

## **Supplementary Material**

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**Table S1** Overview of the red deer sample set used in this research. Filter relatives:  $\hat{p}_i < 0.1825$

Population	Type	Abbreviation	Country	Sampling Period	Sample size	Sample size Filter Relatives	Sample size Males (incl. Filter Relatives)	Enough Samples For All Analysis?
Veluwezoom, Veluwe	Relic	vzm	the Netherlands	2005	9	8	3	Yes
Deelerwoud, Veluwe	Relic	dee	the Netherlands	2005	3	3	2	No
Planken Wambuis, Veluwe	Relic	pwb	the Netherlands	2006	9	8	1	Yes
Kroondomeinen, Veluwe	Estate	kro	the Netherlands	2011	11	9	3	Yes
NP Hoge Veluwe, Veluwe	Estate	hgv	the Netherlands	2011	4	4	4	No
Oostvaardersplassen	Rewilding	ovp	the Netherlands	2007	15	15	8	Yes
West-Czechia	Reference	cze	Czechia	2014	11	10	5	Yes
Kintyre Peninsula, Argyll	Reference	sco	Scotland	1991-2007	43	36	24	Yes
Isle of Rum	Reference	rum	Scotland	1970-2010	100	100	67	Yes

**Table S2** Genetic differentiation  $F_{ST}$  among the red deer genetic clusters identified by the PCoA of Fig. 2B and D. Genetic clusters with less than five unrelated and successfully genotyped individuals are excluded from the analyses. All  $F_{ST}$  deviate significantly from zero ( $P < 0.001$ ). Abbreviations as in Table S1. Hoge Veluwe (hgv) is not included because of low sample size.

	sco	rum	ovp	cze	kro	pwb	Vzm
sco	n.a.	0.06	0.04	0.06	0.08	0.12	0.13
rum	0.06	n.a.	0.05	0.09	0.11	0.14	0.15
ovp	0.04	0.05	n.a.	0.05	0.07	0.11	0.12
cze	0.06	0.09	0.05	n.a.	0.05	0.10	0.12
kro	0.08	0.11	0.07	0.05	n.a.	0.06	0.07
pwb	0.12	0.14	0.11	0.10	0.06	n.a.	0.07
vzm	0.13	0.15	0.12	0.12	0.07	0.07	n.a.

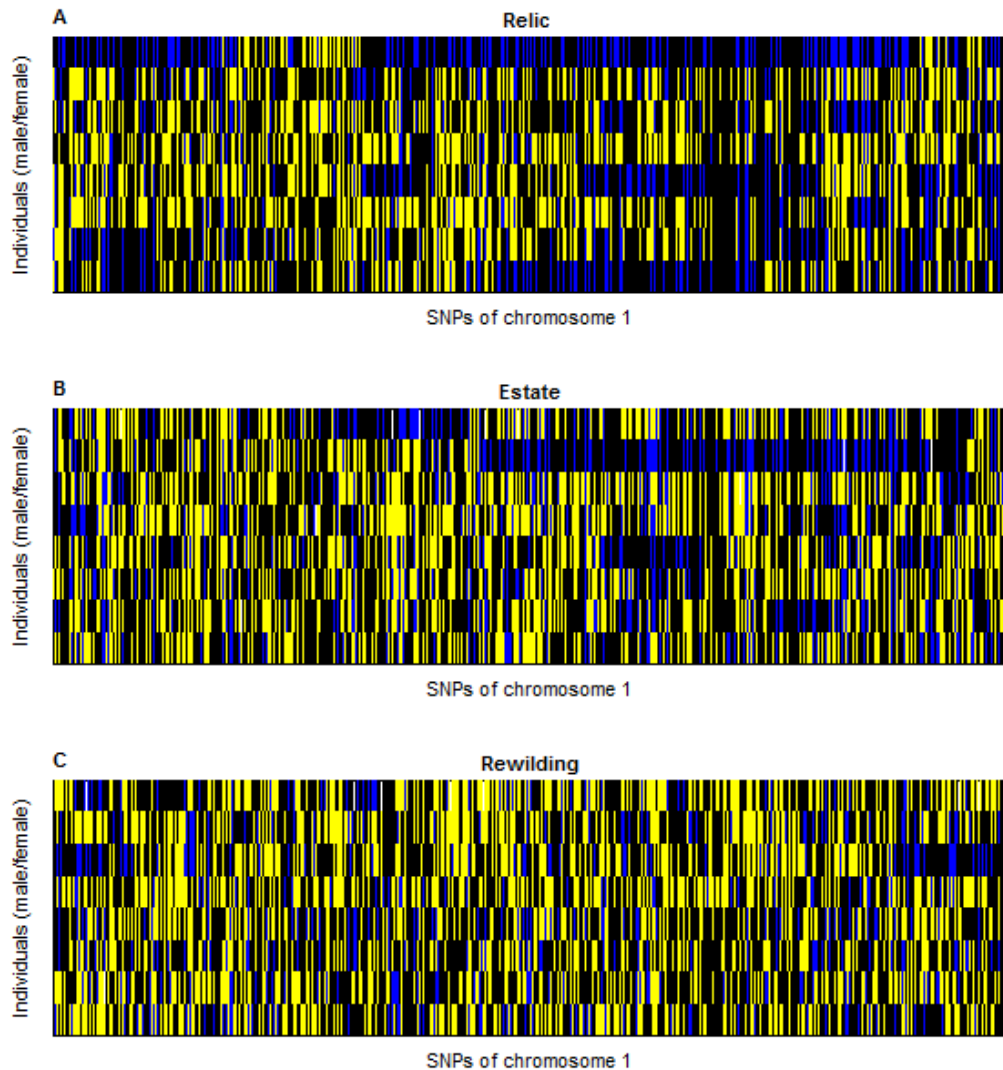
**Table S3** P-values of pairwise comparison tests on Multi Locus Heterozygosity among populations. Pairwise Wilcoxon Rank Sum Test, with Bonferroni corrected p-values. Maximum and minimum median MLH were found in OVP and VZM, respectively.

	Cze	rum	sco	ovp	kro	hgv	vzm
rum	9.6e-06	-	-	-	-	-	-
sco	2.2e-07	1.00000	-	-	-	-	-
ovp (max)	0.00268	0.71364	0.78569	-	-	-	-
kro	1.00000	2.1e-05	6.3e-08	0.00051	-	-	-
hgv	1.00000	0.09431	0.00613	0.13725	1.00000	-	-
vzm (min)	7.9e-05	1.6e-06	3.2e-09	7.2e-06	0.03234	0.15385	-
pwb	0.00074	7.8e-05	3.2e-07	0.00011	0.42616	0.33939	1.00000

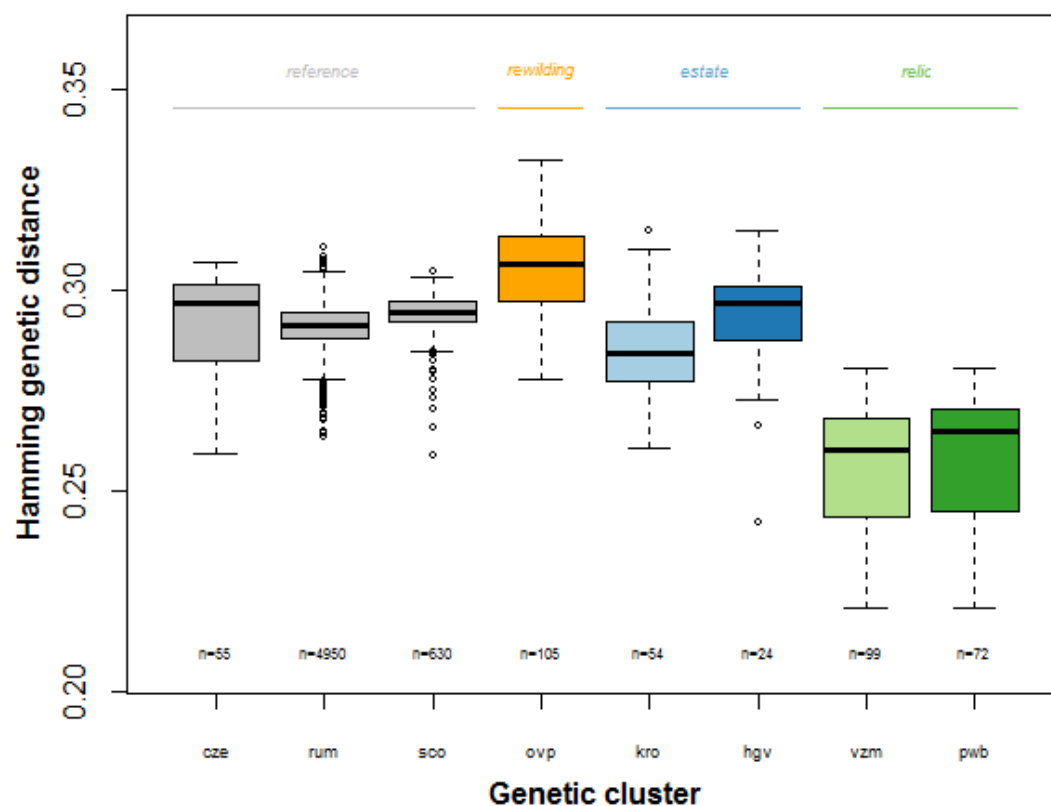
**Table S4** P-values on pairwise comparison test on  $F_{ROH}$  among populations. Pairwise Wilcoxon Rank Sum Test, with Bonferroni corrected p-values. Maximum and minimum median  $F_{ROH}$  were found in SCO and VZM, respectively.

	cze	rum	sco	ovp	kro	hgv	vzm
Rum	0.00183	-	-	-	-	-	-
sco (min)	1.00000	2.0e-14	-	-	-	-	-
ovp	1.00000	1.00000	0.53311	-	-	-	-
kro	0.63290	1.00000	0.00413	1.00000	-	-	-
hgv	0.35374	0.80541	0.15463	1.00000	1.00000	-	-
vzm (max)	0.00227	2.4e-06	1.9e-05	0.00221	0.34908	1.00000	-
pwb	0.00914	0.00073	0.00051	0.07448	1.00000	1.00000	1.00000

**Fig. S1 Visualization of the raw SNP data of an autosomal region of the genome.** SNP genotypes for chromosome 1, shown for eight randomly chosen unrelated individuals per population type (A: Relic, B: Estate, C: Rewilding). Color coding: Black is homozygote major, yellow is heterozygosity, blue is homozygosity minor. Long regions without yellow bins (so homozygote SNPs only) are likely Runs of Homozygosity (to be formally determined based on, inter alia, number of SNPs and genomic length; see methods).

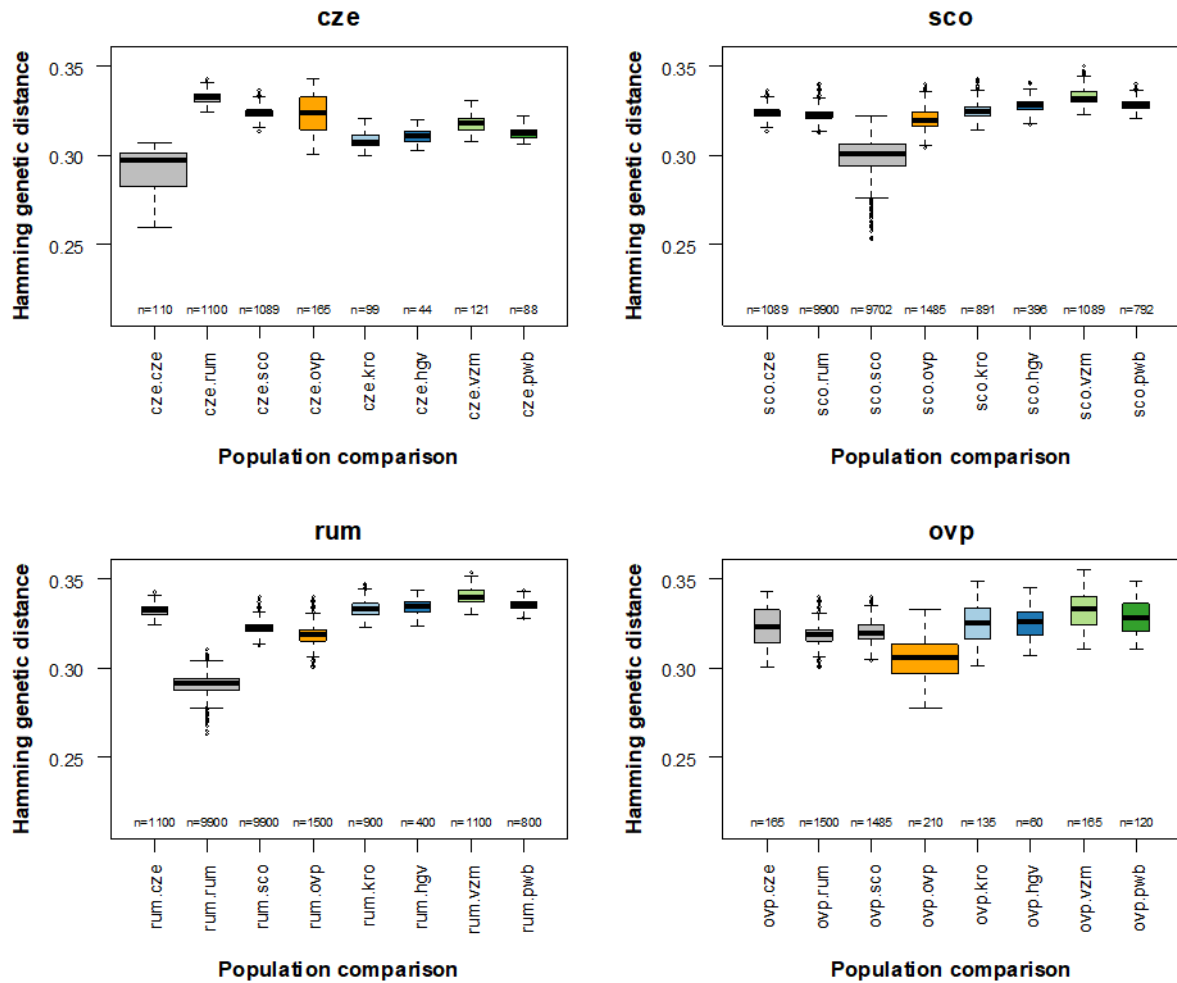


**Fig. S2 Boxplots of individual pairwise Hamming genetic distance within populations.** *Hamming genetic distance is the fraction of alleles that are not identical by state. Vice versa, low Hamming genetic distance implies a high proportion of alleles that are identical by state..*



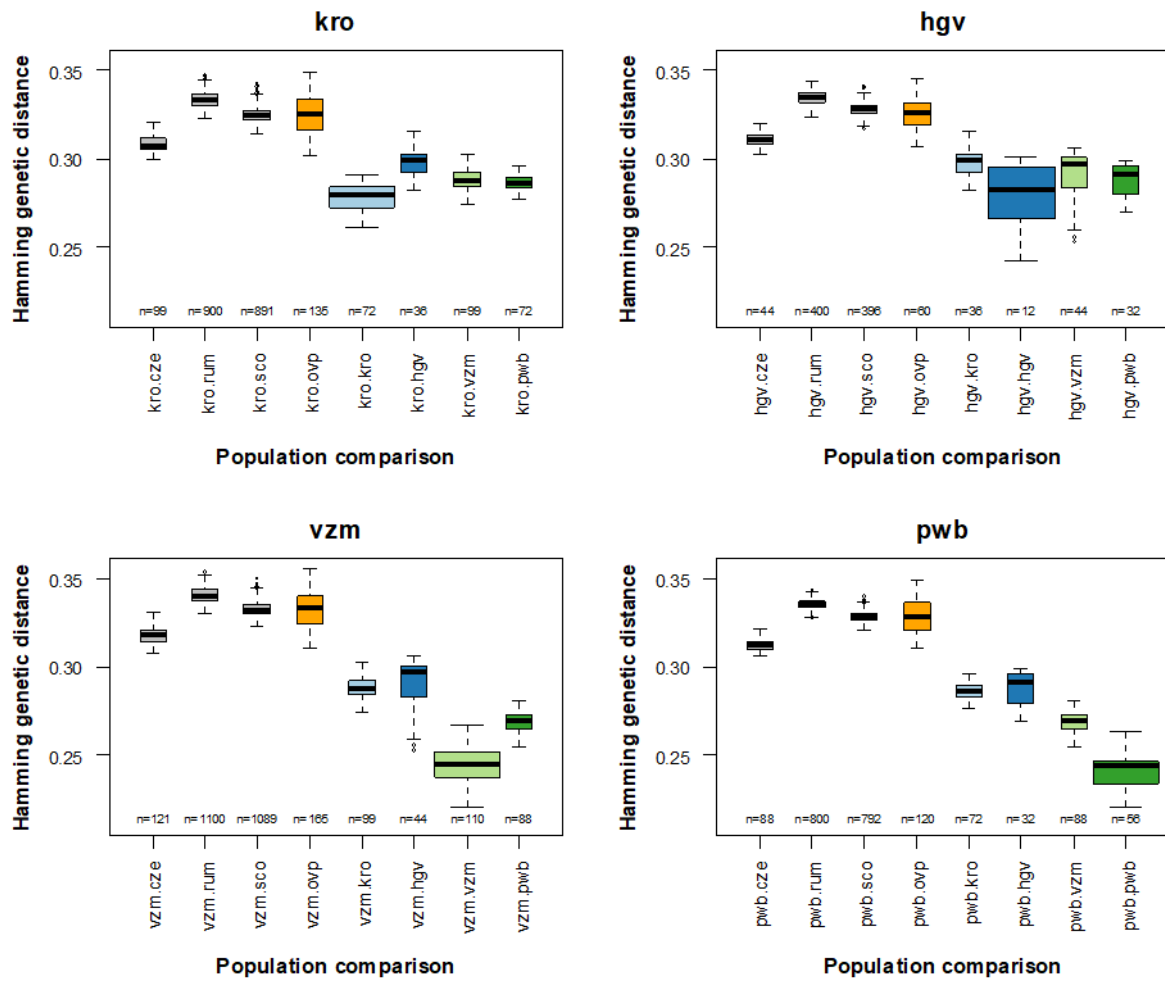
**Fig. S3 Variation in pairwise Hamming genetic distance within and among populations.** Hamming genetic distance is the fraction of alleles that are not identical by state. Per subplot the genetic distances to one fixed focal population is shown. E.g., ‘cze.cze’ = genetic distances of pairs of deer that both belong to the Czechian population; ‘cze.rum’ = genetic distances of deer of Scotland vs. deer of Isle of Rum. Abbreviations are: cze = Czechia, sco = Scottish mainland, ovp = Oostvaardersplassen, kro = Kroondomeinen, hgv = Hoge Veluwe, vzm = Veluwezoom, pwb = Planken Wambuis.

A) Boxplots of Hamming genetic distance. Focal populations: Czechia, Scotland, Isle of Rum, and Oostvaardersplassen



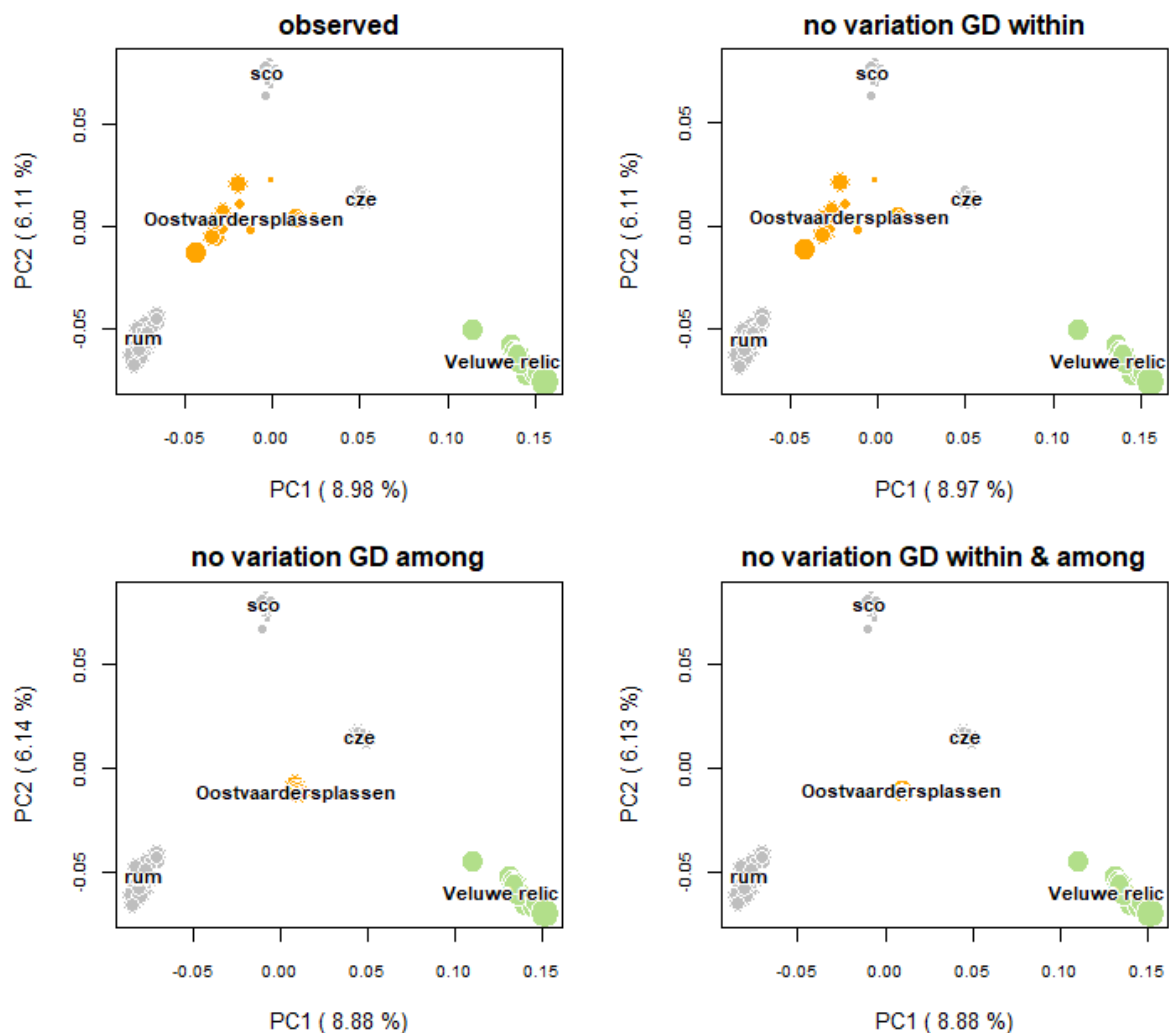
**Fig. S3 continued**

**B) Boxplots of Hamming genetic distance. Focal populations: Kroondomeinen, Hoge Veluwe, Veluwezoom and Planken Wambuis**



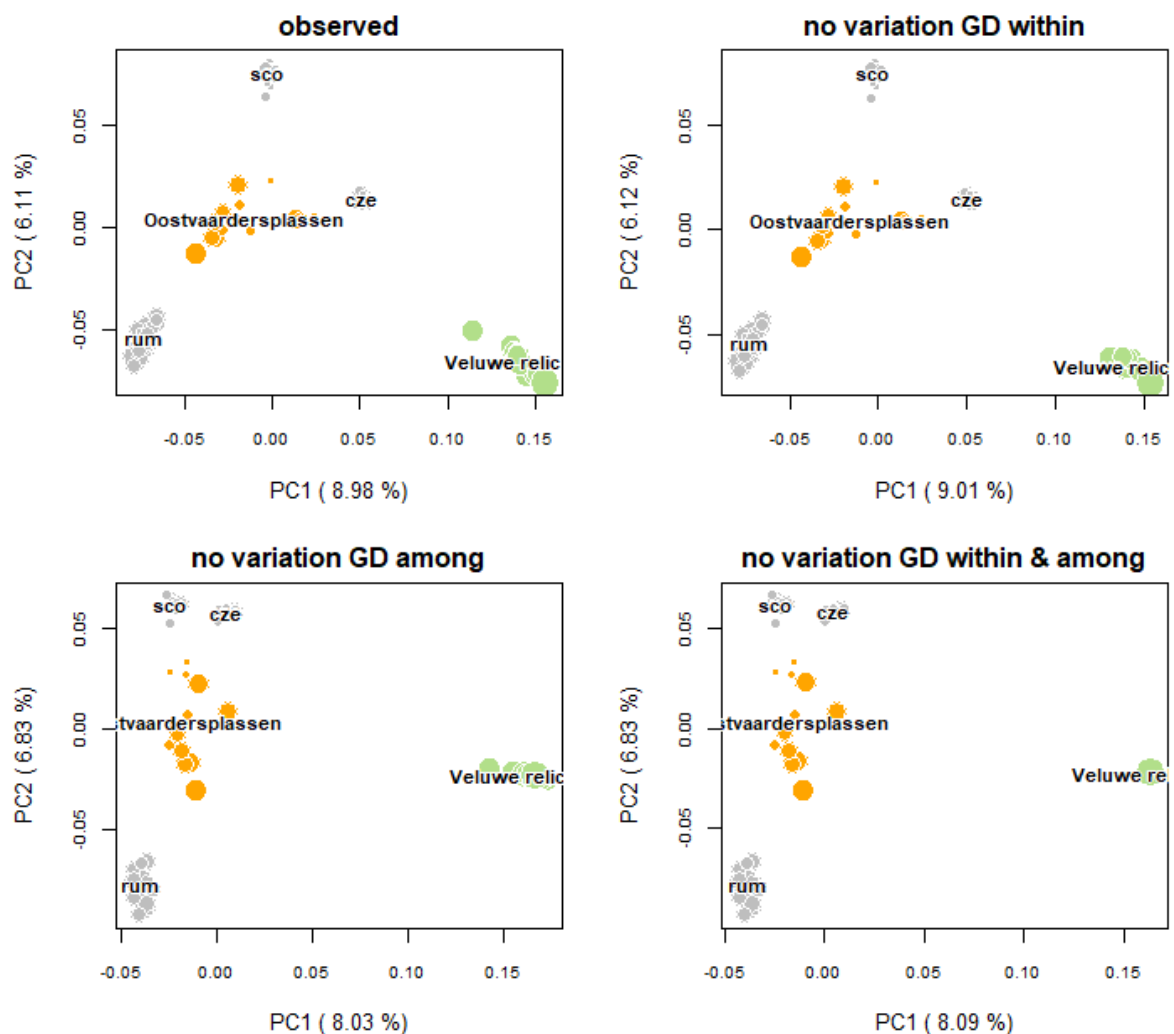
**Fig. S3 continued**

C) Effect of variation in genetic distances on the PCoA ordination of deer of the rewilding Oostvaardersplassen population, in relation to deer of the relic Dutch population Veluwezoom, and deer of Czechia, Isle of Rum and mainland Scotland. 'Observed' shows the PCoA on the true genetic distance matrix. 'No variation GD within' shows the PCoA ordination on a genetic distance matrix in which within-population genetic distances of Oostvaardersplassen are equated to the median. 'No variation GD among' shows the PCoA ordination on a genetic distance matrix in which genetic distances of Oostvaardersplassen deer to deer of other populations are equated to the median. In 'No variation GD within & among', there is no variation in both the within and among genetic distance. These figures show that the scattered ordination of Oostvaardersplassen deer is caused by the variation in genetic distances towards deer of other populations.



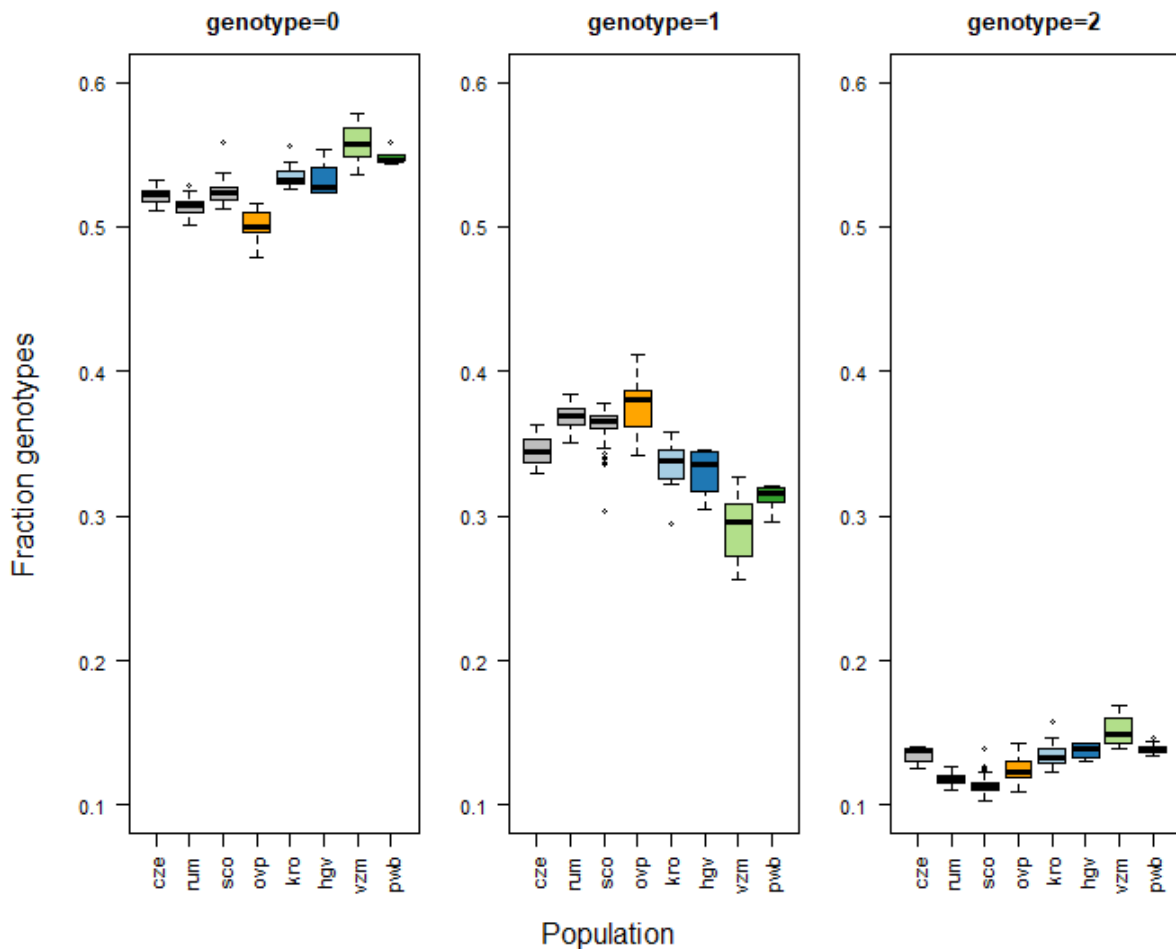
**Fig. S3 continued**

D) Effect of variation in genetic distances on the PCoA ordination of deer of the relic Veluwezoom population, in relation to deer of the Dutch rewilding population Oostvaardersplassen, and deer of Czechia, Isle of Rum and mainland Scotland.. 'Observed' shows the PCoA on the true genetic distance matrix. 'No variation GD within' shows the PCoA ordination on a genetic distance matrix in which within-population genetic distances of Veluwezoom are equated to the median. 'No variation GD among' shows the PCoA ordination on a genetic distance matrix in which genetic distances of Oostvaardersplassen deer to deer of other populations are equated to the median. In 'No variation GD within & among', there is no variation in both the within and among genetic distance. These figures show a scattered ordination may arise due to variation in genetic distances, even when within-population genetic distances are low, and among-population genetic distances are high (as is true for Veluwezoom deer).

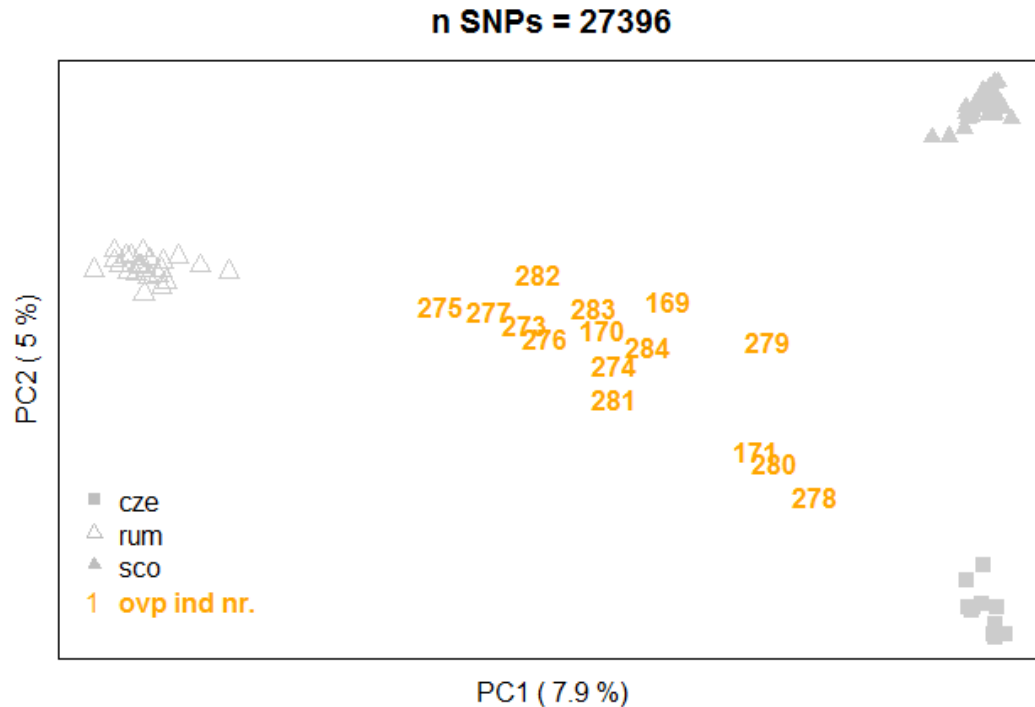


**Fig. S3 continued**

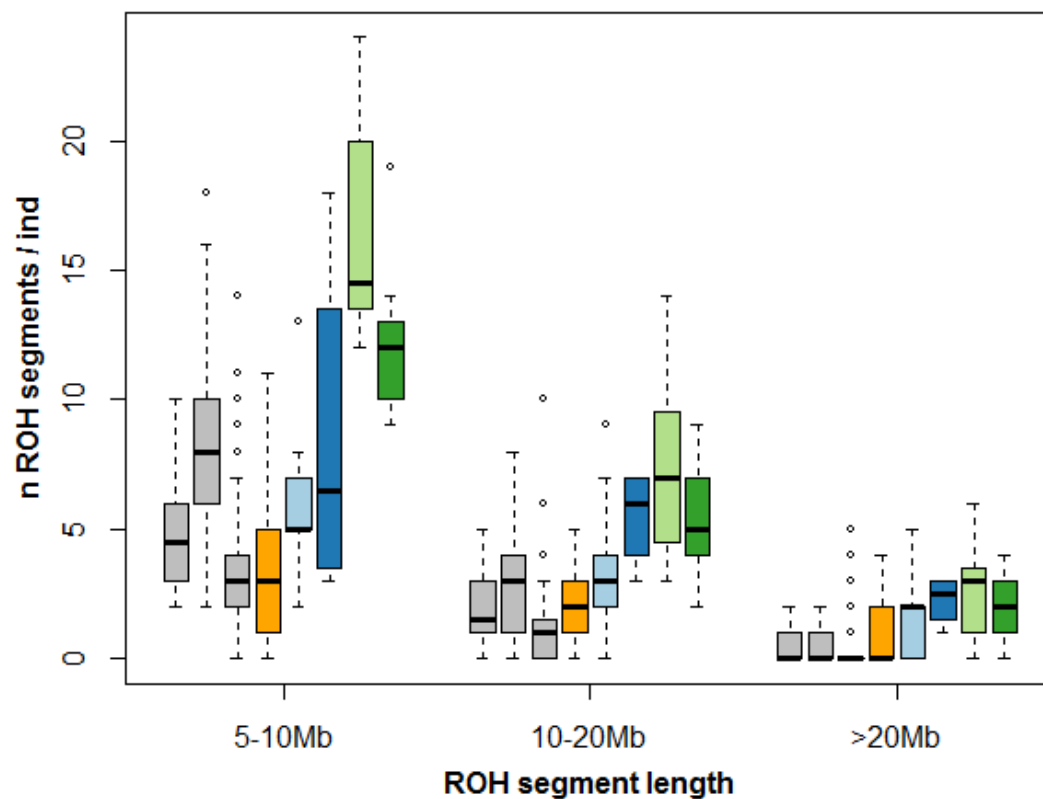
*E) Boxplot of the fraction of loci (per individual, and grouped per population) that have the following genotypes: 0 = homozygous major, 1 = heterozygous, 2 = homozygous minor. Despite similarity in PCoA output (scattered ordination), deer of the rewilding population Oostvaardersplassen and the relic population Veluwezoom differ in genetic composition (Oostvaardersplassen: many heterozygote genotypes, Veluwezoom: many homozygous major and minor genotypes).*



**Fig. S4 Variation among chromosomes in the genetic similarity of red deer of the allochthonous rewilding Oostvaardersplassen to source populations.** (A) PCoA of Oostvaardersplassen and its main restocking source populations: Scotland mainland, Isle of Rum and Czechia. The ordination is based on the full set of SNPs (all chromosomes combined). Individuals 171, 278 and 280 are genetically most similar to Czechia, whereas individuals 275 and 277 are genetically most similar to Isle of Rum.



**Fig. S5 Number of ROHs per individual, stratified by genetic cluster and ROH length.** Populations are in the same order as in Figure 2. The ROH segment length classes of 5-10Mb, 10-20Mb and >20Mb roughly correspond with 5-10, 2-5 and 2 or less generations ago, respectively. Order of populations: CZE, RUM, SCO, OVP, HGV, KRO, VZM and PWB. For abbreviations, see the legend of Fig. 1.



***Fig. S6 Cross-entropy of the LEA ancestry estimation, as a function of  $K$  ancestral populations.***

