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Supplementary material
Appendix 1

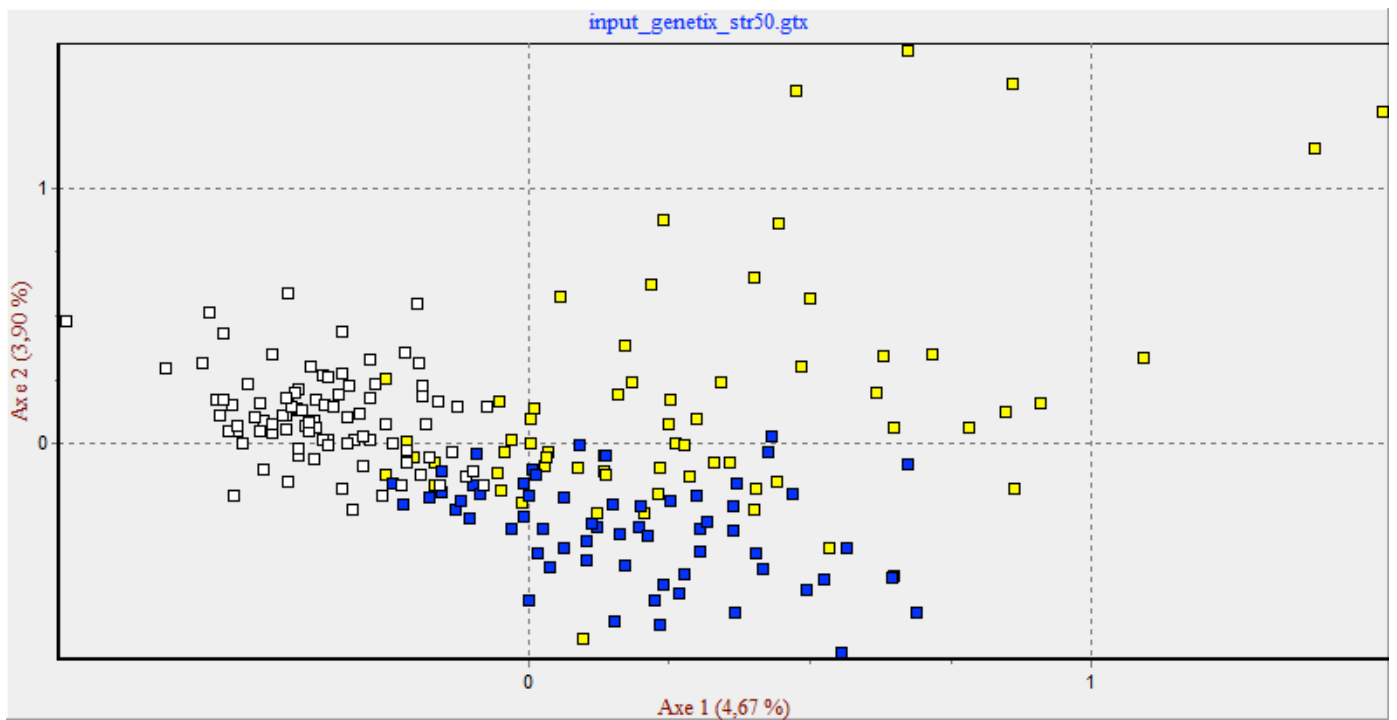


Figure A1. Factorial correspondence analysis (FCA) performed in Genetix 4.05.2. based on 218 individual microsatellite genotypes (11 loci). Squares with different colours indicate individuals assigned to different genetic clusters by Structure 2.3.4. (according to the 50% cut-off criterion): *northern* (yellow), *central* (blue) and *southern* (white). The three clusters show overall segregation, but there is some overlap (mostly of northern eagles with those from the other two clusters).

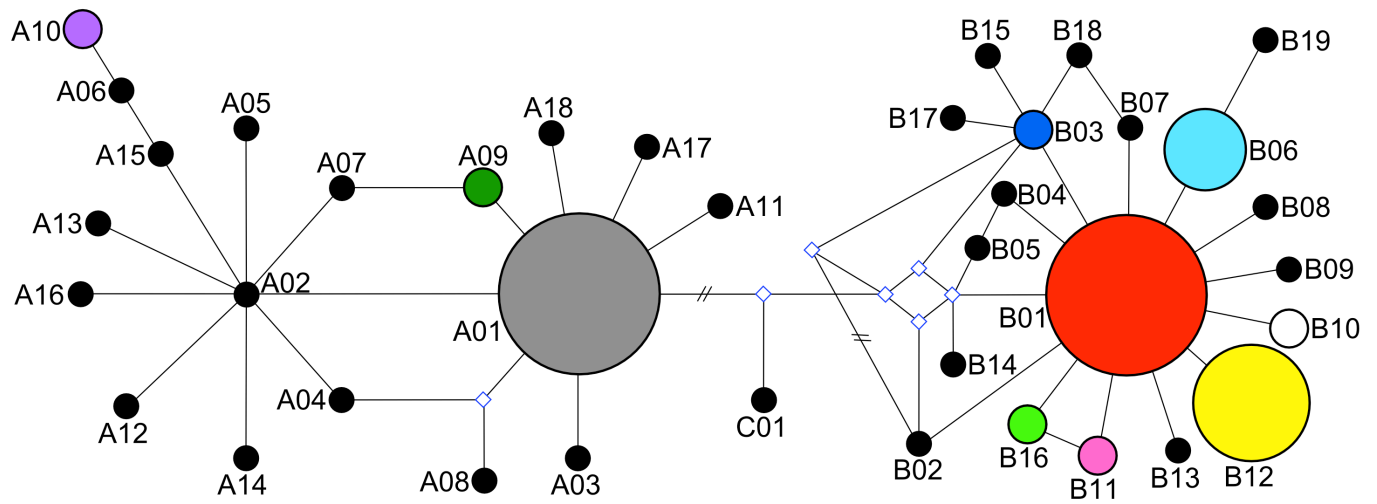


Figure A2. Median-joining network of all 38 mt-hvr1 haplotypes found in the global distribution range of the species. Haplotypes found in the Carpathian Basin so far are in colour, and the area of each circle is proportional to their frequency within the Carpathian Basin. Colour code is identical with Fig 2. Links between the haplotypes indicate a single mutational step except for the ones marked with double dashes where two mutations occurred. In the present study we only found the haplotypes A01, B01, B06 and B12.

Table A1. Ln probability and ΔK values for the number of genetic clusters (K) according to the Structure analysis. Calculation of the ad hoc statistic we used to decide the accepted number of clusters is: $\Delta K = (mL(K + 1) - 2mL(K) + mL(K - 1)) / sdL(K)$, where $L(K)$ is the natural logarithm of the probability that K is the correct number of clusters, m is the mean and SD is the standard deviation of the 10 replicate runs for the same K value. (For the lowest and highest K values in a comparison, ΔK is not applicable.)

Replicate	K=1	K=2	K=3	K=4	K=5	K=6	K=7
1	-6170.2	-6094.3	-6045.7	-6307.2	-6356.6	-6302.9	-6500.0
2	-6169.9	-6092.5	-6039.4	-6092.2	-6420.9	-6143.4	-6488.8
3	-6170.2	-6090.7	-6037.3	-6072.6	-6563.8	-6240.5	-6659.1
4	-6170.0	-6099.4	-6049.8	-6062.5	-6611.8	-6166.0	-6556.6
5	-6170.1	-6095.5	-6037.1	-6080.1	-6259.2	-6161.3	-6350.8
6	-6170.2	-6097.0	-6038.1	-6118.8	-6525.3	-6219.9	-6483.2
7	-6170.2	-6082.1	-6040.9	-6236.1	-6467.1	-6209.3	-6378.0
8	-6169.9	-6089.4	-6043.8	-6079.6	-6339.8	-6186.1	-6415.1
9	-6170.4	-6094.6	-6042.0	-6082.3	-6381.2	-6224.7	-6447.9
10	-6170.1	-6100.5	-6039.5	-6200.5	-6702.9	-6240.3	-6433.9
Mean	-6170.12	-6093.6	-6041.36	-6133.19	-6462.86	-6209.44	-6471.34
SD	0.155	5.348	4.072	84.483	137.538	47.264	89.534
ΔK	-	4.540	35.384	2.815	4.239	10.903	-

Table A2: Percentage of sampled individuals assigned to the genetic clusters proposed by Structure within geographically separable units, using three different cut-off criteria. N: number of sampled individuals. Lower-case letters refer to the three Structure clusters, according to their predominant distribution (**n**: northern, **c**: central, **s**: southern), **u** denotes non-classified individuals. **Spatial units**: clusters suggested by Geneland (Finnish Baltic coast (B. coast), Finnish Lapland and Lithuania (Lap, Lit), Germany and Poland (Ger, Pol), Czech and Carpathian Basin (CB)), sorted into three major groups according to their geographical location (Northern, Central, Southern). The most frequent Structure clusters in each unit are bold. Both Structure and Geneland results are averages across 10 replicate runs.

Spatial unit	N	50% cut-off				60% cut-off				70% cut-off			
		n	c	s	u	n	c	s	u	n	c	s	u
Northern	35	77	11	3	9	71	9	0	20	63	6	0	31
B. coast	23	74	17	0	9	74	13	0	13	61	9	0	30
Lap, Lit	12	83	0	8	8	67	0	0	33	67	0	0	33
Central	71	30	63	1	6	24	59	0	17	18	48	0	34
Ger, Pol	43	12	81	2	5	7	79	0	14	5	72	0	23
Czech	28	57	36	0	7	50	29	0	21	39	11	0	50
Southern (CB)	143	13	9	62	17	8	5	55	32	4	3	43	50

Table A3: Number of individuals within the recolonized regions with probable origin. These birds were classified consistently to one population according to assignment tests of GeneClass2 and genetic clusters suggested by Structure (for the latter, we used three different cut-off criteria: 50, 60 and 70%). **N**: number of sampled individuals in each recolonized region. Lower-case letters in the columns refer to the assumed origin (**n**: northern, **c**: central, **s**: southern).

Recolonized region	N	50% cut-off			60% cut-off			70% cut-off		
		n	c	s	n	c	s	n	c	s
Austria	3	1	0	0	1	0	0	0	0	0
Slovakia	8	0	1	3	0	1	3	0	1	3
Hungary	39	4	0	13	3	0	13	2	0	11
Czech Republic	29	12	2	0	10	2	0	8	1	0
SUM	79	17	3	16	14	3	16	10	2	14

Tables A4-A7: Pairwise F_{ST} (below diagonal) and unbiased Nei's genetic distance (above diagonal) for the Geneland and Structure clusters based on microsatellite data. All comparisons were statistically significant ($p < 0.001$ for Structure and $p < 0.003$ for Geneland clusters). A5-A7: „individual” clusters with 50, 60 and 70% cut-off criteria, respectively.

A4.

Geneland cluster	Finnish Baltic coast	Finnish Lapland and Lithuania	Germany and Poland	Czech	Carpathian Basin
Finnish Baltic coast	0	0.074	0.064	0.104	0.091
Finnish Lapland and Lithuania	0.043	0	0.060	0.030	0.038
Germany and Poland	0.040	0.041	0	0.064	0.062
Czech	0.056	0.018	0.039	0	0.064
Carpathian Basin	0.088	0.051	0.072	0.052	0

A5.

Structure cluster - 50%	<i>northern</i>	<i>central</i>	<i>southern</i>
<i>northern</i>	0	0.070	0.078
<i>central</i>	0.041	0	0.110
<i>southern</i>	0.067	0.099	0

A6.

Structure cluster - 60%	<i>northern</i>	<i>central</i>	<i>southern</i>
<i>northern</i>	0	0.090	0.091
<i>central</i>	0.053	0	0.123
<i>southern</i>	0.084	0.118	0

A7.

Structure cluster - 70%	<i>northern</i>	<i>central</i>	<i>southern</i>
<i>northern</i>	0	0.103	0.111
<i>central</i>	0.059	0	0.145
<i>southern</i>	0.097	0.135	0

Table A8: Number of individuals sampled in the Carpathian Basin that were successfully analysed for both markers (mt-hvr1 and microsatellites), and their assignment to the Structure clusters based on the three cut-off criteria (n: *northern*, c: *central*, s: *southern*).

Haplotype	N	50% cut-off			60% cut-off			70% cut-off		
		n	c	s	n	c	s	n	c	s
A01	24	4	0	18	2	0	16	1	0	14
B01	18	7	0	7	4	0	5	2	0	3
B06	6	1	1	3	1	0	3	1	0	2
B12	12	0	1	10	0	1	10	0	0	9
B03	1	0	1	0	0	1	0	0	0	0
B10	1	0	1	0	0	1	0	0	1	0
SUM	62	12	4	38	7	3	34	4	1	28

Table A9: Number of mt-hvr1 haplotypes occurring in at least two countries and found throughout the breeding range of the species so far. Sampled countries are sorted according to their geographical region: Northern/Central/South-central Europe (NE, CE and SE, respectively), North Atlantic Ocean (NA) and Asia (A). For further information see the original papers (Hailer et al. 2006, 2007, Honnen et al. 2010, Langguth et al. 2013, Ponnikas et al. 2013 and Treinys et al. 2016).

* Haplotypes in Finland were specified based on 473 bp instead of 499 bp. Therefore, B04/B05 and B01/B10/B14 could not be distinguished. As B10 was found only in one Austrian and B14 in one Czech sample, higher proportions of these haplotypes in Finland are unlikely, and we therefore assigned these Finnish birds to B01.

** We did not investigate any Swedish samples for microsatellites. However, based on the similar haplotype-distributions to the neighbouring Finnish areas, the Swedish Lapland and Baltic coast are likely part of the Northern inland and Baltic coast populations, respectively. Moreover, birds originating from the Finnish coast were found as breeders at the Swedish coast (Helander 2003).

*** Classification by Geneland of the only Estonian sample analysed for microsatellites was uncertain. However, mtDNA haplotype composition in Estonia (B03 and B07; Hailer et al. 2007; Langguth et al. 2013) is most similar to that of adjacent population of Finnish Lapland and Lithuania and its geographical position suggests as well that Estonia belongs to this population.

Re-gion	Geneland cluster	Country or part	N	Haplogroup			Frequent haplotypes						Other distributed haplotypes							
				A	B	C	A01	A02	A03	B01	B12	C01	B02	B03	B04	B05	B06	B07	B11	B18
NE	Finnish Lapland and Lithuania	Finnish Lapland	23	8	15	-	8	-	-	9*	-	-	1	-	0-1*	0-1*	2	2	-	-
		Lithuania	45	26	19	-	20	6	-	14	-	-	-	2	-	-	2	1	-	-
		Estonia***	24	5	19	-	4	1	-	9	-	-	-	5	-	-	-	3	-	2
		Overall %	92	42.4	57.6	-	34.8	7.6	-	34.8	-	-	-	1.1	7.61	0-1*	0-1*	4.4	6.5	-
	-	Swedish Lap**	22	12	10	-	12	-	-	5	-	-	1	-	2	1	1	-	-	-
	Finnish Baltic coast	Finnish coast	63	20	31	12	18	2	-	30*	-	12	-	1	-	-	-	-	-	-
Overall %		63	31.7	49.2	19	28.6	3.2	-	47.6	-	19	-	1.6	-	-	-	-	-	-	
-		Swedish coast**	51	24	21	6	22	1	-	21	-	16	-	-	-	-	-	-	-	-
CE	Germany and Poland	Germany	85	78	7	-	17	58	-	7	-	-	-	-	-	-	-	-	-	-
		Poland	55	46	9	-	13	25	-	2	-	-	-	-	-	-	-	-	-	1
		Overall %	140	88.6	11.4	-	21.4	59.3	-	6.4	-	-	-	-	-	-	-	-	-	0.7
	Czech	Czech Republic	9	3	6	-	-	2	-	3	-	-	-	-	-	1	-	1	-	-
N Austria		3	1	2	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	
Overall %		12	33.3	66.7	-	-	25	-	33.3	-	-	-	-	-	8.3	-	8.3	-	-	
SE	Carpathian Basin	SE Austria	10	2	8	-	1	-	-	5	1	-	-	1	-	-	-	-	-	
		Slovakia	6	2	4	-	1	-	-	1	-	-	-	-	-	2	-	1	-	
		Serbia	30	11	19	-	11	-	-	15	2	-	-	-	-	1	-	-	-	
		Hungary	44	18	26	-	18	-	-	9	12	-	-	-	-	5	-	-	-	
		Croatia	9	3	6	-	3	-	-	4	2	-	-	-	-	-	-	-	-	
		Overall %	99	36.4	63.6	-	34.3	-	-	34.3	17.2	-	-	-	1	-	-	8.1	-	1
NA	-	Norway	33	32	1	-	32	-	-	1	-	-	-	-	-	-	-	-	-	
		Greenland	8	8	-	-	7	-	1	-	-	-	-	-	-	-	-	-	-	
		Iceland	26	26	-	-	7	-	19	-	-	-	-	-	-	-	-	-	-	
A	-	Japan	8	-	8	-	-	-	-	7	-	-	-	1	-	-	-	-		
		Kazakhstan	26	3	23	-	-	-	-	13	-	-	-	-	-	1	-	-	-	
		Kola Peninsula	10	1	9	-	1	-	-	2	-	-	2	1	-	1	1	2	-	

Table A10: List of microsatellite alleles and their frequencies within each Geneland and Structure clusters. Geneland clusters: Finnish Baltic coast (BC), Finnish Lapland and Lithuania (FL), Germany and Poland (GP), Carpathian Basin (CB) and Czech (Cz). Structure clusters (with 50, 60 and 70% cut-off criteria): *northern* (n), *central* (c) and *southern* (s).

Alleles with presumable diagnostic information (by presence) on a bird's origin are marked with red (northern), blue (central) or yellow (southern).

Locus	Allele	Geneland clusters					Structure – 50%			Structure – 60%			Structure – 70%		
		Bc	FL	GP	CB	Cz	n	c	s	n	c	s	n	c	s
Aa27	N	23	12	43	76	26	56	56	44	47	49	35	37	39	27
	93	0.02	-	-	-	-	0.01	-	-	0.01	-	-	0.01	-	-
	97	0.04	0.21	0.26	0.16	0.10	0.10	0.23	0.14	0.07	0.22	0.13	0.05	0.24	0.15
	99	0.83	0.58	0.71	0.78	0.52	0.63	0.73	0.83	0.63	0.73	0.84	0.59	0.72	0.83
	101	0.09	0.04	-	-	0.15	0.11	-	-	0.13	-	-	0.15	-	-
	103	0.02	0.17	0.03	0.06	0.23	0.16	0.04	0.03	0.16	0.04	0.03	0.19	0.04	0.02
Aa35	N	23	12	39	140	27	65	58	87	54	50	76	41	38	59
	239	0.11	-	0.01	0.18	-	0.05	0.03	0.23	0.05	0.01	0.25	0.04	-	0.28
	241	0.72	0.83	0.77	0.72	0.96	0.84	0.78	0.68	0.85	0.79	0.67	0.87	0.78	0.64
	245	0.17	0.17	0.22	0.10	0.04	0.12	0.18	0.09	0.10	0.20	0.08	0.10	0.22	0.08
Hal01	N	23	12	35	137	27	61	54	88	51	44	77	40	33	60
	132	0.04	0.17	-	0.01	-	0.04	-	0.01	0.05	-	0.01	0.06	-	0.01
	134	0.13	0.25	0.46	0.61	0.46	0.26	0.51	0.66	0.22	0.55	0.68	0.19	0.52	0.68
	136	0.15	0.08	0.0	0.08	0.11	0.16	0.02	0.06	0.17	-	0.05	0.19	-	0.04
	138	0.33	0.29	0.41	0.30	0.19	0.28	0.38	0.27	0.27	0.34	0.27	0.28	0.42	0.27
	140	0.33	0.17	0.11	0.004	0.22	0.23	0.08	-	0.25	0.10	-	0.25	0.06	-
	142	0.02	0.04	-	0.01	0.02	0.03	0.01	-	0.04	0.01	-	0.04	-	-
Hal04	N	23	12	39	142	27	66	57	90	54	48	78	41	38	61
	150	-	-	0.01	-	0.19	0.07	0.01	-	0.07	0.01	-	0.09	0.01	-
	154	-	0.08	-	0.03	-	0.04	0.02	-	0.05	-	-	0.05	-	-
	156	0.09	0.04	0.06	0.11	0.11	0.10	0.05	0.11	0.10	0.04	0.12	0.11	0.05	0.12
	158	0.07	0.17	0.10	0.01	0.04	0.05	0.07	0.01	0.05	0.07	0.01	0.04	0.09	0.02
	160	0.85	0.71	0.82	0.71	0.54	0.68	0.81	0.72	0.68	0.84	0.70	0.67	0.83	0.66
	162	-	-	-	0.13	0.13	0.06	0.04	0.16	0.06	0.03	0.17	0.05	0.01	0.20
Hal09	N	23	12	40	142	26	65	57	90	53	47	7	40	38	61
	127	0.04	0.13	0.09	0.04	0.06	0.10	0.05	0.01	0.10	0.04	0.01	0.13	0.05	-
	133	0.22	0.25	0.29	0.32	0.33	0.28	0.31	0.32	0.25	0.30	0.35	0.24	0.30	0.36
	137	0.02	0.08	0.01	0.01	-	0.05	0.01	-	0.06	0.01	-	0.04	0.01	-
	139	0.04	0.08	0.01	0.02	-	0.04	-	0.02	0.04	-	0.01	0.05	-	0.02
	141	0.30	0.25	0.31	0.36	0.31	0.26	0.32	0.39	0.30	0.35	0.40	0.28	0.32	0.43
	143	0.13	0.17	0.26	0.12	0.31	0.18	0.25	0.09	0.16	0.26	0.09	0.18	0.28	0.07
	145	0.24	0.04	0.03	0.12	-	0.08	0.06	0.16	0.08	0.04	0.15	0.10	0.04	0.11
Hal10	N	23	12	40	143	28	66	59	90	54	49	78	41	37	61
	232	0.48	0.08	0.54	0.16	0.23	0.24	0.61	0.06	0.20	0.63	0.04	0.22	0.65	0.02
	234	0.04	0.04	0.01	0.05	-	0.02	0.01	0.07	0.03	0.01	0.08	0.04	0.01	0.08
	236	0.07	0.29	0.03	0.14	0.18	0.20	0.06	0.08	0.21	0.05	0.08	0.20	0.04	0.06
	238	0.41	0.58	0.41	0.65	0.57	0.52	0.32	0.79	0.55	0.31	0.80	0.55	0.30	0.84
	240	-	-	0.01	-	0.02	0.02	-	-	0.01	-	-	-	-	-
Hal13	N	23	12	40	143	26	65	58	90	53	48	78	41	37	61
	149	0.28	0.08	-	0.01	-	0.14	0.01	-	0.16	0.01	-	0.15	-	-
	151	0.13	0.04	-	0.08	-	0.07	-	0.08	0.08	-	0.07	0.07	-	0.06
	155	0.04	-	0.08	0.01	0.08	0.02	0.09	0.01	0.03	0.09	0.01	0.04	0.08	-
	157	0.33	0.63	0.35	0.58	0.37	0.40	0.35	0.70	0.35	0.36	0.71	0.35	0.36	0.74
	159	-	-	0.04	0.02	0.08	0.05	0.03	0.01	0.06	0.04	0.01	0.06	0.04	-
	161	0.17	0.25	0.51	0.29	0.35	0.25	0.51	0.19	0.24	0.48	0.19	0.22	0.51	0.19
	163	0.04	-	0.03	-	0.04	0.05	-	-	0.06	-	-	0.07	-	-
	165	-	-	-	0.01	0.10	0.03	0.01	0.02	0.04	0.01	0.01	0.04	-	0.02
IEAAAG04	N	23	12	39	142	26	64	59	89	52	50	78	41	38	61
	198	-	0.08	0.03	-	-	0.03	-	-	0.03	-	-	0.04	-	-

	202	-	0.04	-	0.05	-	0.03	-	0.05	0.03	-	0.05	0.02	-	0.03
	206	0.61	0.42	0.58	0.68	0.62	0.62	0.61	0.66	0.61	0.60	0.65	0.57	0.57	0.66
	210	0.20	0.21	0.26	0.26	0.27	0.20	0.24	0.28	0.21	0.24	0.28	0.21	0.26	0.30
	214	0.07	0.08	0.13	0.01	0.10	0.04	0.14	0.01	0.03	0.15	0.01	0.04	0.16	0.02
	218	0.13	0.17	-	-	-	0.06	0.01	-	0.08	0.01	-	0.10	0.01	-
	222	-	-	0.01	-	0.02	0.02	-	-	0.02	-	-	0.02	-	-
IEAAAG05	N	23	12	41	142	26	65	60	88	53	50	77	41	39	61
	113	-	-	0.01	-	0.04	0.02	0.01	-	0.01	0.01	-	0.01	-	-
	121	-	0.04	-	-	-	0.01	-	-	0.01	-	-	0.01	-	-
	125	0.11	0.04	-	-	-	0.05	-	-	0.05	-	-	0.06	-	-
	133	0.02	0.04	-	0.02	0.02	0.05	-	0.01	0.05	-	0.01	0.05	-	-
	137	0.04	0.04	0.29	0.01	0.10	0.02	0.24	0.01	0.02	0.29	-	0.02	0.29	-
	141	0.04	0.08	0.06	0.08	0.27	0.17	0.05	0.06	0.18	0.05	0.06	0.17	0.05	0.07
	145	0.39	0.29	0.45	0.61	0.27	0.27	0.51	0.68	0.27	0.50	0.68	0.26	0.53	0.69
	149	0.28	0.04	0.04	0.03	0.21	0.13	0.09	0.03	0.14	0.07	0.03	0.16	0.06	0.03
	153	0.04	0.17	0.11	0.07	0.10	0.12	0.09	0.03	0.13	0.07	0.04	0.12	0.06	0.02
	157	-	0.13	0.01	0.01	-	0.03	-	0.02	0.02	-	0.01	0.02	-	0.02
	161	0.02	0.08	-	0.06	-	0.05	-	0.05	0.06	-	0.05	0.06	-	0.06
	165	-	-	0.01	-	-	-	0.01	-	-	0.01	-	-	-	-
	169	0.04	-	0.01	0.07	-	0.04	-	0.09	0.04	-	0.10	0.02	-	0.11
	180	-	-	-	0.01	-	0.01	-	0.01	-	-	0.01	-	-	-
	188	-	0.04	-	-	-	0.01	-	-	0.01	-	-	0.01	-	-
	206	-	-	-	0.01	-	0.02	-	0.01	0.02	-	-	0.01	-	-
	210	-	-	-	0.01	-	0.01	-	0.01	-	-	-	-	-	-
IEAAAG12	N	23	12	43	97	25	59	56	64	51	49	53	39	38	43
	95	0.04	0.04	-	0.01	0.02	0.03	0.01	-	0.04	0.01	-	0.04	-	-
	97	0.48	0.50	0.50	0.60	0.60	0.63	0.43	0.61	0.65	0.43	0.65	0.63	0.43	0.67
	101	0.09	0.04	-	-	-	0.03	-	-	0.04	-	-	0.04	-	-
	104	0.15	0.21	0.15	0.15	0.20	0.13	0.19	0.16	0.12	0.19	0.13	0.15	0.18	0.14
	108	0.09	0.17	0.16	0.18	0.14	0.14	0.18	0.16	0.11	0.16	0.15	0.12	0.16	0.14
	112	0.15	-	0.19	0.06	0.04	0.03	0.20	0.07	0.04	0.20	0.07	0.01	0.22	0.05
	116	-	0.04	-	-	-	0.01	-	-	0.01	-	-	0.01	-	-
IEAAAG14	N	23	12	36	140	24	63	54	89	52	46	77	41	37	60
	174	0.30	0.46	0.43	0.36	0.48	0.43	0.45	0.31	0.46	0.49	0.32	0.46	0.49	0.32
	178	0.24	0.42	0.29	0.34	0.29	0.30	0.21	0.39	0.28	0.22	0.38	0.26	0.23	0.36
	182	0.46	0.13	0.28	0.28	0.23	0.26	0.33	0.28	0.26	0.29	0.27	0.28	0.28	0.30
	186	-	-	-	0.02	-	0.01	-	0.02	-	-	0.02	-	-	0.03

Table A11: Number of private alleles in each Geneland and Structure cluster and geographical region.

Type	Unit name	Private allele
Structure 50%	northern	11
	central	1
	southern	0
Structure 60%	northern	13
	central	1
	southern	2
Structure 70%	northern	17
	central	0
	southern	1
Geneland	Finnish Baltic coast	1
	Finnish Lapland and Lithuania	3
	Germany and Poland	1
	Czech	0
	Carpathian Basin	4
Geographical regions	East European Plain	7
	Great European Plain	5
	Carpathian Basin	4

Table A12: Optimized volumes of microsatellite primers for PCR reactions and fragment length analysis on a sequencer. Different amplification reactions are separated by dotted lines. All primers were diluted to 5 pikomol/ μ l and forward primers were 5'-labeled with fluorescent dyes. Each PCR reaction was performed in 16 μ l volume. All primers were published earlier (Hal: Hailer et al. 2005), Aa: Martínez-Cruz et al. 2002; IEAAAG: Busch et al. 2005).

Primer pair	Dye	PCR reactions		Run in sequencer		
		primer (μ l)	type	Volume of PCR product(μ l)	Run	
IEAAAG04	FAM	1.6	multiplex	9	1	
Hal04	NED	2.4				
Hal01	FAM	2.3	multiplex	7		
IEAAAG12	HEX	1.7				
IEAAAG14	VIC	2.57	singleplex	4.5		
IEAAAG05	PET	2.57	singleplex	4.5		
Hal13	VIC	1.8	multiplex	9		
Hal10	NED	2.2				
Hal09	FAM	2.57	singleplex	6		2
Aa35	PET	2.1	multiplex	10		
Aa27	FAM	1.9				