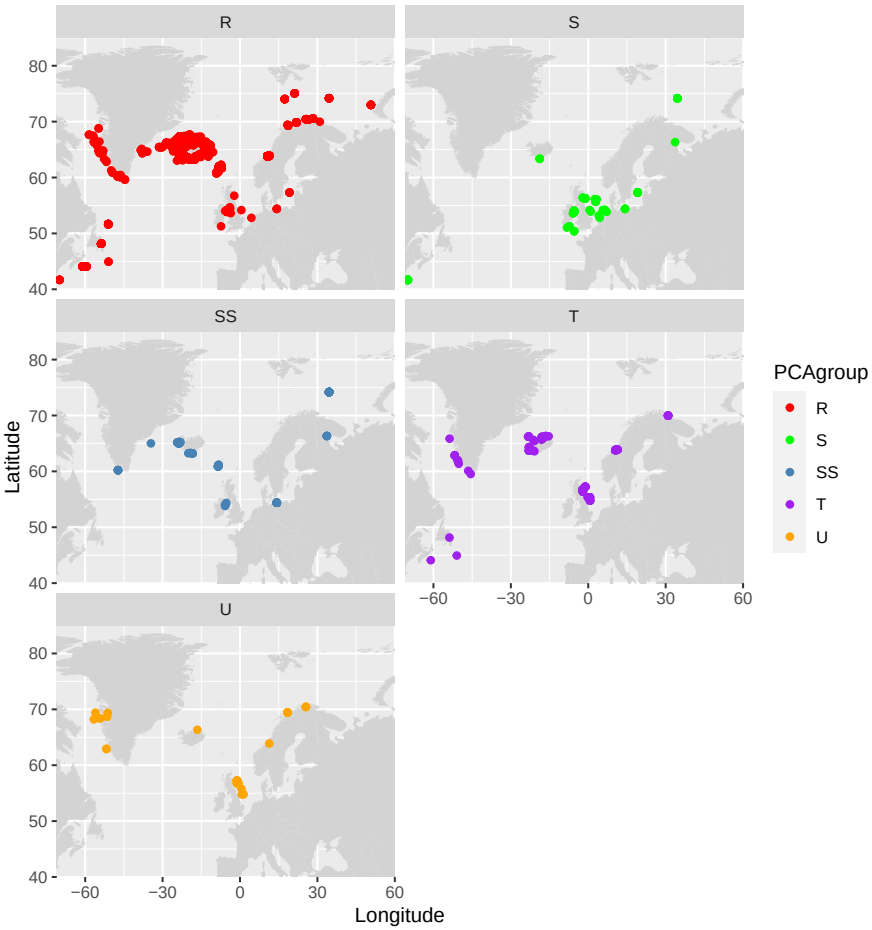

Fig. 3 Widespread but spotty. The geographic distribution of individuals from the five PCA groups on a map of the North-Atlantic.

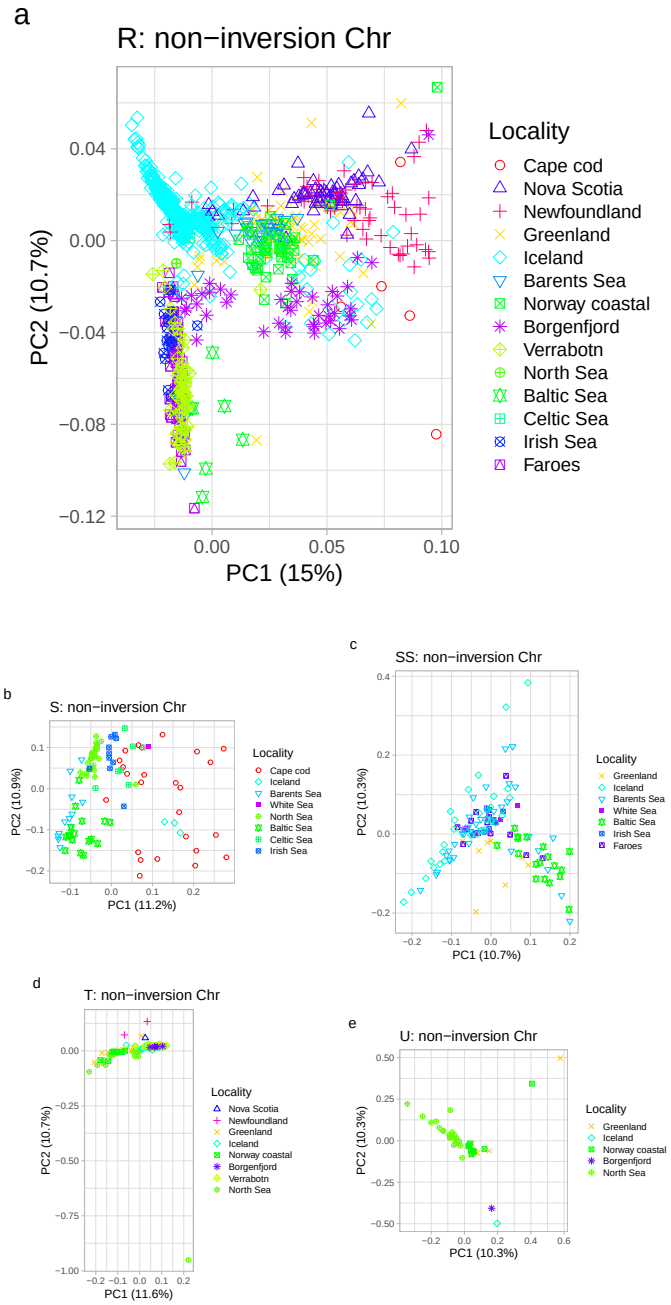

Fig. 4 Principal component analysis of non-inversion chromosomes by PCA group and geographic locality. PCA of PCA group R (**a**), S (**b**), SS (**c**), T (**d**), and U (**e**) labeled by geographic locality ranging from Cape Cod to Faroe Islands. The R group PCA was based on 1218 individuals after removing 52 outliers that compress the PCA results.

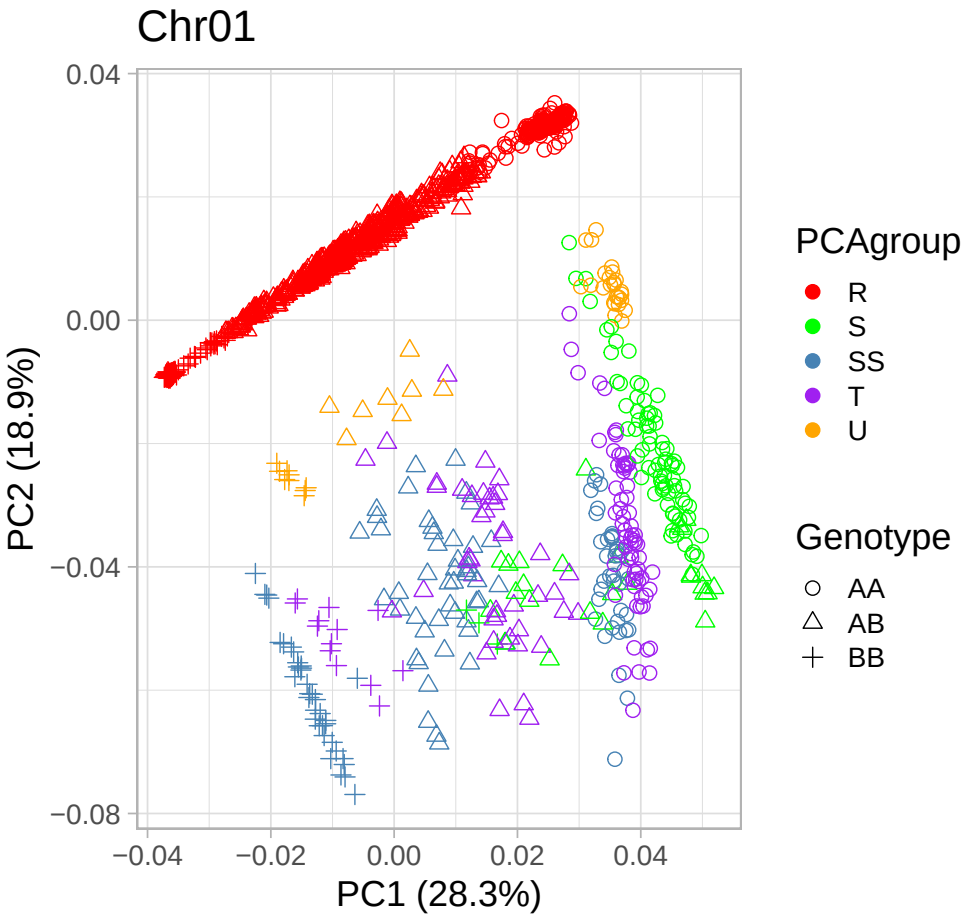

Fig. 5 Principal components analysis of inversions on Chr01. PCA of inversion region between co-ordinates 11,763,336 and 27,936,178 labeled by PCA group (Fig. 1) and inversion genotype (Fig. D6). Bi-allelic SNPs with singletons excluded and LD pruned 1,260,547 SNPs.

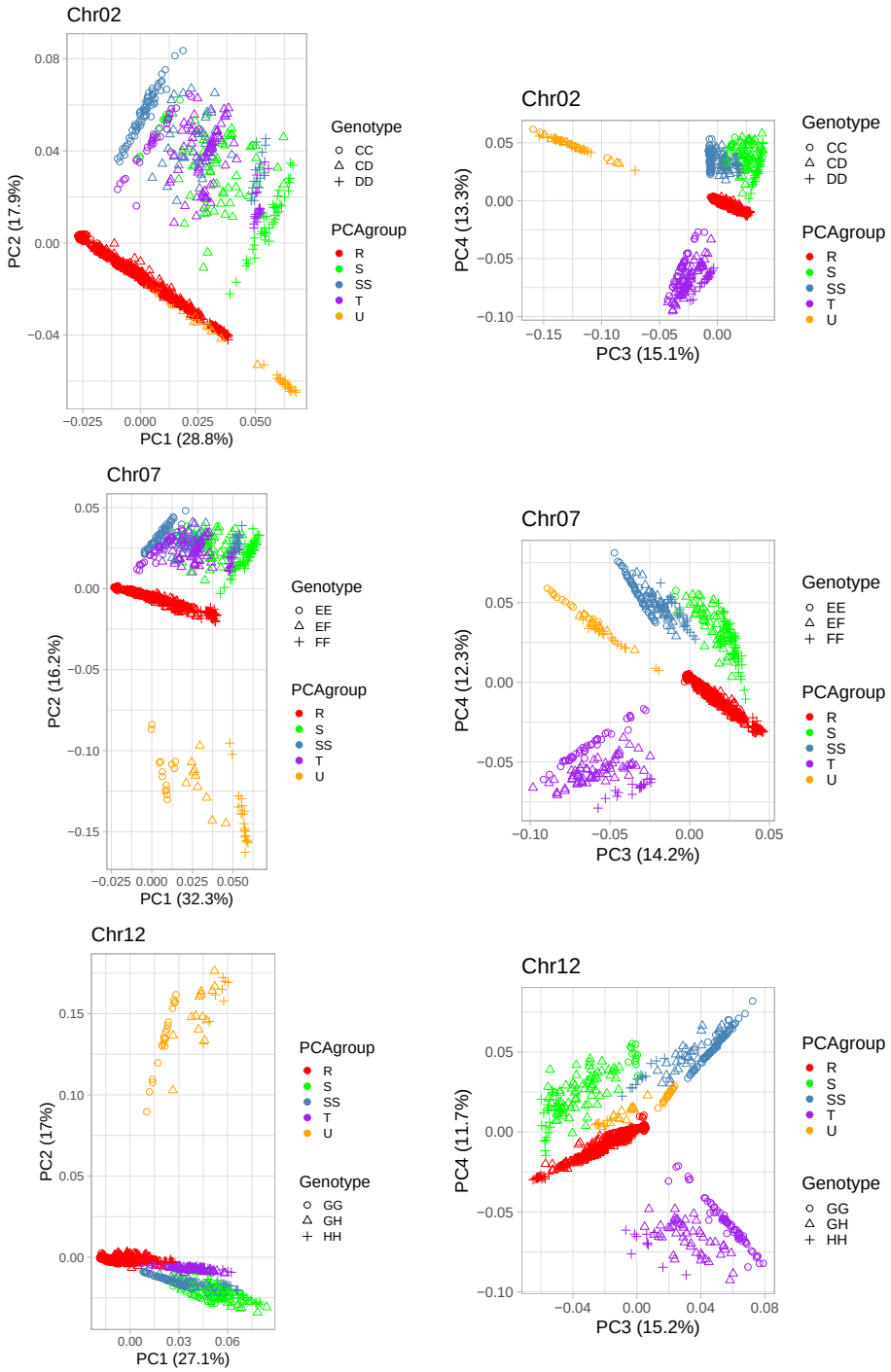

Fig. 6 Principal components analysis of inversions on chromosomes 02, 07, and 12 are classified by PCA group and inversion. PC2 on PC1 and PC4 on PC3 explain 75, 75, and 71% of variation respectively. Chromosomal coordinates inside inversions: 579,097 to 4,096,513 for Chr02, 17,004,745 to 25,979,379 for Chr07, and 1,001,809 to 13,965,277 for Chr12.

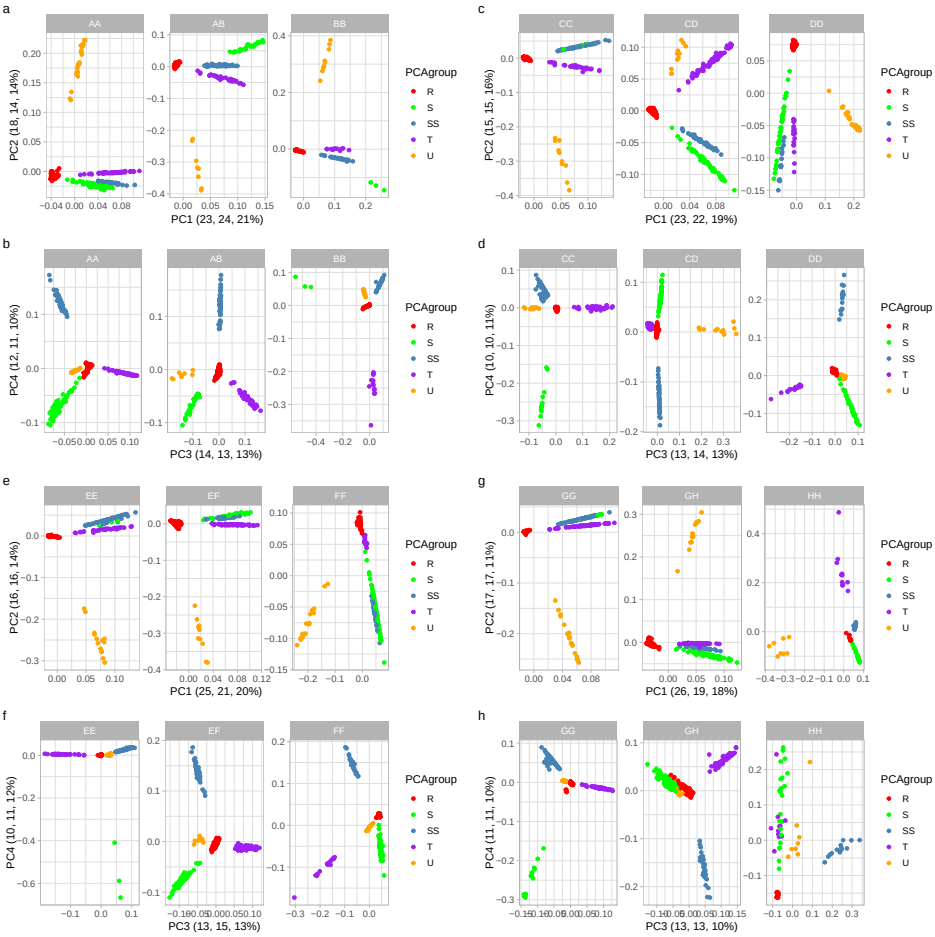

Fig. 7 Differentiation of haplotypic genotypes of inversion chromosomes. Principal components analysis of inversion genotypes labeled by PCA group. PC2 on PC1 and PC4 on PC3 for Chr01 (**a** and **b**), Chr02 (**c** and **d**), Chr07 (**e** and **f**), and Chr12 (**g** and **h**) respectively. The percentage of variation explained for the three genotypes, and each PC is given in parentheses. Chromosomal coordinates inside inversions: 11,763,336 to 27,936,178 for Chr01, 579,097 to 4,096,513 for Chr02, 17,004,745 to 25,979,379 for Chr07, and 1,001,809 to 13,965,277 for Chr12. Bi-allelic SNPs with singletons excluded and LD pruned.

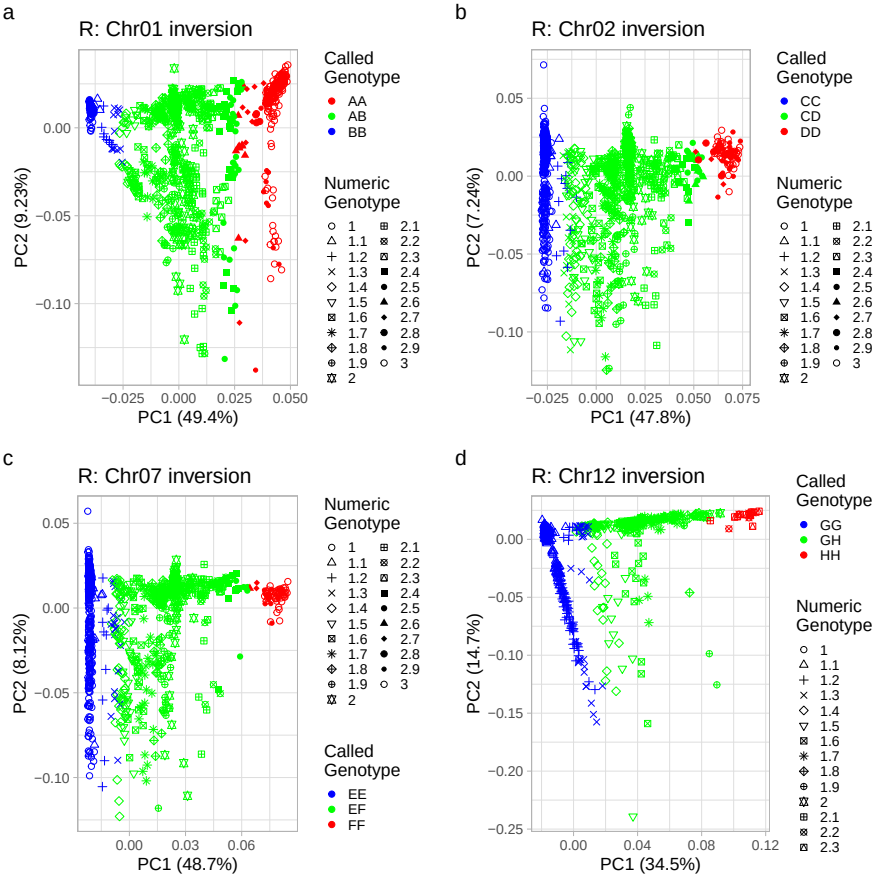

Fig. 8 Principal component analysis of inversion chromosomes of PCA group *R* labelled by called and numeric genotype. Called and numeric genotypes based on inversion genotyping (Fig. D6).

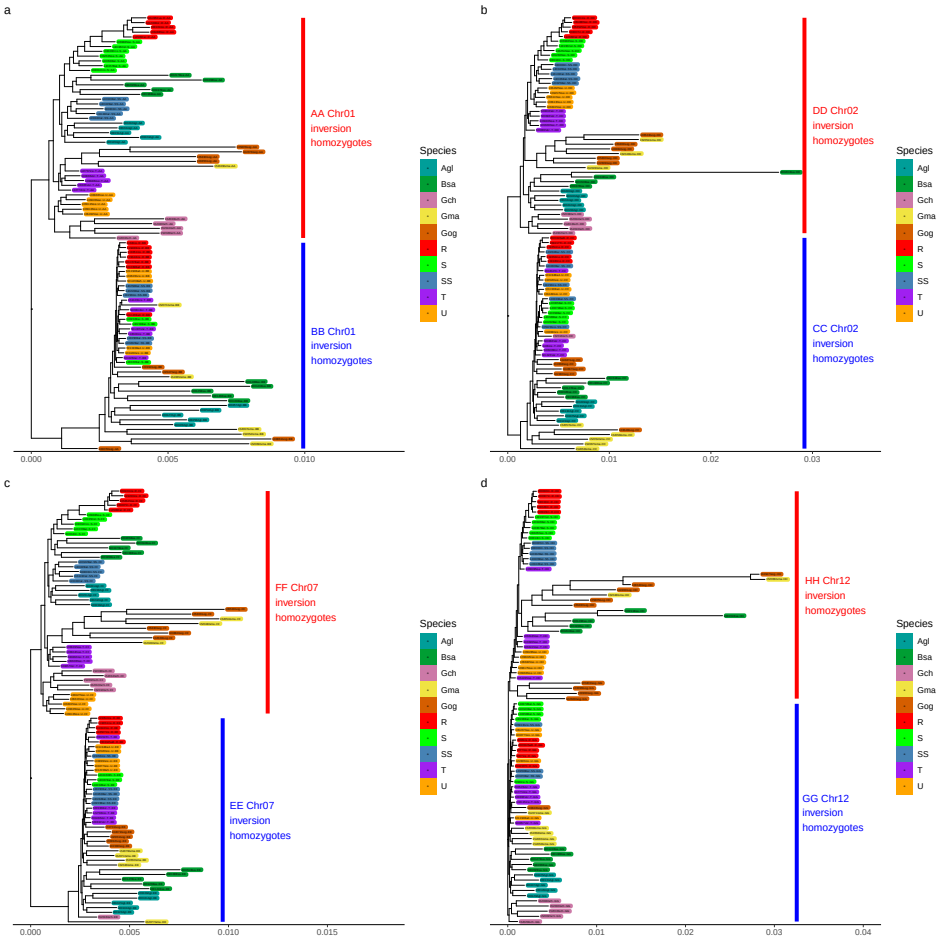

Fig. 9 Trans-species polymorphism of inversion haplotypic genotypes. Maximum likelihood phylogenetic trees of inversion homozygotes of PCA groups and related gadid taxa for Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**). Best substitution model estimated with `ModelFinder` [57] and trees estimated with `iqtree` [58]. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The blue and red bars indicate homozygotes for the reference and the alternative allele, respectively. Chromosomal coordinates inside inversions: 11,763,336 to 27,936,178 for Chr01, 579,097 to 4,096,513 for Chr02, 17,004,745 to 25,979,379 for Chr07, and 1,001,809 to 13,965,277 for Chr12. All trees are subtrees excluding outgroups from trees in Fig. E13.

7 Methods

7.1 Sampling

We obtained tissue samples from our large DNAfish tissue sample base that has been already been described [8, 10]. We sampled $n = 1688$ Atlantic cod from throughout its distributional range in the North Atlantic Ocean (Fig. 3). of Atlantic cod from throughout its distributional range in the North For comarison and phylogenetic analysis of inversion polymorphisms we sampled Pacific cod and walleye pollock of the Pacific Ocean, Greenland cod at western Greenland, and Polar cod and Arctic cod of the Arctic ocean. We used single specimens of haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) as outgroups for phylogenetic analysis.

7.2 Ethics statement

The DNAfish tissue sample collection was built with ethical board review as already described [8, 10].

7.3 Molecular analysis

Genomic DNA was isolated and shipped to the Bauer Core Facility at Harvard University. Libraries were made with Kapa HyperPrep Plus kit (Kapa Biosystems) for enzymatic DNA fragmentation and adapter ligation indexed using IDT (Integrated DNA Technologies) unique dual 8 bp indexes for Illumina, and sequenced on NovaSeq (Illumina) S4 flow cells with a 2×150 bp read format as already described [8, 10].

7.4 Bioinformatic analysis

The sequencing returned de-multiplexed `fastq` files for different runs of each individual. Data processing followed the Genome Analysis Toolkit (GATK) best practices [23] as implemented in the fastq_to_vcf pipeline of Alison Shultz (github.com/ajshultz/comp-pop-gen). Using the pipeline the raw reads were adapter trimmed using `NGmerge` [59], the trimmed `fastq` files aligned to the gadMor3.0 chromosome-level reference genome assembly (NCBIaccessionID:GCF_902167405.1) using `bwa mem` [60], and the resulting `bam` files deduplicated, sorted, and indexed with `gatk` [23] as already described [8, 10]. To go from `bam` to `VCF` files we used snpArcher https://github.com/harvardinformatics/snpArcher, which implements GATK best practices for variant calling [23].

7.4.1 Population analysis

For subsequent population-level analysis, we relied heavily on `samtools` and `BCFtools` [61], `VCFtools` [62], `plink` [63, 64], and `linux` command line tools (`bash`, `awk`, and `sed`) to manipulate data, select or combine individual chromosomes or inversion regions. We selected bi-allelic SNPs (`bcftools view`

`-m2 -M2 -v snps`). For analysis of the population structure and admixture, we first removed singletons (with minor allele count greater than 2 `plink --mac 2`) followed by LD pruning (`plink --indep-pairwise 50 10 0.1`) 50 kb segments with 10 kb window and $r^2 < 0.1$) followed by random thinning of remaining SNPs for computationally heavy analysis (`plink --thin 0.10`, keeping 10% of remaining SNPs, or `--thin 0.01` for even more thinning). We studied local heterogeneity in the genome and looked for inversion using the `lostruct` `R` package [24]. We studied population structure performing principal component analysis of various chromosomes or chromosomal regions, such as inversions. (PCA, `plink --pca`) and analyzed and plotted results with the `tidyverse` environment [65] in `R`. We inferred population structure and individual ancestries with `admixture` [25]. We ran 10 replicates for each $k = 2 \ldots 10$ ancestral populations. We analyzed and visualized the `admixture` runs with `pong` [29].

7.4.2 Inversion and non-inversion chromosomes

Four chromosomes (Chr01, Chr02, Chr07, and Chr12) carry large inversions that are clearly visible in the genome and have been described and tied to ecotypic variation and selection [10–12, 66, 67]. We refer to them as the inversion chromosomes. The remaining 19 chromosomes may have smaller structural variations and are subject to selective sweeps and rapid LD decay [10]. We refer to them as the non-inversion chromosomes.

7.4.3 Genotyping inversions

To genotype inversions, we used a well-defined population from the the south coast of Iceland, which we already had genotyped using PCA and analysis of site frequency spectra [10]. We took x individuals of one homozygote and y individuals of the other, used `plink --count-geno` to count the number of individuals of each genotype at all sites within the inversion or the whole chromosome, and defined as diagnostic sites, all sites that returned exactly x homozygotes for the reference allele and y individuals homozygous for the alternative allele (or y and x if unsure which group is which homozygote). We then used the diagnostic sites thus defined for the South/south-east population in Iceland [10], which belongs to the R PCA group, to genotype the inversions among the PCA groups and among the other gadid taxa. Some of the sites that were diagnostic for R were not diagnostic for, say, the SS group, but the genotyping works. We gave homozygous reference allele (0/0), a numeric score of 1, heterozygotes (0/1), a score of 2, and homozygous alternative allele (1/1), a score of 3. We found the numeric genotype score as the marginal mean over sites for each individual and plotted a heatmap with hierarchical clustering of individuals using the `pheatmap` `R` package [68]. Because of the population structure within taxa, differences among taxa, and the recombinational breakup of inversion, the diagnostic sites based on the local Icelandic population did not yield 'pure' composite genotypes in some cases. To call the composite genotype of an inversion in an individual we inspected the mean

numerical genotype, the heatmap, and the hierarchical clustering and drew a line to separate homozygotes from heterozygote. To determine the robustness of the method we took individuals from the *SS* PCA group called as homozygous based on the *R* PCA group and used the method to find sites that were diagnostic for the inversion within *SS*. We then genotyped *SS* using the sites diagnostic for *SS*. The differences were minor and always involved individuals that were on the boundary of being called homozygous or heterozygous.

7.4.4 Analysis of hybridization and phylogeny

We studied the relationships of inversion haplotypes among the PCA groups and the various gadid taxa. We randomly selected five homozygotes for each haplotype with the highest sequence coverage (fewer than five if the haplotype frequency was low). We used the `vcf_to_treemix.py` script from the Pop-Gen Pipeline Platform `PPP` [69] to generate input for studying population splits and a mixture of the inversion regions of the inversion chromosomes (Chr01, Chr02, Chr07, Chr12) for the PCA groups and gadid taxa with `treemix` [70]. In some instances, five homozygous individuals were not found in the sample, so we used what we had. We ran maximum likelihood `treemix` analysis with migration edges $m = 0 \ldots 5$ treating each inversion genotype as a population of five individuals. We used the individuals selected for Chr01 inversion to do the analysis for non-inversion chromosomes Chr04, Chr17, and Chr23. We analyzed and visualized the results with the `plot_treemix` `R` function from the `popcorn` package [71].

We generated nucleotide sequences for the individuals of PCA groups and gadid taxa selected for the `treemix` analysis with `vcf_to_seq.py` script from `PPP`. We also included sequences for the haddock and whiting outgroups. We estimated the most appropriate evolutionary model with `ModelFinder` [57] and maximum likelihood phylogenetic trees with `iqtree2` [58] and 1000 replicates with the ultrafast bootstrap [72]. We analyzed and visualized the trees with `ggtree` [73, 74].

7.5 Computations

The computations in this paper were run on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University. Computations were run on the Mimir bioinformatics server and the Assa bioinformatics computer at the University of Iceland.

Acknowledgments. We thank Kristján Kristinsson and the Icelandic Marine Research Institute, Grant Pogson, Mike Canino, and Darin Jones and Mike Levine at NOAA/NMFS/AFSC, Snæbjörn Pálsson, JS Christiansen, SE Fevolden, and the TUNU-I Expedition (the University of Tromsø, Norwegian College of Fishery Science, Institute of Aquatic BioSciences), Greenland Institute of Natural Resources and crew of R/V Paamiut, Lilja Jansen, Svava Ingimarsdóttir, Gudni Magnús Eiríksson, the Faroese Fisheries Laboratory and crew of R/V Magnus Heinason, Remment ter Hofstede and the Netherlands

Institute for Fisheries Research, and Óskar Gudmundsson on Sigrún RE-303 for help with sampling and Jarle Mork for samples from the J.Mork collection. We thank Matthew Haynsen for help with molecular work. We acknowledge technical help with the molecular analysis by The Bauer Core Facility at Harvard University.

Declarations

- Funding: The work was supported by an Icelandic Research Fund Grant of Excellence no. 185151-051 to EÁ, KH, Alison Etheridge, Wolfgang Stephan, and Bjarki Eldon.
- Conflict of interest/Competing interests (check journal-specific guidelines for which heading to use): The authors declare no conflict of interest or competing interests.
- Ethics approval: The samples used for this study were from our large DNAfish tissue collection, the sampling of which was approved by appropriate ethics review boards as appropriate and already described [8, 10].
- Consent to participate: Not applicable
- Consent for publication: Not applicable
- Availability of data and materials: The `bam` alignment files will be available in the NCBI short read sequence archive as bioproject XXX at the time of publication.
- Code availability: Code for snpArcher variant calling is available on `github`: https://github.com/harvardinformatics/snpArcher.
- Authors' contributions: EÁ, KH: designed experiment, secured funding, performed molecular analysis, analyzed data, wrote and revised the paper. AWE performed data analysis and read and commented on the paper. ALG performed data analysis and read and commented on the paper. TS secured computing, analyzed data, read and revised the paper.

Appendix A Sampling localities

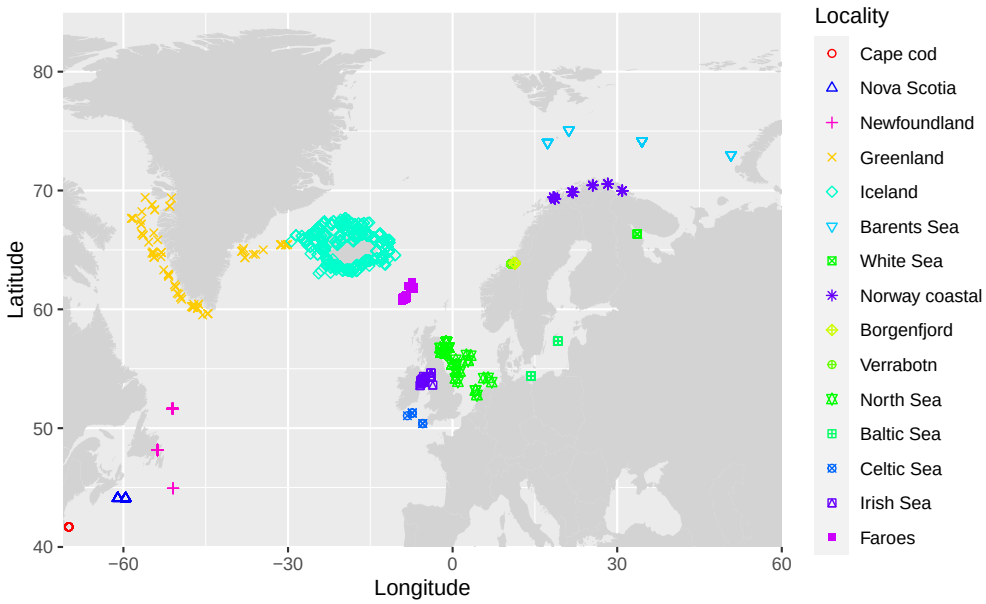

Fig. A1 Sample localities of Atlantic cod *Gadus morhua* on a map of the North Atlantic Ocean. Localities range from Cape Cod in the west to the Faroe Islands in the east.

Appendix B Local structure of the genome

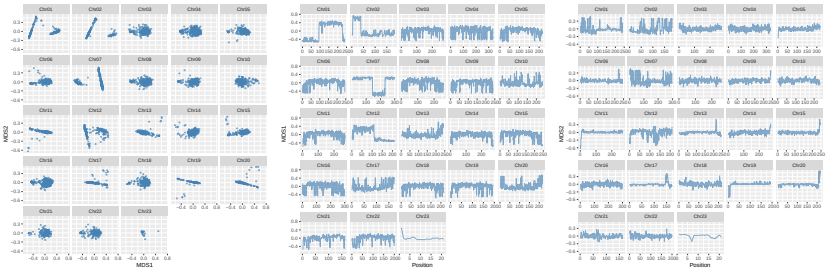

Fig. B2 Local structure of the genome. Local PCA using `lostruct` [24] of all chromosomes of Atlantic cod. Matrix of distances of principal component analysis of local chromosomal segments analyzed with multidimensional scaling (MDS). MDS coordinate 2 on MDS coordinate 1 (left), and MDS1 and MDS2 on chromosomal location (middle and right).

Appendix C Population structure

C.1 Principal components analysis of individual non-inversion chromosomes

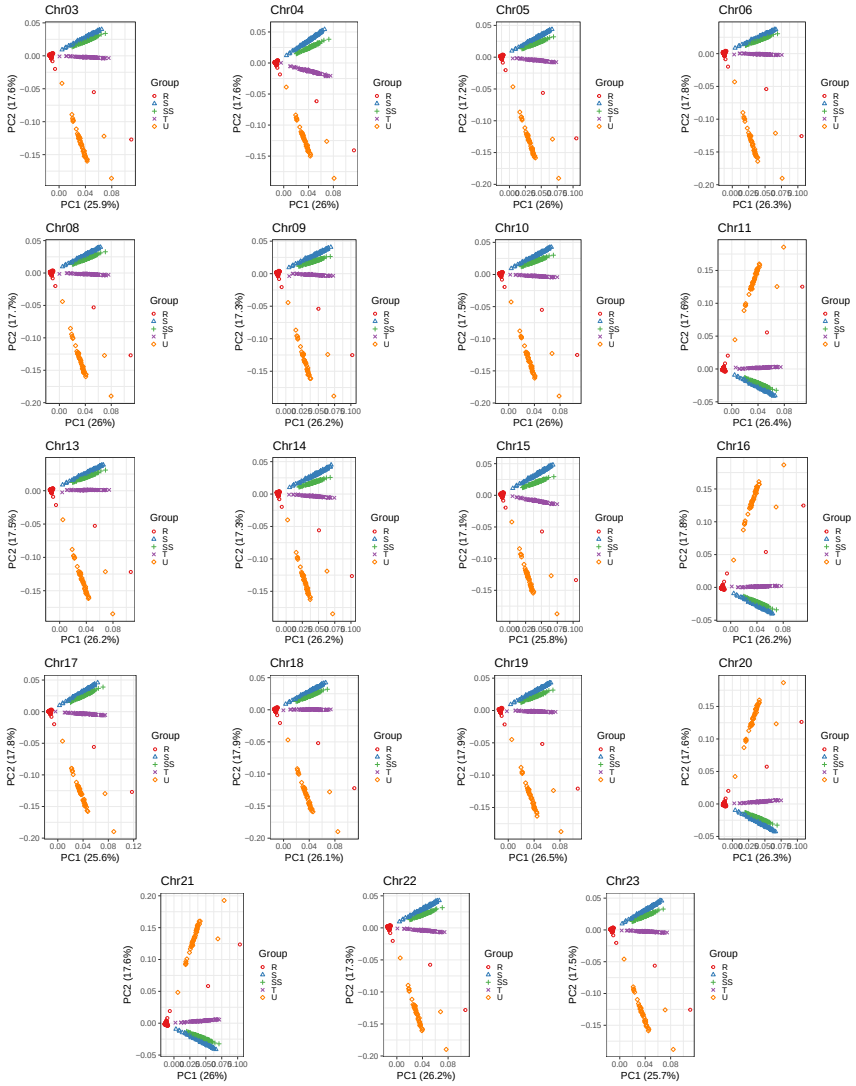

Fig. C3 Principal component analysis of individual non-inversion chromosomes.

C.2 Admixture analysis of non-inversion chromosomes

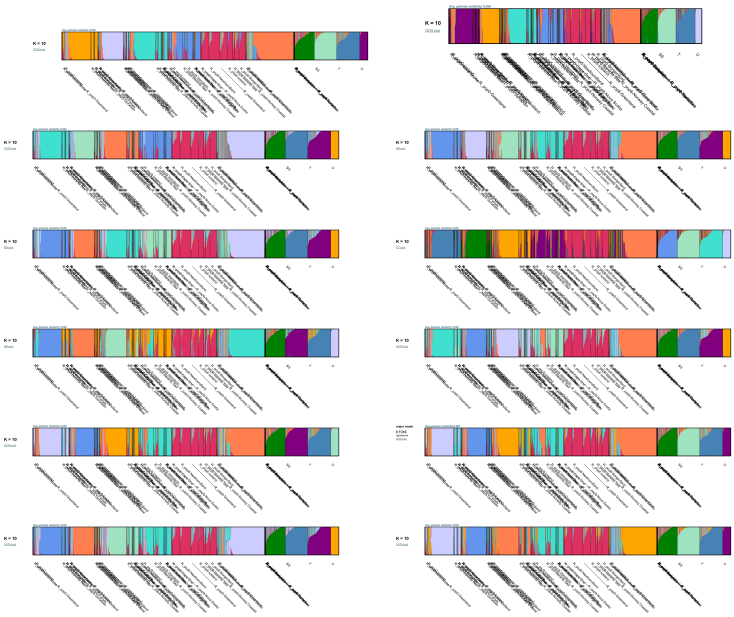

Fig. C4 Admixture analysis of individual non-inversion chromosomes. Pong [29] display of 10 admixture runs for representative non-inversion chromosomes 03, 05 06, 08, 09, 17, 18, 19, 20, 21, 22, and 23. The PCA groups *S*, *SS*, *T*, and *U* are clearly separate and different from *R*. The *R* group is heterogeneous. *R* labelled with admixture population and geographic location ranging from Cape Cod to the Faroe Islands. All 10 replicate admixture runs agree for every $k = 2 \ldots 10$. Individual non-inversion chromosomes show the same pattern as analysis of non-inversion chromosomes combined (Fig. 2).

Appendix D Inversion genotypes

D.1 Admixture analysis of inversion chromosomes

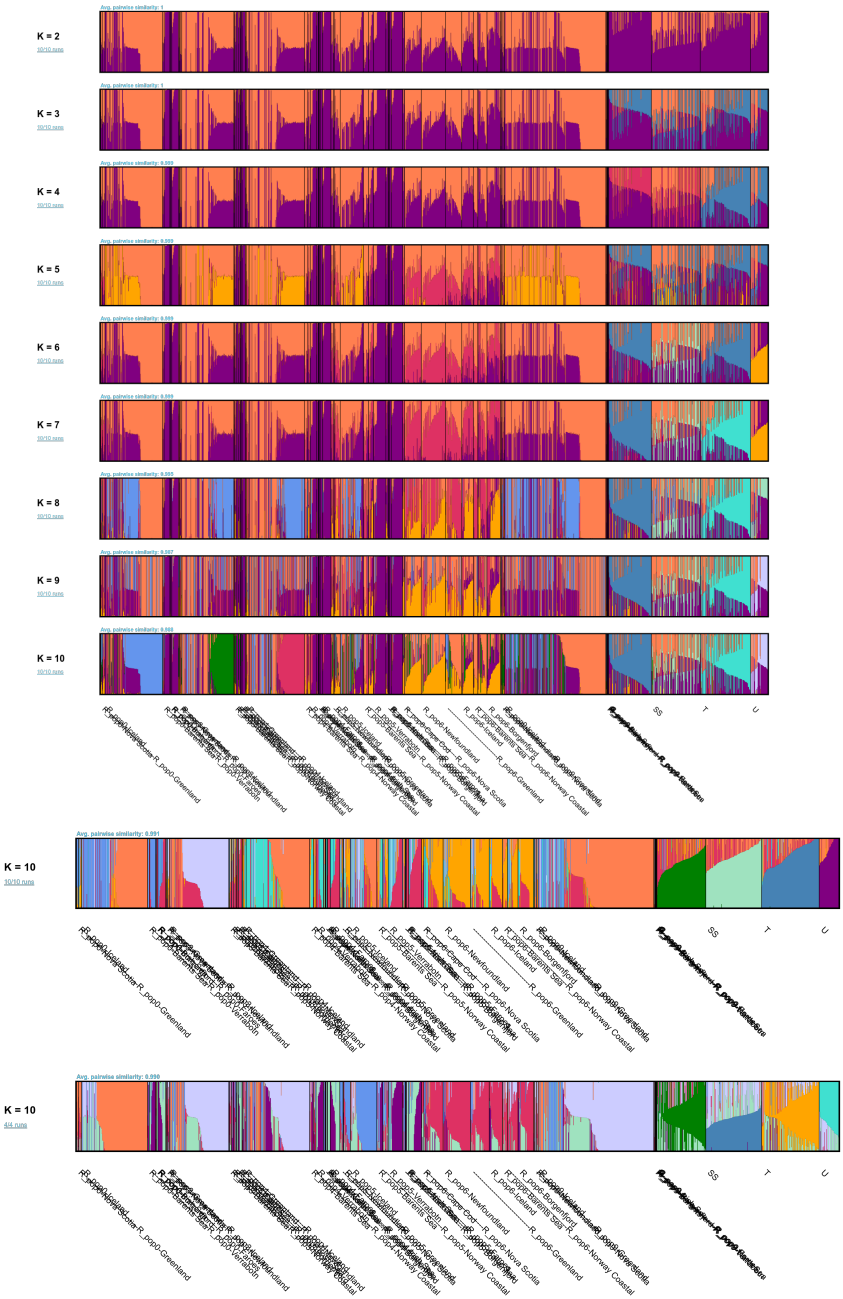

Fig. D5 Admixture analysis of inversion chromosomes 01, 02, and 07. Pong [29] display of 10 replicate admixture runs for Chr01 for $k = 2 \ldots 10$ (top). The PCAgroups S, SS, T, and U are clearly separate and different from R. The R group is heterogeneous. All groups are segregating for the inversion. All ten admixture runs agree for every $k = 2 \ldots 10$. Results of 10 replicate admixture runs of Chr02 for $k = 10$ (middle), and of Chr07 (bottom).

D.2 Genotyping inversions based on a local Icelandic population

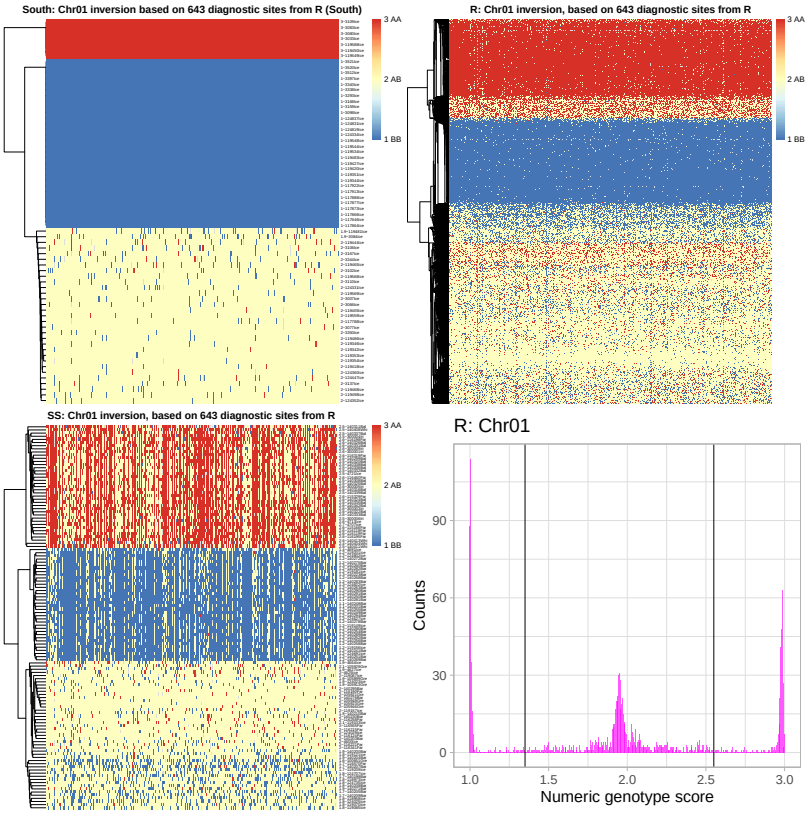

Fig. D6 Diagnostic sites and method of inversion genotyping. Diagnostic sites obtained based on a local population from south/south-east coast of Iceland that is *R* group and already genotyped by PCA and site-frequency spectra [10] (top left). For each site, homozygotes for reference allele are given a numeric score 1 (blue), homozygotes for alternative allele a score of 3 (red), and heterozygotes a 2 (yellow) score. A numeric genotype score is the average over all sites (columns). Results plotted with `pheatmap` [68] and individuals clustered with hierarchical clustering. Diagnostic sites from the south population [10] used to genotype all *R* individuals (top right), and all *SS* individuals (bottom left). Distribution of numeric genotype score for all *R* individuals (bottom right). Vertical lines delimiting called genotypes are determined by inspection of hierarchical clustering and color.

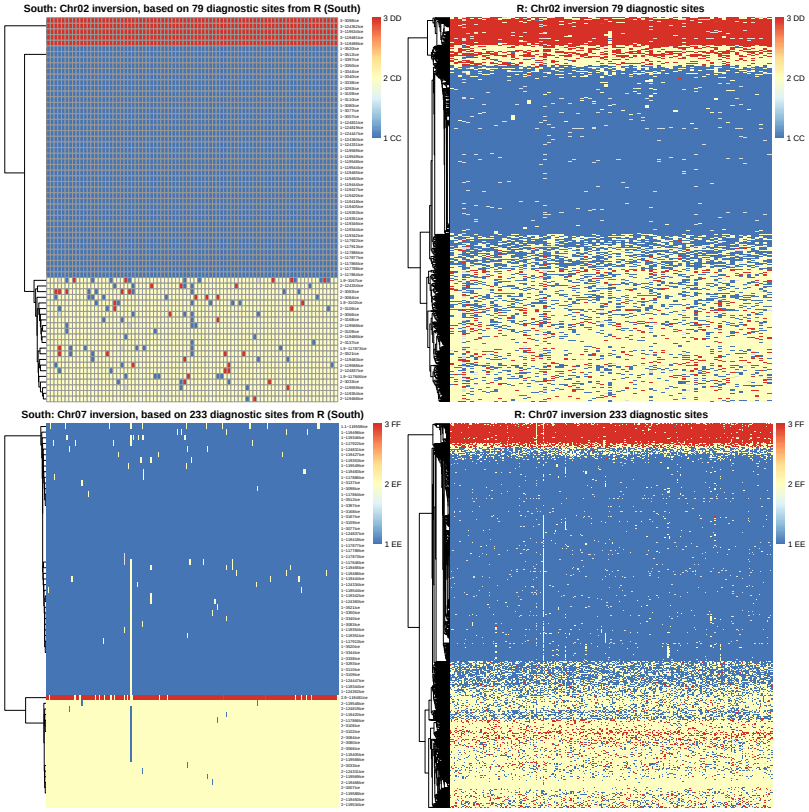

Fig. D7 Diagnostic sites and genotyping of inversions on Chr02 and Chr07. Diagnostic sites obtained for Chr02 (top left) and Chr07 (bottom left) based on a local population from south/south-east coast of Iceland that is R group and already genotyped by PCA and site-frequency spectra [10]. Genotyping of al R individuals for Chr02 (top right) and Chr07 (bottom right).

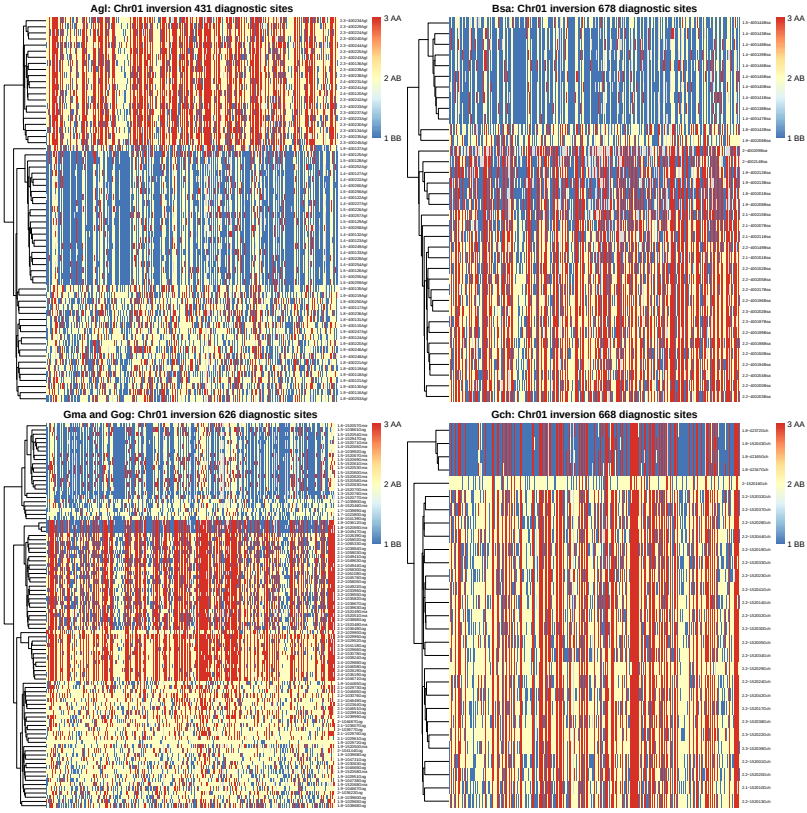

Fig. D8 Genotyping samples of other gadids for Chr01. Samples of Polar cod (Agl), Arctic cod (Bsa), Pacific cod (Gma), Greenland cod (Gog), and walleye pollock (Gch) genotypes for Chr01 inversion using diagnostic sites from *R*.

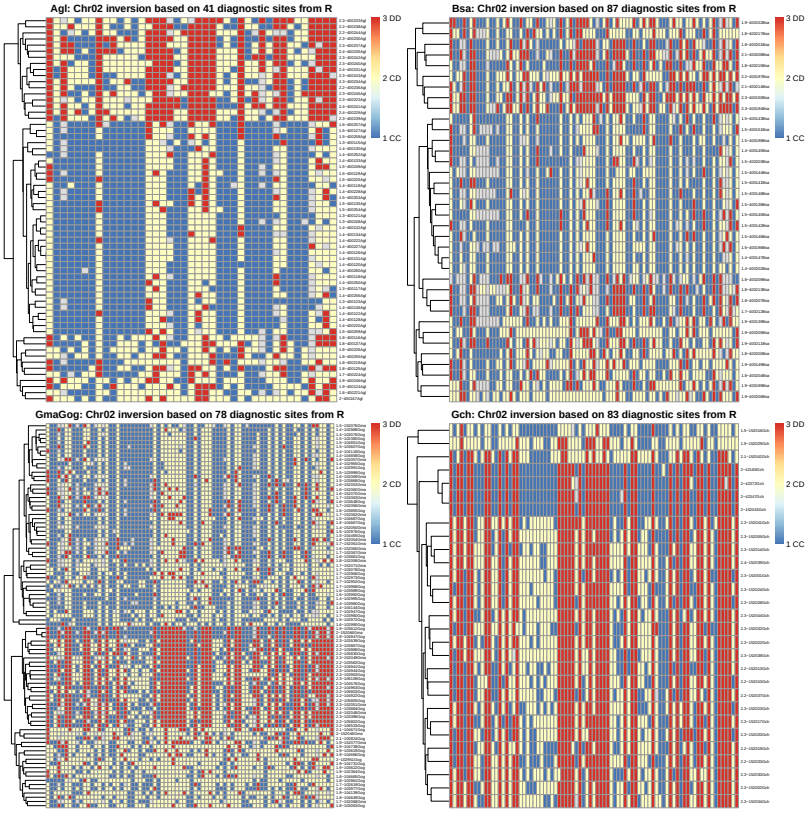

Fig. D9 Genotyping samples of other gadids for Chr02. Samples of Polar cod (Agl), Arctic cod (Bsa), Pacific cod (Gma), Greenland cod (Gog), and walleye pollock (Gch) genotypes for Chr02 inversion using diagnostic sites from *R*.

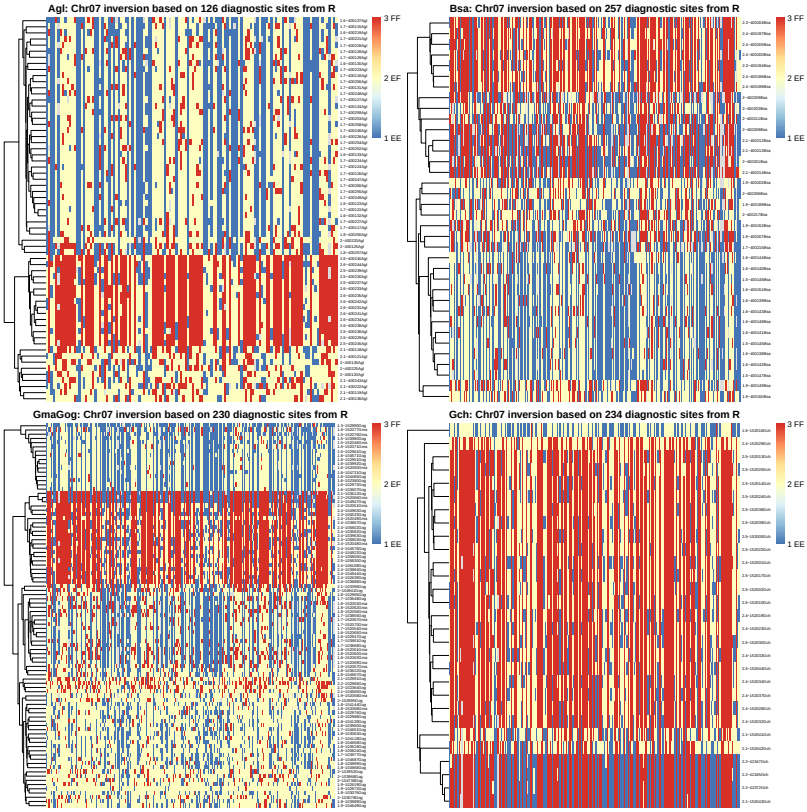

Fig. D10 Genotyping samples of other gadids for Chr07. Samples of Polar cod (Agl), Arctic cod (Bsa), Pacific cod (Gma), Greenland cod (Gog), and walleye pollock (Gch) genotypes for Chr07 inversion using diagnostic sites from *R*.

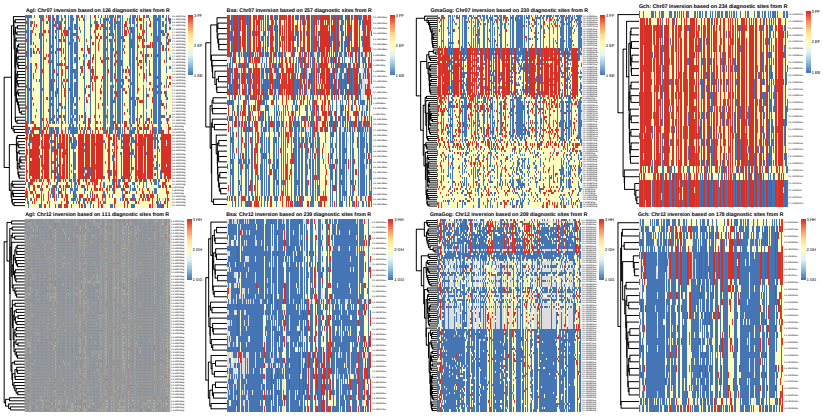

Fig. D11 Test Genotyping samples of other gadids for Chr12. Samples of Polar cod (Agl), Arctic cod (Bsa), Pacific cod (Gma), Greenland cod (Gog), and walleye pollock (Gch) genotypes for Chr12 inversion using diagnostic sites from *R*.

D.3 Robustness of genotyping

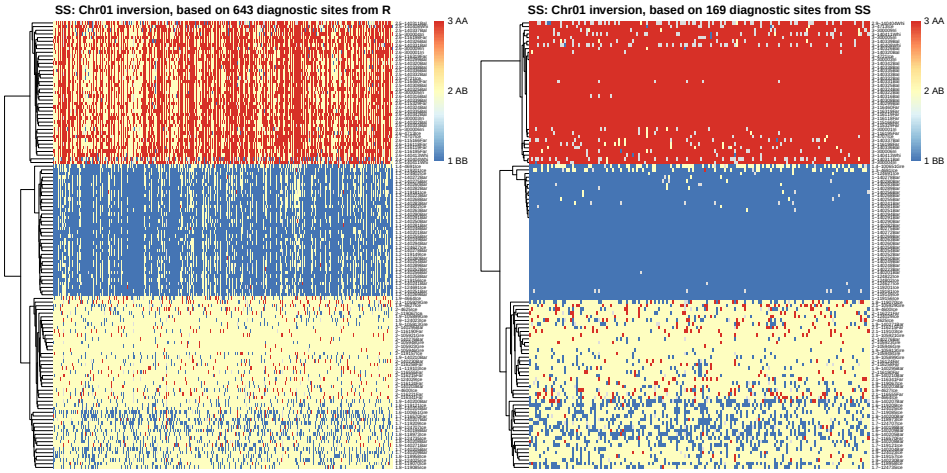

Fig. D12 Robustness of genotyping of Chr01 in *SS*. The *SS* group genotyped with diagnostic sites based on *R* (left) and with diagnostic sites based on *SS* itself (right).

D.4 Interchromosomal genotypic associations of inversions

Table D1 Association of haplotypic genotypes across chromosomes in PCAgroup *R*. All comparisons of chromosomal association were highly significant by X^2 test ($P << 0.001$).

		Chr01			Chr02			Chr07		
		AA	*AB*	*BB*	*DD*	*CD*	*CC*	*FF*	*EF*	*EE*
Chr02	*DD*	71	11	7						
	CD	150	408	86						
	CC	49	291	197						
Chr07	*FF*	59	4	2	44	15	6			
	EF	147	311	46	31	372	101			
	EE	64	395	242	14	257	430			
Chr12	*HH*	16	1	0	11	5	1	11	4	2
	GH	161	130	11	58	190	54	45	180	77
	GG	93	579	279	20	449	482	9	320	622

Table D2 Association of haplotypic genotypes across chromosomes in the South/south-east population in Iceland [10]. The association comparing Chr01 and Chr12 was statistically significant by Fisher's exact test ($P = 0.001$); none of the other comparisons were significant.

		Chr01			Chr02			Chr07		
		AA	*AB*	*BB*	*DD*	*CD*	*CC*	*FF*	*EF*	*EE*
Chr02	*DD*	0	3	2						
	CD	4	11	7						
	CC	3	17	21						
Chr07	*FF*	0	1	0	1	0	0			
	EF	4	10	5	1	8	10			
	EE	3	20	25	3	14	31			
Chr12	*GH*	4	1	1	0	4	2	0	1	5
	GG	3	30	29	5	18	39	1	18	43

Table D3 Test of Hardy-Weinberg equilibrium of haplotypic genotype frequencies at inversion chromosomes in PCA group *R*. Observed (Obs.) and expected (Exp.) genotype frequencies, X^2 test statistic distributed as χ^2, significance P, and allele frequency of alternative allele, p.

	Chr01			Chr02			Chr07			Chr12		
	AA	*AB*	*BB*	*DD*	*CD*	*CC*	*FF*	*EF*	*EE*	*HH*	*GH*	*GG*
Obs.	270	710	290	89	644	537	65	504	701	17	302	951
Exp.	307.6	634.8	327.6	133.0	556.0	581.0	79.1	475.8	715.1	22.2	291.6	956.2
X^2	17.8, $P < 0.001$			31.8, $P < 0.001$			4.48, $P < 0.001$			1.63, $P > 0.2$		
p	0.49			0.32			0.25			0.13		

Appendix E Divergence of inversion haplotypes related to ecotypic variation

E.1 Trans-species and trans-oceanic supergenes

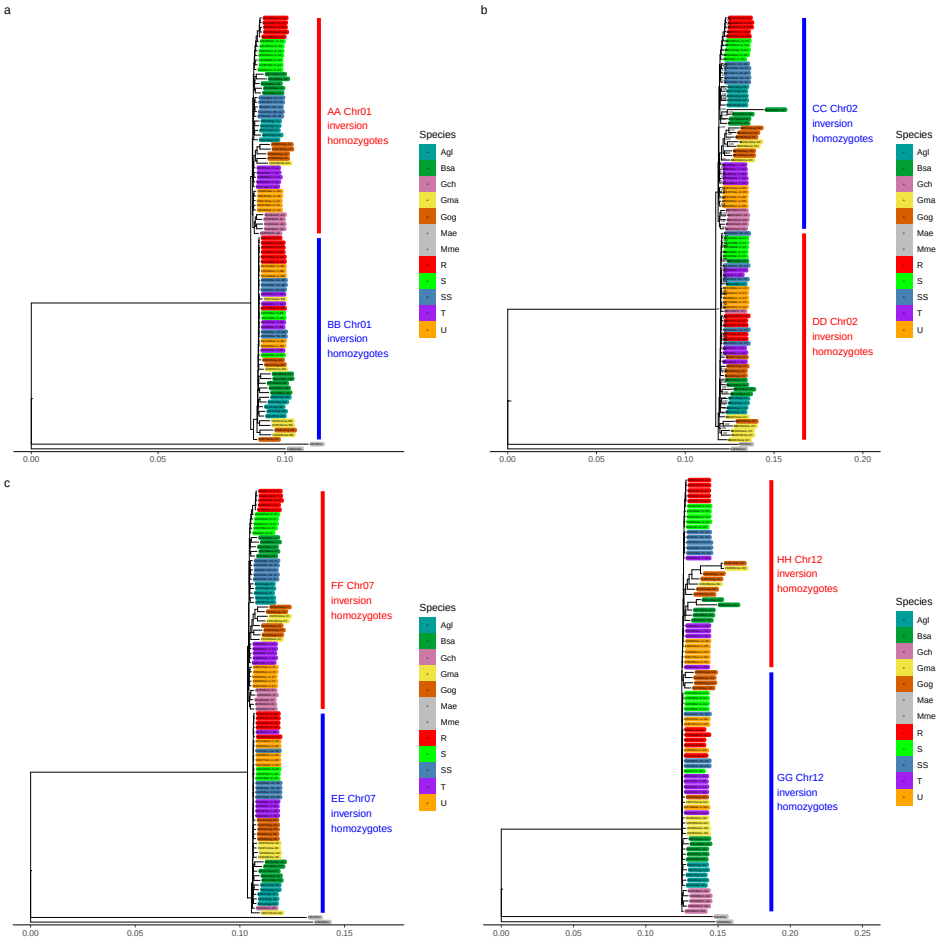

Fig. E13 Trans-species polymorphism of inversion genotypes with outgroup. Maximum likelihood phylogenetic trees of inversion homozygotes of PCAgroups and related gadid taxa for Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**). Best substitution model estimated with `ModelFinder` [57] and trees estimated with `iqtree` [58]. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The outgroup taxa are haddock (Mae *Melanogrammus aeglefinus*) and whiting (Mme *Merlangius merlangus*). The blue and red bars indicate homozygotes for the reference and the alternative allele, respectively. Chromosomal coordinates inside inversions: 11,763,336 to 27,936,178 for Chr01, 579,097 to 4,096,513 for Chr02, 17,004,745 to 25,979,379 for Chr07, and 1,001,809 to 13,965,277 for Chr12.

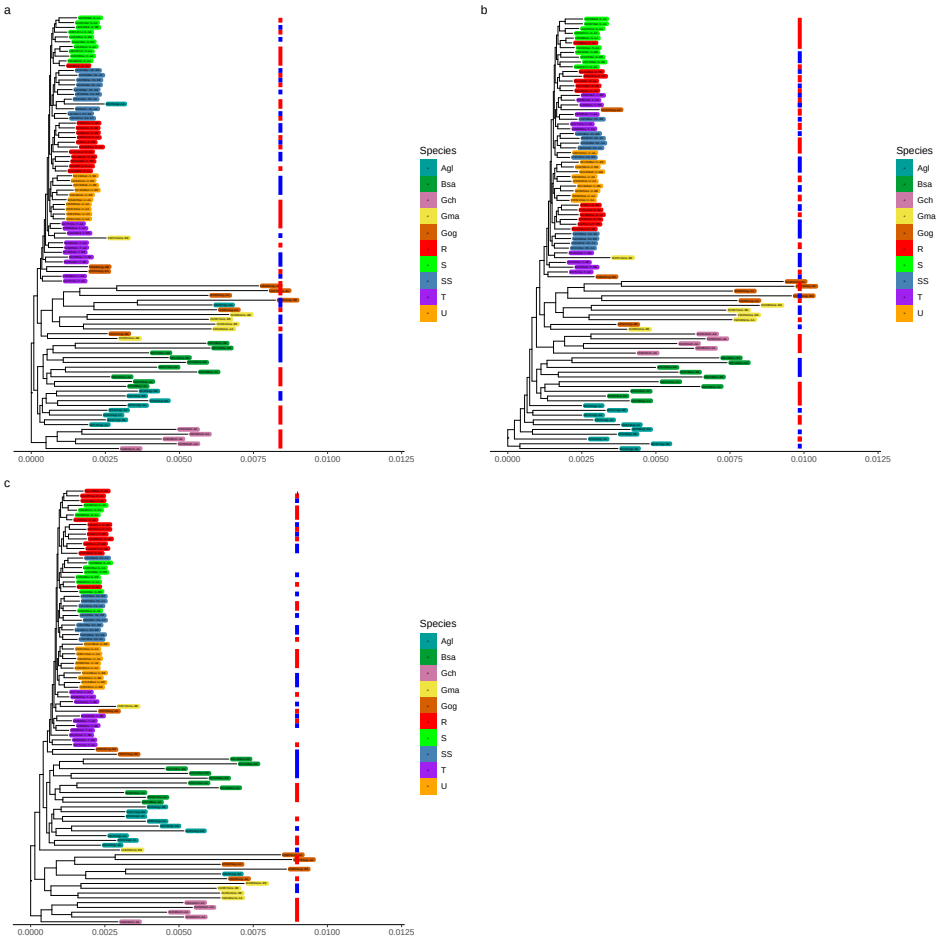

Fig. E14 Phylogeny of non-inversion chromosomes. Maximum likelihood phylogenetic trees of Chr04, Chr17, and Chr23 (**a**, **b**, and **c** respectively) for individuals homozygous for *AA* and *BB* genotypes at Chr01 (Fig. 9 **a**). Best substitution model estimated with `ModelFinder` [57] and trees estimated with `iqtree` [58]. The blue and red bars indicate homozygotes for the reference and the alternative allele respectively at Chr01.

E.2 Treemix network analysis of trans-species inversion genotypes

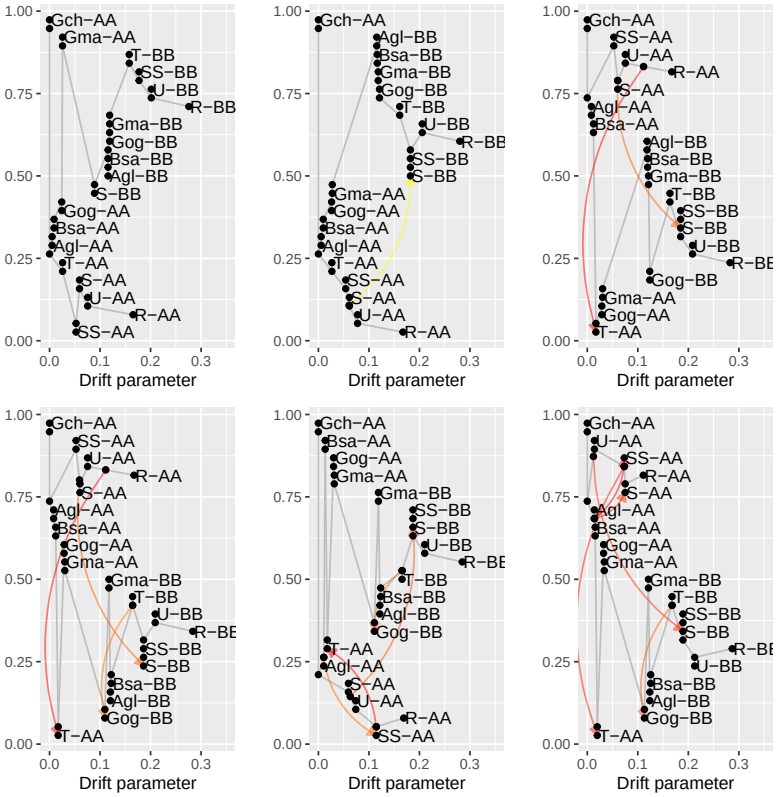

Fig. E15 Treemix network analysis of species and PCA groups of inversions on Chr01. Maximum likelihood treemix network analysis [70] of homozygous genotypes of PCA groups and gadid taxa for Chr01 (individuals studied in Fig. E13 **a**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

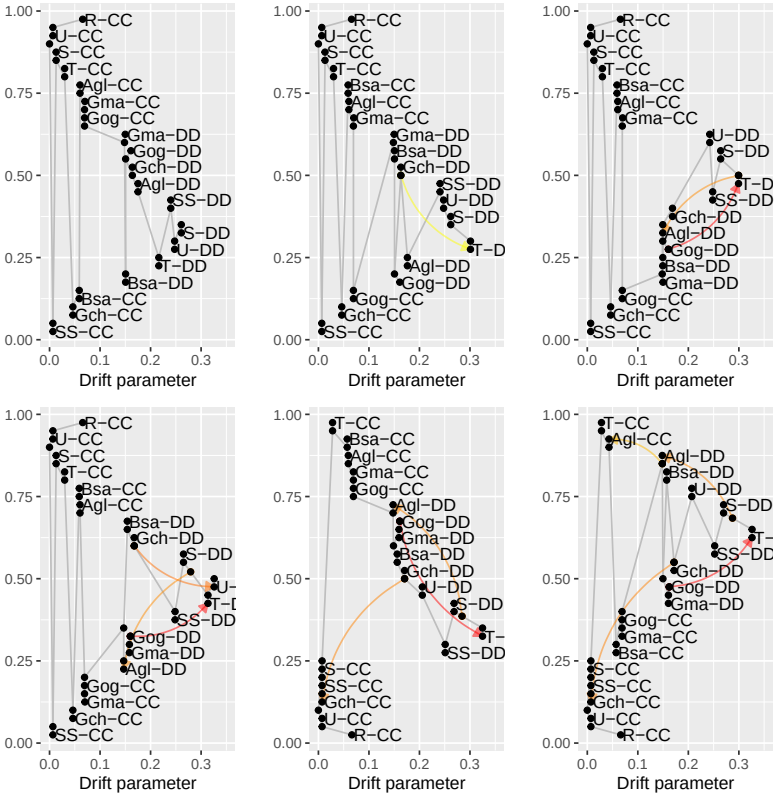

Fig. E16 Treemix network analysis of species and PCA groups of inversions on Chr02. Maximum likelihood treemix network analysis [70] of homozygous genotypes of PCA groups and gadid taxa for Chr02 (individuals studied in Fig. E13 **b**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

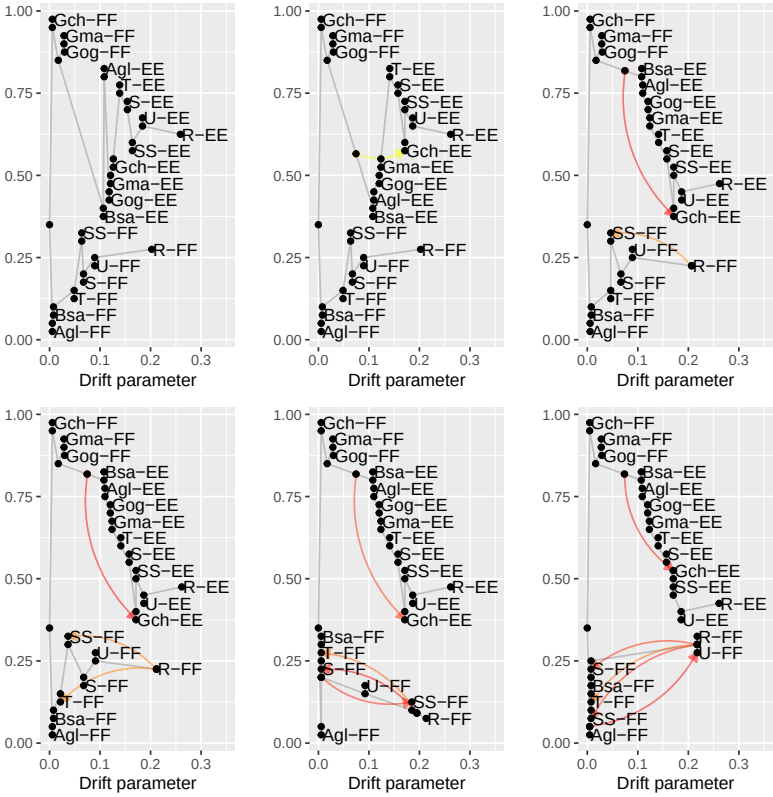

Fig. E17 Treemix network analysis of species and PCA groups of inversions on Chr07. Maximum likelihood treemix network analysis [70] of homozygous genotypes of PCA groups and gadid taxa for Chr07 (individuals studied in Fig. E13 **c**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

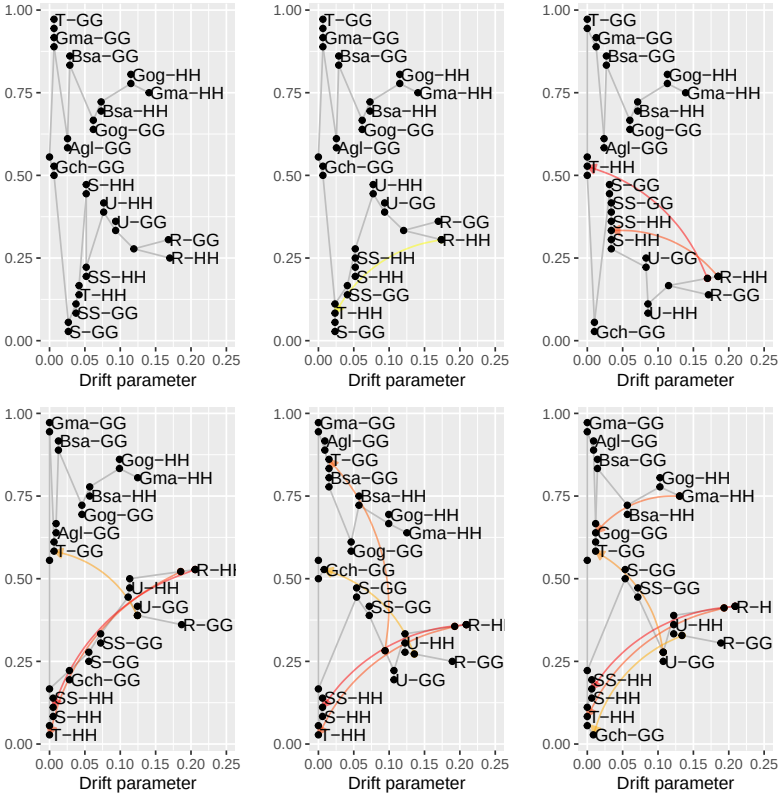

Fig. E18 Treemix network analysis of species and PCA groups of inversions on Chr12. Maximum likelihood treemix network analysis [70] of homozygous genotypes of PCA groups and gadid taxa for Chr12 (individuals studied in Fig. E13 **d**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

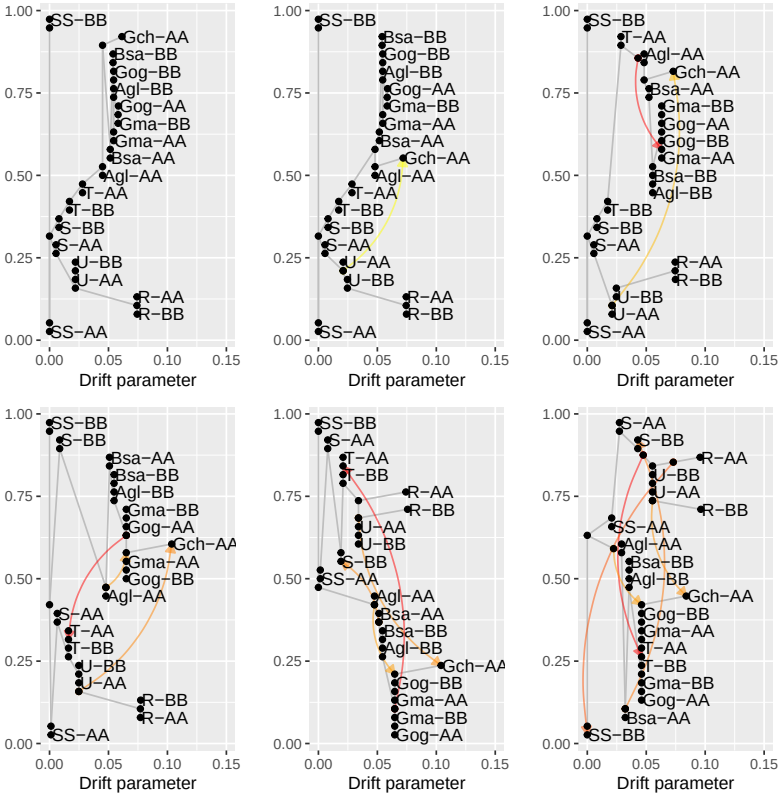

Fig. E19 Treemix network analysis of species and PCA groups of non-inversions Chr04. Maximum likelihood treemix network analysis [70] of non-inversion Chr04 using individuals of homozygous genotypes for Chr01 (individuals studied in Fig. E13 **a**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

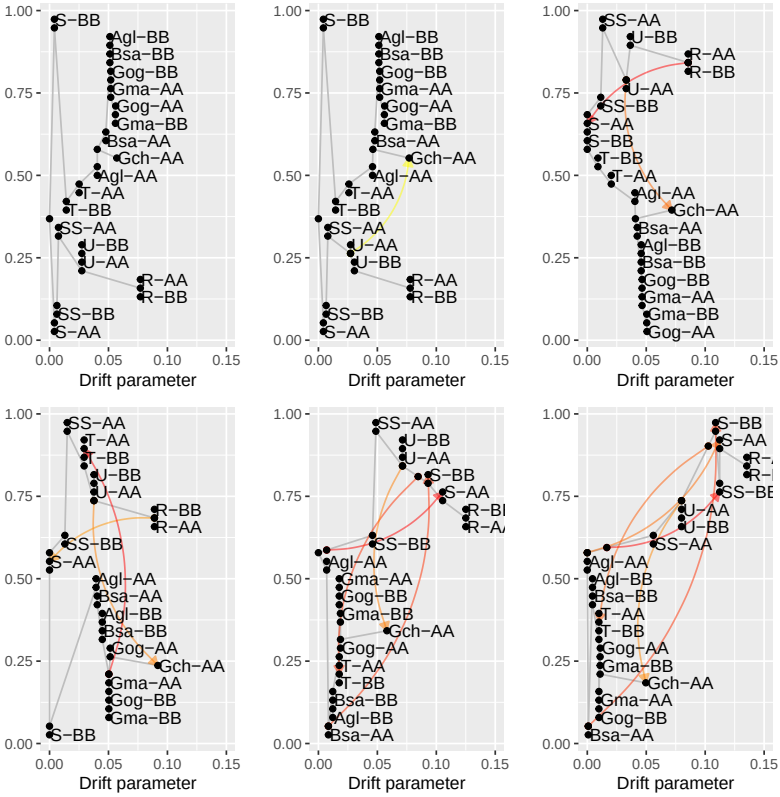

Fig. E20 Treemix network analysis of species and PCA groups of non-inversions Chr17. Maximum likelihood treemix network analysis [70] of non-inversion Chr04 using individuals of homozygous genotypes for Chr01 (individuals studied in Fig. E13 **a**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

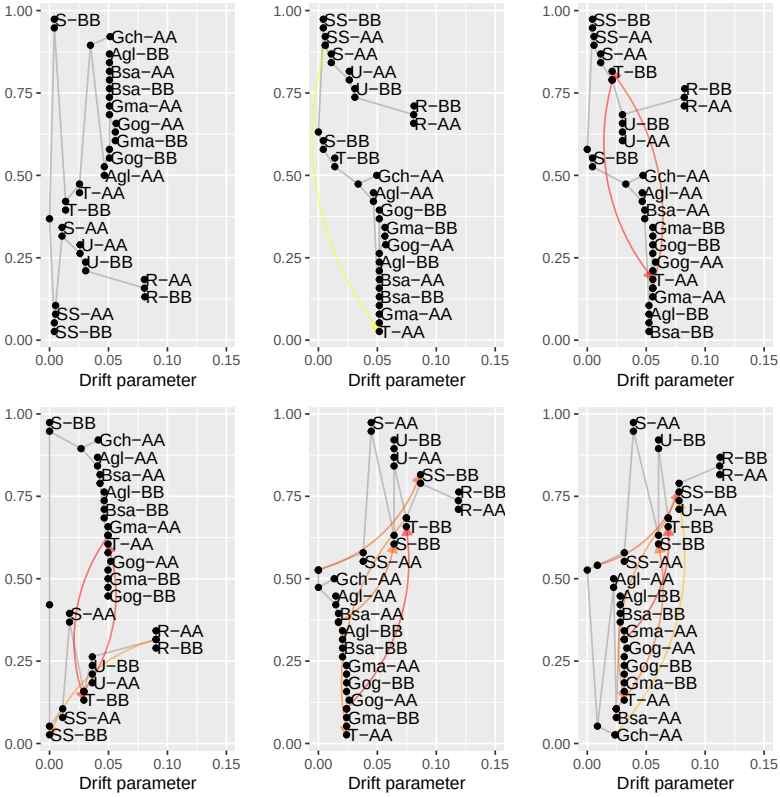

Fig. E21 Treemix network analysis of species and PCA groups of non-inversions Chr23. Maximum likelihood treemix network analysis [70] of non-inversion Chr04 using individuals of homozygous genotypes for Chr01 (individuals studied in Fig. E13 **a**), usually five to six individuals per group. The PCA groups *R*, *S*, *SS*, *T*, and *U* of Atlantic cod (*Gadus morhua*) and the gadid taxa Polar cod (Agl, *Arctogadus glacialis*), Arctic cod (Bsa *Boreogadus saida*), walleye pollock (Gch *G. chalcogrammus*), Pacific cod (Gma *G. macrocephalus*), and Greenland cod (Gog *G. ogac*). The number of migration edges $m = 0 \ldots 5$ (top left to bottom right).

Appendix F Spotty but widespread geographical distribution

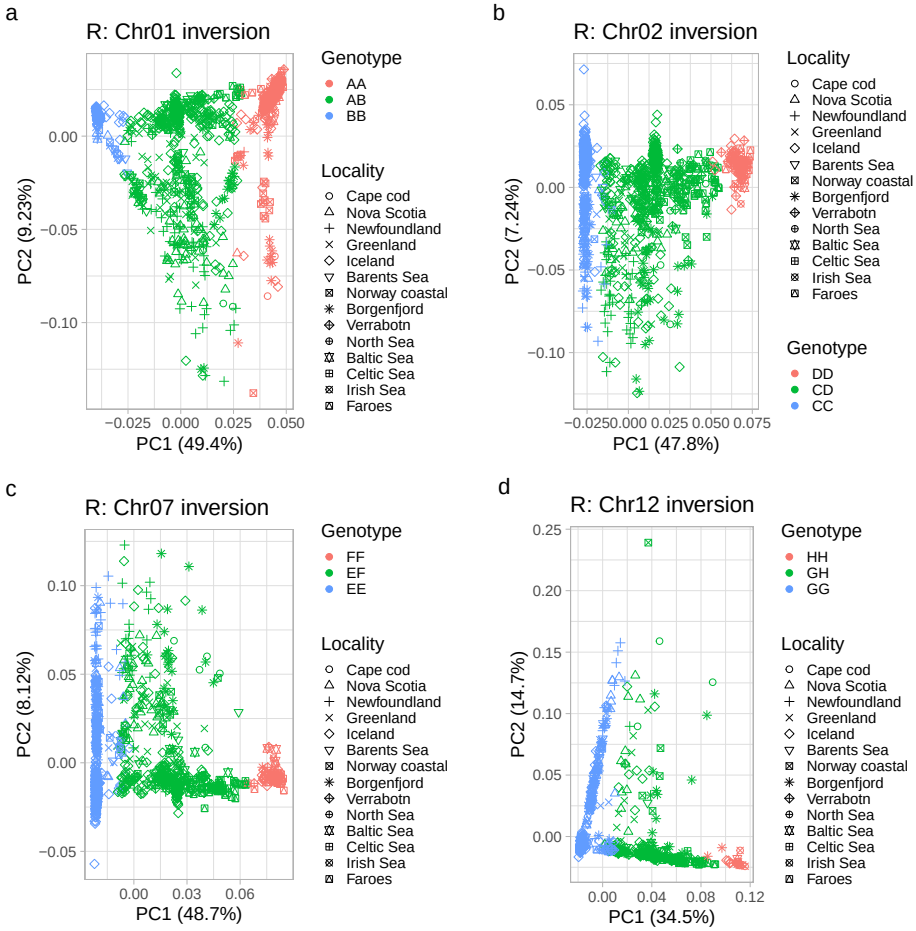

Fig. F22 Principal component analysis of inversion chromosomes of *R* group labeled by called genotype and locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

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Appendix G Recombinational spectrum of inversion genotypes and geographical distribution

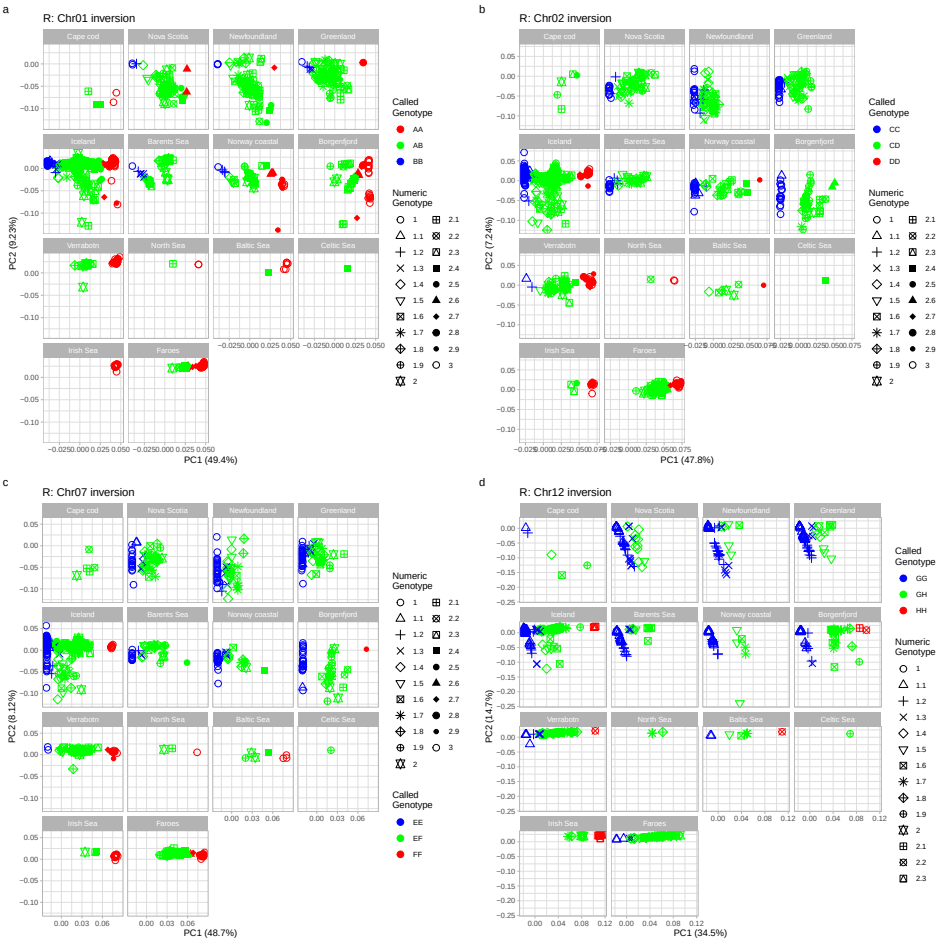

Fig. G23 Principal component analysis of inversion chromosomes of *R* group labeled by called and numeric genotype faceted by locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

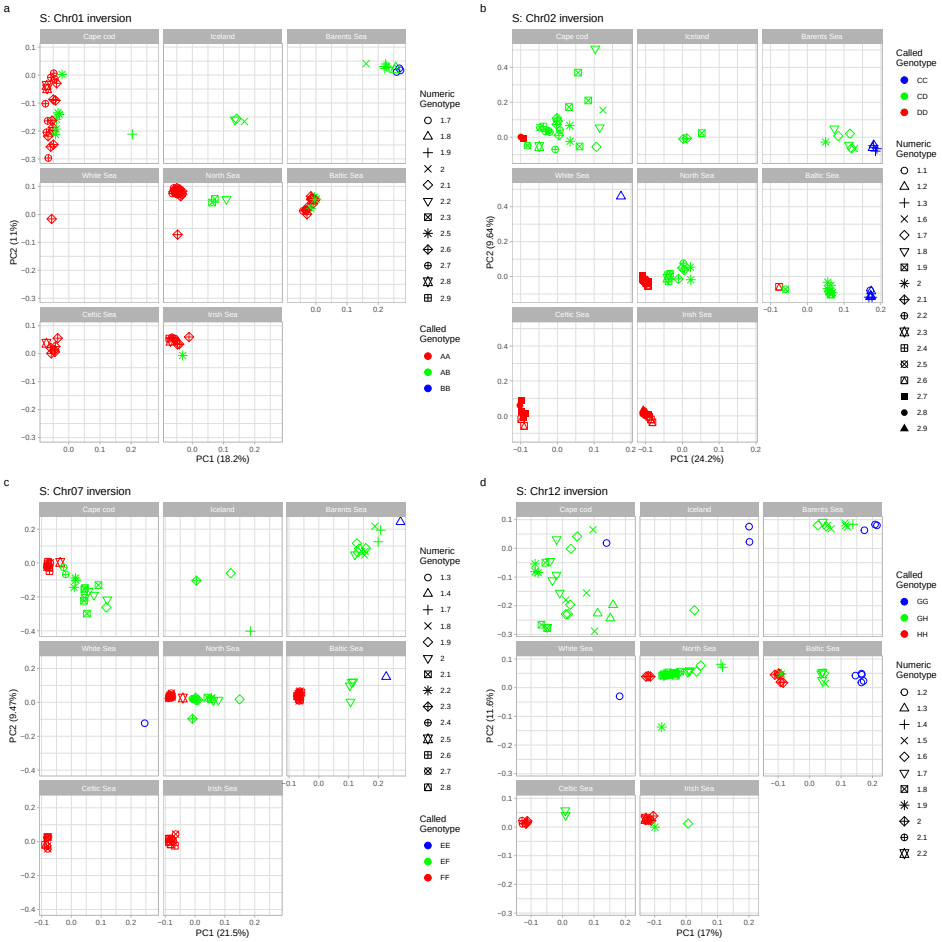

Fig. G24 Principal component analysis of inversion chromosomes of *S* group labeled by called and numeric genotype faceted by locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

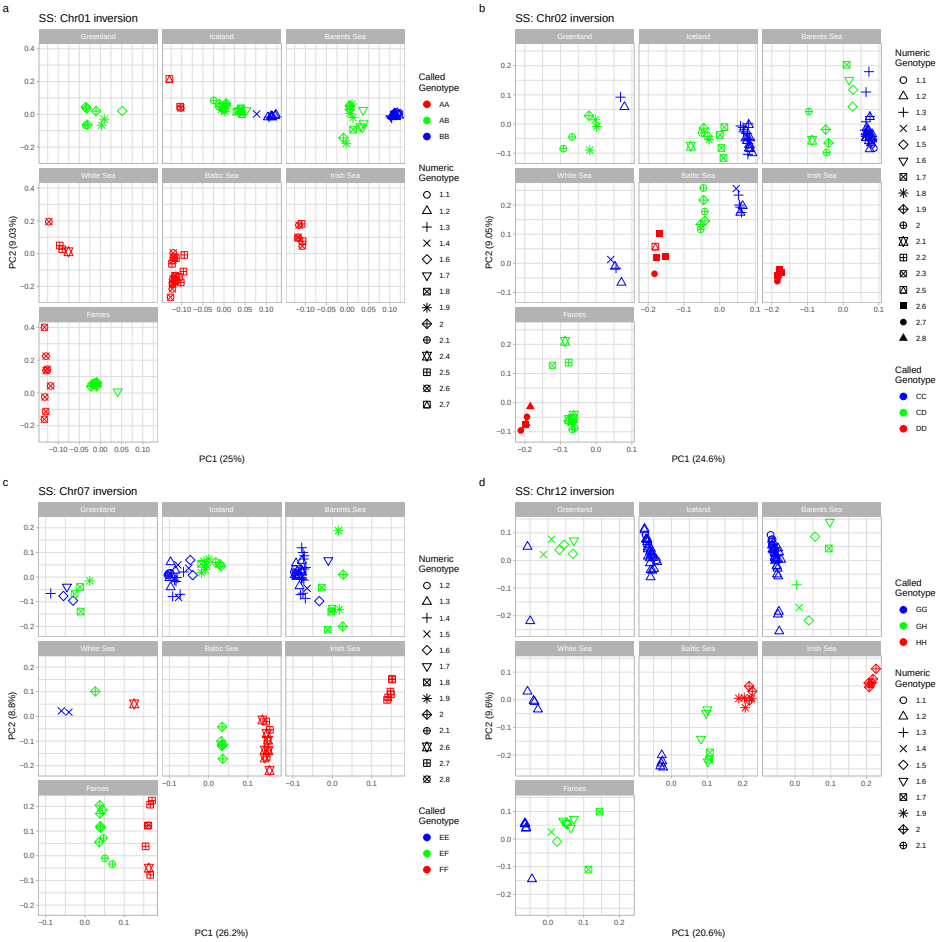

Fig. G25 Principal component analysis of inversion chromosomes of *SS* group labeled by called and numeric genotype faceted by locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

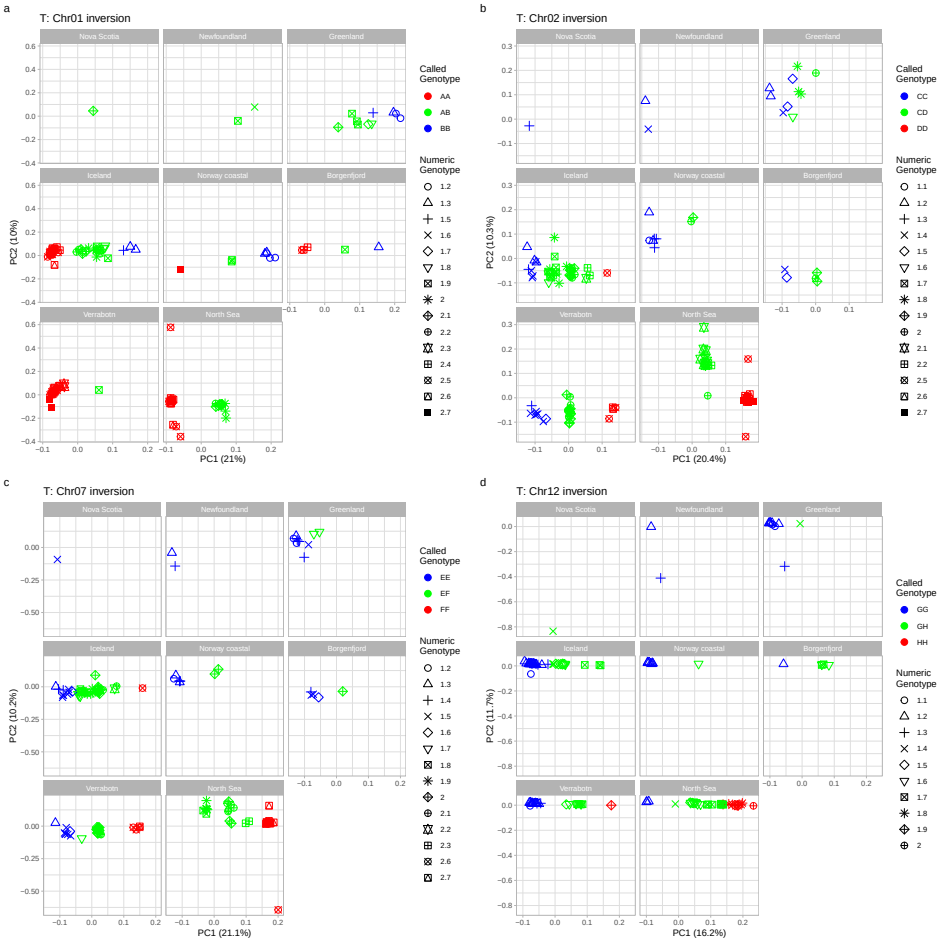

Fig. G26 Principal component analysis of inversion chromosomes of *T* group labeled by called and numeric genotype faceted by locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

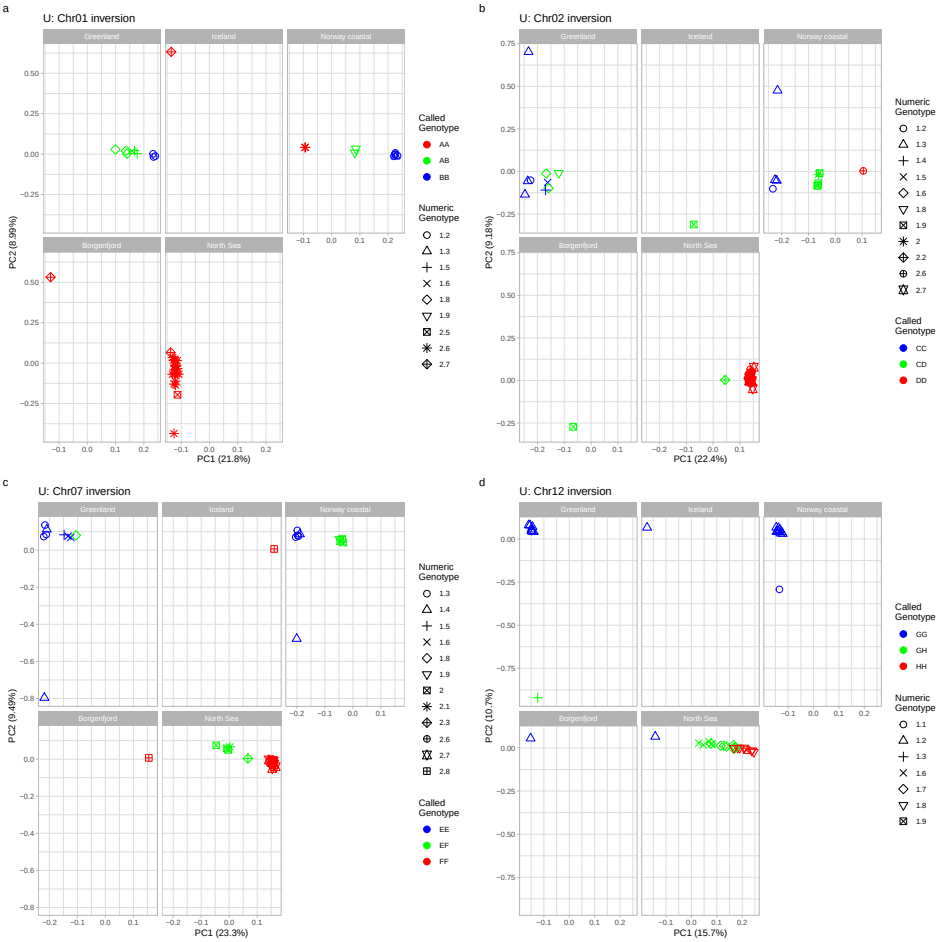

Fig. G27 Principal component analysis of inversion chromosomes of *U* group labeled by called and numeric genotype faceted by locality. Genotypes of inversions on Chr01 (**a**), Chr02 (**b**), Chr07 (**c**), and Chr12 (**d**), labelled [10] and called as described D.2. Localities arranged from Cape Cod in the west to the Faroe Islands in the east.

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