Supporting Information:

Ancient DNA reveals the Arctic origin of Viking Age cod from Haithabu, Germany

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Supplementary Figure Legends

Fig. S1. aDNA fragmentation and mis-incorporation patterns of sequencing read data from 15 Atlantic cod samples. Patterns were obtained using MapDamage v. 2.0.6 after down-sampling BAM files to 1,000,000 reads. For visualization purposes, we only show the typical increase in C->T mis-incorporations due to cytosine deamination at the 5′-end of DNA fragments and the corresponding increase of G->A mis-incorporations at the 3′-end.

Fig. S2. ADMIXTURE ancestry components for modern and ancient Atlantic cod specimens. Population structure was investigated using models with a variable number of clusters (k). Model fit was assessed by calculating the cross-validation (CV) error, with a lower CV error indicating a better fit. Linkage group (LG) 01, 02, 07, 12 and unplaced scaffolds were excluded from these analyses (see text for explanation).

Fig. S3. Principle component analysis of genomic inversions in Atlantic cod. Ancient specimens (*stars*) were projected onto the first two principle components calculated using individuals from modern populations (*circles*). The first principle component (PCA 1) separates genomic variation within each of the four mega-base long regions (LG01; 9.1 - 26.2 Mbp, LG02; 18.5 - 24 Mbp, LG07; 13.6 - 23 Mbp and LG12; 1.3 -13.6 Mbp) into distinct clusters (*grey dotted ovals*) that reflect the bi-allelic segregation of the three major inversion genotypes (AA; collinear, AB; heterozygote and BB; inverted). The number of SNPs (*n*) used per region is indicated. Mean heterozygosity values per genotype (*presented in grey under each genotype*; estimated by calculating the inbreeding coefficient F using a method of moments as implemented in VCFTOOLS v0.1.14) show the marked decrease in F-values for the AB genotypes due to heterozygote excess. Ancient samples follow the tri-modal cluster pattern of the modern individuals.

Supplementary Tables

Table S1. Specimen ID, location, estimated date and bone type of ancient Atlantic cod samples. All bones were morphologically identified as Atlantic cod (*Gadus morhua*). WGS shotgun libraries were paired-end sequenced, and we report the number of collapsed reads, their clonality, their endogenous DNA content (defined as the unique, non-repetitive fraction of reads aligning towards the gadmor2 reference genome with a minimum MapQ value of 25), the average insert length and the fold coverage obtained for the nuclear genome. Four other specimens (not shown) were extracted of which two did not yield libraries, and two had endogenous DNA content below 1%.

Specimen	Country	Location	Date (CE)	Bone type	Reads (millions)	Clonality (%)	Endogenous DNA (%)	Average insert length (bp)	Fold coverage
COD003	Germany	Schleswig	c.1100-1200	Vertebra	52	14	33	69	1.9
COD023	Germany	Schleswig	c.1200-1280	Articular	67	6	37	51	2.0
COD027	UK	Orkney	c.1000-1200	Premaxilla	59	9	42	75	2.9
COD028	UK	Orkney	c.1000-1200	Premaxilla	84	4	15	72	1.4
COD029	UK	Orkney	c.1000-1200	Premaxilla	58	5	33	71	2.1
COD030	UK	Orkney	c.1000-1200	Premaxilla	55	8	25	72	1.6
COD034	UK	Orkney	c.1000-1200	Premaxilla	49	23	27	72	1.5
COD053	Germany	Haithabu	c.800-1066	Cleithrum	83	8	28	47	1.7
COD054	Germany	Haithabu	c.800-1066	Cleithrum	58	8	37	67	2.3
COD061	Germany	Haithabu	c.800-1066	Vertebra	72	11	24	40	1.1
COD062	Germany	Haithabu	c.800-1066	Vertebra	76	8	25	46	1.4
COD063	Germany	Haithabu	c.800-1066	Articular	95	6	32	46	2.2
COD076	Norway	Bjørkum	c.700-950	Dentary	74	27	18	48	1.0
COD086	Norway	Oslo	c.1025-1175	Ceratohyal	59	12	46	78	3.4
COD092	Norway	Oslo	c.1025-1175	Cleithra	72	16	21	47	1.2

Table S2. Population, specimen ID and sample date of modern Atlantic cod specimens. We report if sampling took placed during spawning, the number of reads obtained and the resulting fold coverage for the nuclear genome.

Population	Specimen_ID	Sample Date	Spawning population	Number of reads (millions)	Fold coverage
Eastern Baltic	ARK_4001	2012, May	Yes	40	7.4
Eastern Baltic	ARK_4002	2012, May	Yes	20	3.8
Eastern Baltic	ARK_4003	2012, May	Yes	38	6.9
Eastern Baltic	ARK_4006	2012, May	Yes	34	6.3
Eastern Baltic	ARK_4007	2012, May	Yes	34	6.4
Eastern Baltic	ARK_4008	2012, May	Yes	47	8.8
Eastern Baltic	ARK_4010	2012, May	Yes	31	5.9
Eastern Baltic	ARK_4011	2012, May	Yes	49	9.1
Eastern Baltic	ARK_4012	2012, May	Yes	35	6.6
Eastern Baltic	ARK_4013	2012, May	Yes	37	6.9
Eastern Baltic	ARK_4014	2012, May	Yes	34	6.1
Eastern Baltic	ARK_4015	2012, May	Yes	44	8.3
Eastern Baltic	ARK_4016	2012, May	Yes	29	5.1
Eastern Baltic	ARK_4017	2012, May	Yes	45	8.5
Eastern Baltic	ARK_4018	2012, May	Yes	33	6.1
Eastern Baltic	ARK_4019	2012, May	Yes	47	8.8
Eastern Baltic	ARK_4020	2012, May	Yes	23	4.1
Eastern Baltic	ARK_4021	2012, May	Yes	57	10.6
Eastern Baltic	ARK_4022	2012, May	Yes	34	6.4
Eastern Baltic	ARK_4024	2012, May	Yes	31	5.8
Eastern Baltic	ARK_4030	2012, May	Yes	34	6.5
Eastern Baltic	ARK_4032	2012, May	Yes	91	17.2
Eastern Baltic	ARK_4039	2012, May	Yes	63	11.9
Eastern Baltic	ARK_4043	2012, May	Yes	68	12.8
Eastern Baltic	BOR_90E	2011, April	Yes	44	8.3
Eastern Baltic	BOR_91E	2011, April	Yes	47	9.0
Eastern Baltic	BOR_74E	2011, May	Yes	45	8.6
Eastern Baltic	BOR_79E	2011, May	Yes	50	9.6
Eastern Baltic	BOR_60E	2012, April	Yes	46	8.7
Eastern Baltic	BOR_611E	2012, May	Yes	45	8.5
Eastern Baltic	BOR_613E	2012, May	Yes	43	8.2
Eastern Baltic	BOR_614E	2012, May	Yes	42	7.9
Eastern Baltic	BOR_615E	2012, May	Yes	42	8.0
Eastern Baltic	BOR_617E	2012, May	Yes	41	7.8
Eastern Baltic	BOR_621E	2012, May	Yes	41	7.8
Eastern Baltic	BOR_624E	2012, May	Yes	42	8.1
Eastern Baltic	BOR_630E	2012, May	Yes	40	7.5
Eastern Baltic	BOR_655E	2012, May	Yes	73	13.8
Eastern Baltic	BOR_659E	2012, May	Yes	59	11.1
Eastern Baltic	BOR_664E	2012, May	Yes	66	12.5
Eastern Baltic	BOR_AL713	2012, May	Yes	44	8.4
Eastern Baltic	BOR_739E	2012, May	Yes	57	10.8
Eastern Baltic	BOR_749E	2012, May	Yes	59	11.2
Eastern Baltic	BOR_760E	2012, May	Yes	6	1.1
Eastern Baltic	BOR_AL724	2012, May	Yes	49	9.3
Eastern Baltic	BOR_AL736	2012, May	Yes	44	8.4
Eastern Baltic	BOR_AL741	2012, May	Yes	46	8.7
Eastern Baltic	BOR_AL777	2012, May	Yes	43	8.3
Iceland	I50_02	2003, April	Yes	49	10.9
Iceland	I50_03	2003, April	Yes	43	9.7
Iceland	I50_04	2003, April	Yes	44	9.8
Iceland	I50_05	2003, April	Yes	46	10.3
Iceland	I50_06	2003, April	Yes	32	7.1
Iceland	I50_07	2003, April	Yes	42	9.4
Iceland	I50_08	2003, April	Yes	35	7.7
Iceland	I50_09	2003, April	Yes	51	11.2
Iceland	I50_10	2003, April	Yes	43	9.6
Iceland	I50_11	2003, April	Yes	50	11.1
Iceland	I50_12	2003, April	Yes	39	8.8
Iceland	I50_13	2003, April	Yes	48	10.7
Iceland	I50_14	2003, April	Yes	26	5.7
Iceland	I50_15	2003, April	Yes	41	9.3
Iceland	I50_16	2003, April	Yes	49	11.0
Iceland	I50_17	2003, April	Yes	43	9.5
Iceland	I50_18	2003, April	Yes	46	10.1

Iceland	I50_19	2003, April	Yes	52	11.4
Iceland	I50_20	2003, April	Yes	54	12.1
Iceland	I50_23	2003, April	Yes	58	12.9
Iceland	I50_26	2003, April	Yes	53	10.3
Iceland	I50_38	2003, April	Yes	64	12.3
Iceland	I50_41	2003, April	Yes	92	19.1
Iceland	I50_42	2003, April	Yes	52	11.6
Lofoten	LOF_A_14_01	2014, August	No	60	11.4
Lofoten	LOF_A_14_03	2014, August	No	54	10.2
Lofoten	LOF_A_14_04	2014, August	No	47	9.0
Lofoten	LOF_A_14_05	2014, August	No	47	8.9
Lofoten	LOF_A_14_06	2014, August	No	52	10.0
Lofoten	LOF_A_14_08	2014, August	No	43	8.2
Lofoten	LOF_A_14_09	2014, August	No	50	9.5
Lofoten	LOF_A_14_10	2014, August	No	48	9.1
Lofoten	LOF_A_14_11	2014, August	No	53	10.1
Lofoten	LOF_A_14_16	2014, August	No	54	10.1
Lofoten	LOF_A_14_17	2014, August	No	60	11.4
Lofoten	LOF_A_14_18	2014, August	No	52	9.9
Lofoten	LOF_A_14_19	2014, August	No	54	10.3
Lofoten	LOF_A_14_20	2014, August	No	43	8.2
Lofoten	LOF_A_14_21	2014, August	No	52	9.8
Lofoten	LOF_A_14_22	2014, August	No	48	9.1
Lofoten	LOF_A_14_23	2014, August	No	46	8.8
Lofoten	LOF_A_14_24	2014, August	No	49	9.3
Lofoten	LOF_A_14_25	2014, August	No	59	11.2
Lofoten	LOF_A_14_26	2014, August	No	51	9.7
Lofoten	LOF_A_14_27	2014, August	No	65	12.4
Lofoten	LOF_A_14_28	2014, August	No	49	9.3
Lofoten	LOF_A_14_29	2014, August	No	46	8.7
Lofoten	LOF_A_14_30	2014, August	No	44	8.3
Lofoten	LOF_A_14_33	2014, August	No	46	8.8
Lofoten	LOF_A_14_41	2014, August	No	45	8.6
Lofoten	LOF_A_14_43	2014, August	No	195	37.0
North East Arctic	LOF_M_14_26	2014, March	Yes	46	8.7
North East Arctic	LOF_M_14_27	2014, March	Yes	47	8.9
North East Arctic	LOF_M_14_28	2014, March	Yes	44	8.3
North East Arctic	LOF_M_14_29	2014, March	Yes	46	8.6
North East Arctic	LOF_M_14_30	2014, March	Yes	43	8.1
North East Arctic	LOF_M_14_31	2014, March	Yes	50	9.4
North East Arctic	LOF_M_14_32	2014, March	Yes	50	9.4
North East Arctic	LOF_M_14_33	2014, March	Yes	50	9.4
North East Arctic	LOF_M_14_35	2014, March	Yes	42	8.0
North East Arctic	LOF_M_14_36	2014, March	Yes	53	10.0
North East Arctic	LOF_M_14_43	2014, March	Yes	88	16.7
North East Arctic	LOF_M_14_44	2014, March	Yes	47	9.0
North East Arctic	LOF_M_14_45	2014, March	Yes	45	8.6
North East Arctic	LOF_M_14_46	2014, March	Yes	57	10.7
North East Arctic	LOF_M_14_47	2014, March	Yes	50	9.4
North East Arctic	LOF_M_14_50	2014, March	Yes	39	7.4
North East Arctic	LOF_M_14_51	2014, March	Yes	43	8.1
North East Arctic	LOF_M_14_52	2014, March	Yes	44	8.3
North East Arctic	LOF_M_14_53	2014, March	Yes	24	4.5
North East Arctic	LOF_M_14_54	2014, March	Yes	43	8.2
North East Arctic	LOF_M_14_55	2014, March	Yes	46	8.7
North East Arctic	LOF_M_14_56	2014, March	Yes	55	10.5
North East Arctic	LOF_M_14_62	2014, March	Yes	50	9.4
North East Arctic	LOF_M_14_68	2014, March	Yes	38	7.2
Øresund	ORE_301	2012, March	Yes	44	8.2
Øresund	ORE_302	2012, March	Yes	42	7.7
Øresund	ORE_303	2012, March	Yes	40	7.6
Øresund	ORE_308	2012, March	Yes	33	6.0
Øresund	ORE_309	2012, March	Yes	45	8.4
Øresund	ORE_310	2012, March	Yes	39	7.4
Øresund	ORE_313	2012, March	Yes	105	19.8
Øresund	ORE_314	2012, March	Yes	34	6.0
Øresund	ORE_315	2012, March	Yes	25	4.7
Øresund	ORE_316	2012, March	Yes	49	9.3
Øresund	ORE_317	2012, March	Yes	38	7.1
Øresund	ORE_318	2012, March	Yes	36	6.6
Øresund	ORE_322	2012, March	Yes	33	6.0
Øresund	ORE_323	2012, March	Yes	52	9.7
Øresund	ORE_325	2012, March	Yes	46	8.6
Øresund	ORE_326	2012, March	Yes	39	7.2
Øresund	ORE_331	2012, March	Yes	50	9.3

Øresund	ORE_332	2012, March	Yes	49	9.2
Øresund	ORE_333	2012, March	Yes	48	8.9
Øresund	ORE_336	2012, March	Yes	41	7.7
Øresund	ORE_341	2012, March	Yes	45	8.4
North Sea	SOD_01	2002, March	Yes	43	8.3
North Sea	SOD_02	2002, March	Yes	46	8.8
North Sea	SOD_03	2002, March	Yes	44	8.3
North Sea	SOD_04	2002, March	Yes	55	10.4
North Sea	SOD_06	2002, March	Yes	45	8.7
North Sea	SOD_07	2002, March	Yes	46	8.8
North Sea	SOD_08	2002, March	Yes	42	8.1
North Sea	SOD_09	2002, March	Yes	49	9.4
North Sea	SOD_10	2002, March	Yes	42	8.1
North Sea	SOD_13	2002, March	Yes	43	8.3
North Sea	SOD_14	2002, March	Yes	48	9.2
North Sea	SOD_15	2002, March	Yes	45	8.5
North Sea	SOD_17	2002, March	Yes	46	8.7
North Sea	SOD_19	2002, March	Yes	47	8.8
North Sea	SOD_20	2002, March	Yes	39	7.2
North Sea	SOD_21	2002, March	Yes	45	8.3
North Sea	SOD_22	2002, March	Yes	46	8.8
North Sea	SOD_23	2002, March	Yes	44	8.4
North Sea	SOD_25	2002, March	Yes	45	8.6
North Sea	SOD_26	2002, March	Yes	53	10.0
North Sea	SOD_27	2002, March	Yes	51	9.0
North Sea	SOD_28	2002, March	Yes	46	8.6
North Sea	SOD_29	2002, March	Yes	46	8.8
North Sea	SOD_30	2002, March	Yes	42	7.6

DNA extraction and library creation of ancient Atlantic cod samples

All extraction and library protocols were performed in a dedicated laboratory at the Department of Biosciences, University of Oslo following strict aDNA precautions (79, 80). This is a laboratory that is physically separated from the modern DNA laboratories and in which no modern samples have ever been processed. Extraction of ancient Atlantic cod bones (n = 19) used a combined bleach and pre-digestion protocol (54). Each sample was exposed to UV for 10 minutes on each side, resulting in a total dosage of 4800 J/m2 before being cut and milled to powder. Then, for each sample, two times 150-200 mg of bone powder (milled in a Retsch MM400) was incubated in 0.5% bleach solution for 15 min (94). The samples were subsequently washed with H_2O and the remaining bone powder was exposed to a 30-minute pre-digestion treatment followed by an overnight, second digestion using a freshly prepared digestion buffer (95). Following the second digestion, the two eluates were combined and concentrated (Amicon-30kDA Centrifugal Filter Units) after which DNA was extracted using Qiagen Minelute columns according to manufacturer's instructions. DNA was eluted in 60 µl pre-heated (60°C) EB buffer with a 15 min incubation at 37°C. Negative controls were included in all extraction experiments. Blunt-end Illumina libraries were built (81), following (20) and ligated DNA was amplified using sample-specific seven base-pair indexes in the P7 primer to allow multiplexing. PCRs were done in 15 µl (2.5 U PfuTurbo Cx Hotstart DNA Polymerase (Agilent Technologies), 1x buffer, 0.2 mM per dNTP, 0.2 µM P7 index primer, 0.2 µM P5 IS4 primer and 0.4 mg/ml BSA) for 13 cycles (2 min at 95°C, 13 cycles of 30s at 95°C, 30s at 60°C and 70s at 72°C with a final extension of 10 min at 72°C). Amplified products were cleaned using Agencourt® AMPure XP beads at a 1:1.7 ratio, eluted in 30 µl in EB buffer and quantified using a Bioanalyzer 2100 (Agilent). Libraries were sequenced on an Illumina Hiseq 2500 at the Norwegian Sequencing Centre (125 bp paired-end) and demultiplexed allowing zero mismatches in the index tag.