



Fragmentation and Translocation Distort the Genetic Landscape of Ungulates: Red Deer in the Netherlands

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Many ungulate populations have a complex history of isolation and translocation. Consequently, ungulate populations may have experienced substantial reductions in the level of overall gene flow, yet simultaneously have augmented levels of long-distance gene flow. To investigate the effect of this dual anthropogenic effect on the genetic landscape of ungulates, we genotyped 35K SNPs in 47 red deer (*Cervus elaphus*) of Netherlands, including putative autochthonous relic populations as well as allochthonous populations established in private estates and rewilding areas. We applied F_{ST} and ordination analyses to determine the meta-population genetic structure and thereby the occurrence of hybridization. At population level, we investigated levels of inbreeding through individual-based diversity measures, including Runs of Homozygosity. We documented that both spatial genetic structure and within-population genetic variation differed markedly from patterns assumed from present-day abundance and distribution. Notwithstanding the small spatial scale, red deer populations formed distinct genetic clusters, and some had higher genetic similarity to distant than to nearby populations. Moreover, the putative autochthonous relic deer populations had much reduced levels of polymorphism and multi-locus heterozygosity, despite relatively large current population sizes. Accordingly, genomes of these deer contained a high proportion of long (>5 Mb) Runs of Homozygosity. Whereas the observed high levels of inbreeding warrant defragmentation measures, the presence of adjacent autochthonous and allochthonous genetic stocks imply that facilitation of gene flow would cause genetic homogenization. Such distortions of the genetic landscape of ungulates creates management dilemmas that cannot be properly anticipated without baseline genetic monitoring.

Keywords: red deer, ungulate, gene flow, translocation, single nucleotide polymorphisms, genetic monitoring

INTRODUCTION

In the Anthropocene, ungulates are subject to both population isolation and translocation. Because of eradication and overexploitation, many ungulates occur in small or bottlenecked autochthonous population relics that are further isolated by anthropogenic barriers (Linnell and Zachos, 2011; Deinet et al., 2013; Ripple et al., 2015). Being such iconic elements of the ‘the world around us,’ ungulates have a long history of translocation (Iacolina et al., 2019). Translocations may come in the form of supplementation of existing populations or (re)introduction of new populations, and frequently involve immigrants from non-native, distant stocks (Seddon et al., 2012; Iacolina et al., 2018, 2019). The dual anthropogenic influences of isolation and translocation have contrasting effects on the key evolutionary process of gene flow, as it is both impeded as well as augmented. This means that the genetic landscape (e.g., Söderquist et al., 2017 and Wang et al., 2019) of ungulate populations is altered by inbreeding (i.e., mating among closely related individuals) as well as outbreeding (i.e., mating among distantly related individuals). Consequently, ungulate populations are at risk of inbreeding depression (i.e., lowered fitness of inbred individuals) on the one hand (Ralls et al., 2018) and outbreeding depression (i.e., lowered fitness of hybrids) on the other (Frankham et al., 2011). Furthermore, outbreeding results in loss of genetic integrity and in genetic homogenization, i.e., an increase of genetic similarity of populations (Kolodny et al., 2019).

The occurrence of inbreeding depression in isolated wildlife populations is now well established (Hasselgren and Norén, 2019). This outcome is especially true for ungulates, for which genomic approaches developed for domestic counterparts could be adopted. The genomes of over 20 ungulates have now been assembled (Martchenko et al., 2018). The genomic consequences of population isolation have been investigated in a variety of species, showing that many populations have individuals with genomes containing frequent and long Runs of Homozygosity (e.g., wild boar (*Sus scrofa*), ibex (*Capra ibex*), bighorn sheep (*Ovis canadensis*), Soay sheep (*Ovis aries*), and red deer (*Cervus elaphus*); Béréños et al., 2016; Grossen et al., 2018, 2019; Hasselgren and Norén, 2019). A negative relationship between inbreeding and individual survival and reproductive performance was reported in red deer of the Isle of Rum, Scotland (Huisman et al., 2016). Moreover, in a recent study involving 26 European ibex populations, growth rate appeared to be substantially lower in inbred than in non-inbred populations (Bozzuto et al., 2019).

The general consensus is that in contemporary ungulate populations outbreeding, and the consequences thereof, is less adverse and pressing than inbreeding (Edmands, 2007; Pekkala et al., 2014, Bell et al., 2019). Recently, however, the debate began on whether the consequences of outbreeding are underestimated (Kolodny et al., 2019; Bell et al., 2019). There is equivocal evidence that hybrids may have lowered relative fitness (Marshall and Spalton, 2000; Bell et al., 2019). Such outbreeding depression may arise because of genetic incompatibility and reduced local adaptation. Apart from this, genetic homogenization may reduce the genetic diversity at a species-wide scale (Kolodny et al., 2019),

in particular when migrants swamp the genetic variation of the native stock (Bell et al., 2019; Harris et al., 2019). Species exhibit geographic variation in phenotypic traits. Ungulates, in particular, have substantial regional variation in morphology (body size, size of horns and antlers, coat pattern and coloration) and behavior (timing of mating season, vocalization) (e.g., Putman and Flueck, 2011; Castelló, 2016). Underlying this geographic variation in phenotypes may be a complex and often little understood spatial genetic structuring (e.g., Moodley and Bruford, 2007). Translocation and subsequent hybridization of ungulates therefore may have both genetic and conspicuous phenotypic effects.

In practice, ungulate management policies often account poorly for both inbreeding and outbreeding. “Genetic legislation” or guidelines on inbreeding and outbreeding are absent or non-normative (Hoban et al., 2013; Ralls et al., 2018). The main genetic guideline, the 100/1000 Ne rule (or 50/500, Frankham et al., 2014), has gained a foothold, but in practice actual effective population sizes are seldom estimated. Indeed, genetic diversity measures are not incorporated in IUCN assessments (Vitorino et al., 2019). Similarly, the IUCN guidelines on translocation (“Guidelines for Reintroductions and Other Conservation Translocations”) do not contain normative or quantitative targets (IUCN/SSC, 2013). Although recommended (e.g., IUCN/SSC, 2013), genetic monitoring, whether pre- or post-intervention, is rarely applied (Hoban et al., 2013). Considering the long history of diverse anthropogenic impacts, the demographic history of contemporary ungulate populations is often complex. Present-day abundance and distribution may therefore give little indication of genetic status.

The simultaneous risk of inbreeding and outbreeding in contemporary ungulate populations is exemplified by the red deer of Netherlands. After a complex and partially known history of eradication, overexploitation, fragmentation and translocation, red deer of Netherlands now occur in remnant populations with different genetic ancestry. By 1900, the red deer population was likely reduced to perhaps a few tens of individuals, which occurred in the pastoral lands of the Veluwe moraine forest and heaths. To improve hunting opportunities, Dutch royalty and local landlords translocated red deer from across the European continent to estates (Rijk and Pelzers, 1991; van den Hoorn, 1992). Fences around the estates, and later busy provincial and national roads, probably hindered gene flow between the introduced allochthonous estate population and the presumably relic, autochthonous population. Within the framework of the “rewilding movement”, red deer were introduced in a disjunct area of The Oostvaardersplassen around 1990. This area was in a newly reclaimed polder about 30 km removed from the Veluwe area. These deer were translocated from various stocks, predominantly Scotland and Czechia. Across the species range, deer including red deer show considerable phenotypic variation in morphology (body size, antler size and shape) and behavior (e.g., male rutting roars) (Mystkowska, 1966; Geist, 1998, pp. 170-222; Putman and Flueck, 2011; Volodin et al., 2018). This phenotypic variation is partially heritable (Kruuk et al., 2002; Coulson et al., 2003; Flueck and Smith-Flueck, 2011). In Netherlands, hunters maintain that up until

today the allochthonous and autochthonous red deer populations differ in timing of mating activity and morphology (in particular, body and antler size; **Figure 1**). These differences may, however, also be caused by local variation in population density as well as in resource quality the latter because of contrasting soils and management regimes (including forage improvement and supplementary feeding). For example, the Oostvaardersplassen area has very fertile marine clay soils while the Veluwe area is on nutrient-poor leached Pleistocene cover sands.

To what extent gene flow may have homogenized the populations with distinct genetic ancestry, or whether anthropogenic barriers still prevent this homogenization is unknown. In addition, the level of inbreeding in the various populations is poorly understood. The various red deer populations underwent bottlenecks or founder effects, and today vary from a few hundred to thousands of animals. All populations accord with Dutch nature legislation based on European directives, which prescribes that to guarantee a 'favorable conservation status,' ungulate populations should have a minimum size of 150 (assuming a minimum effective population size of 50, and an effective to census population size ratio of 0.33; Groot Bruinderink et al., 2000). The first microsatellite study was suggestive of the existence of separate

genetic clusters, but did not detect genetic erosion (Groot et al., 2016). Concerns about inbreeding have triggered defragmentation measures in the past few decades, including the removal of fences and the construction of highway wildlife overpasses. The need for such measures, as well as the potential hybridization consequences, are poorly understood. In Dutch ungulate management, the allochthonous ancestry of some red deer populations is not part of the decision-making process (Spek, 2014).

Here, we report on a case study of the alteration of the genetic landscape of ungulates caused by population isolation and translocation, through analyses of 35K SNPs of red deer populations of Netherlands. Specifically, at meta-population level, we applied genetic ordination to study the occurrence of hybridization and assess its potential. At the population level, we investigated levels of inbreeding through Runs of Homozygosity. We expected a distortion of the null model of Isolation by Distance, as evidenced by (i) distinct genetic clusters at small spatial scales, and (ii) genetic similarity between distant, rather than nearby, populations. Furthermore, we expected (iii) relic autochthonous populations to be inbred, because of their bottlenecked history. In contrast, we expected allochthonous populations, which were established by founders of diverse genetic ancestry, to be outbred. Nonetheless, if fences around estates cause isolation, then autochthonous estate populations also would be expected to have relatively low genetic variation. We test the overall hypothesis that genetic status of red deer can be derived from contemporary abundance and distribution patterns in a human-dominated landscape.

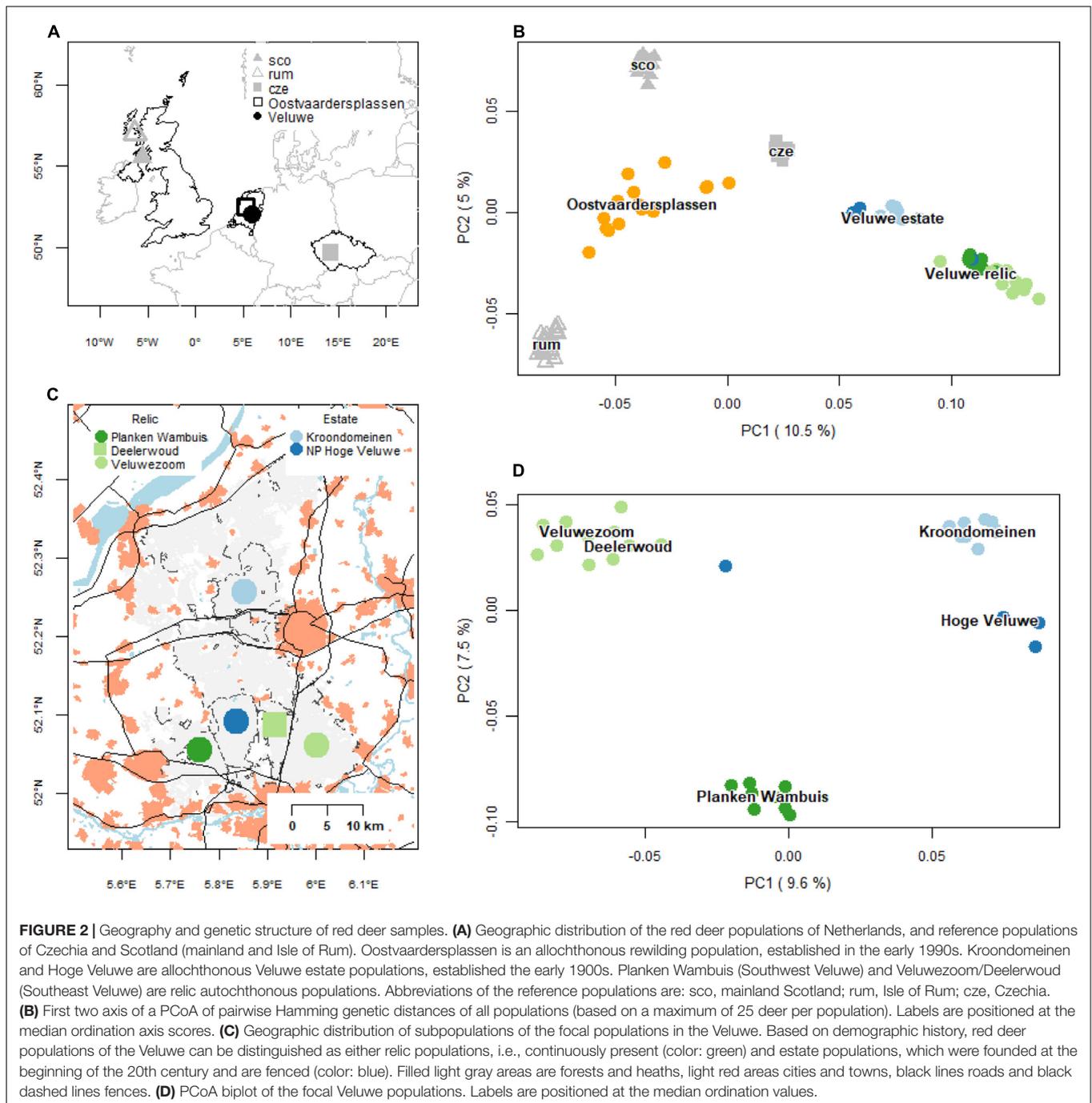
MATERIALS AND METHODS

Sample Collection, DNA Extraction and Genotyping

We assembled a SNP dataset from red deer populations from Netherlands with Czechia and Scotland as references (**Figure 2A** and **Supplementary Table 1**). Within Netherlands, we sampled red deer of the two main populations: the Veluwe and the Oostvaardersplassen (**Figure 2C**). In the Veluwe, we sampled two allochthonous populations from (formerly) fenced estates (NP Hoge Veluwe, Kroondomeinen), and two relic autochthonous populations, in the southwest (Planken Wambuis) and southeast (Veluwezoom and Deelerwoud, referred to as Veluwezoom). In addition, we sampled deer from the rewilding population at the Oostvaardersplassen. To avoid potential sampling bias, we excluded 1st and 2nd degree relatives (following Anderson et al., 2010, see subsequent calculation). This resulted in the following sample sizes: 15 deer from the Oostvaardersplassen, 13 from the Veluwe estate populations and 19 from the relic Veluwe populations. In addition 10, 36 and 100 samples were used for the reference populations of Czechia, mainland Scotland and Isle of Rum, respectively (**Supplementary Table 1**). To decrease the bias due to unequal sample size, a maximum of 25 samples from the Scottish populations were randomly selected for some of the analyses (PCoA, rarefaction, F_{ST}).



FIGURE 1 | Difference in the shape of red deer antlers of relic and estate populations of Netherlands. **Above:** Antlers characteristic for deer of the allochthonous estate Hoge Veluwe (outside) and the autochthonous relic population. The antlers are from deer that are approximately seven years old. The antlers differ, inter alia, in the shape of the crown, which is a fork in the relic population, but tends to form palms in the estate. **Below:** Antlers collected in the estate Hoge Veluwe in 1985 (**left**) and 2013 (estimated age of the deer: both eight years). The characteristic shape of tines and palms is retained over generations.



Collection and SNP genotyping of the Scottish deer is described in Senn and Pemberton (2009) and Huisman et al. (2016). Samples of red deer of Netherlands and Czechia were SNP genotyped specifically for this study. These samples, mostly tongue and ear tissue, were obtained shortly after death from animals that were culled for population management purposes, died from traffic collisions or because of natural mortality (the latter in the Oostvaardersplassen). Samples were genotyped with the cervine 50 K Illumina Infinium iSelect HD Custom BeadChip (Brauning et al., 2015; Rowe et al., 2015). Chromosome and

chromosome positions of SNP loci were based on the linkage map by Johnston et al. (2017). After quality control, which included filtering on a minimum individual and SNP call rate of 0.98 and a minor allele frequency of > 0.01 , 35,522 SNPs remained, of which 33,688 were autosomal. These remaining SNPs had a median density of one SNP per 53Kbp. For all analyses except ROH detection, we excluded SNPs that were in Linkage Disequilibrium (LD; $r^2 > 0.2$) (Anderson et al., 2010). After LD pruning, the number of remaining autosomal SNPs was 27,396 (median spacing of 55Kbp).

Genome-Wide SNP Analyses

Data management and standard genetic analyses were conducted with a combination of the software PLINK1.9 (Purcell et al., 2007) and R (R Core Team, 2019), specifically the package Aegenet (Jombart and Ahmed, 2011). To ensure independence of markers, we pruned SNPs with PLINK using a LD threshold of $r^2 = 0.2$. We used the PLINK pairwise IBD estimator to calculate π_{hat} , which is the proportion of IBD between pairs of individuals (Purcell et al., 2007). We did this for various genetic clusters separately because the method assumes that samples do not show population stratification. We pruned individuals such that the maximum estimated π_{hat} was 0.1.

To study genetic structure, we estimated genetic dissimilarity at the individual and population level. As individual measure we used the Hamming genetic distance estimator, which we calculated with the R package poppr (Kamvar et al., 2015). The Hamming genetic distance measure is simply the inverse of the proportion of alleles that are shared (i.e., that are Identical-by-State). For dissimilarity at the population level, we calculated the fixation index F_{ST} (Weir and Cockerham, 1984) with the R package StAMPP (Pembleton et al., 2013). The F_{ST} estimator used by this program is accurate even when sample size is small (minimally 5 individuals), provided that the number of markers is large ($\sim 10,000$ loci or more) and differentiation is weak ($F_{\text{ST}} < 0.10$) (Willing et al., 2012). Significance of F_{ST} -values was tested by bootstrapping loci 1,000 times (Pembleton et al., 2013). To study the main genetic partitioning among samples, we applied multidimensional reduction of the pairwise genetic distance matrices by use of PCoA (Principal Coordinate Analysis) as implemented in the R package Ape (Paradis et al., 2004). Furthermore, we evaluated the most likely number of clusters and estimated individual ancestry coefficients using the least-square method incorporated in the R package LEA (function 'snmf'; Frichot and Francois, 2015). We used the aforementioned PCoA and LEA ancestry analysis for direct gene flow estimation through the proportion of misassignments (Paetkau et al., 2004). The medium-density SNP dataset gave resolution to detect first generation hybrids. Hence, we calculated migration rates for the current and previous generation (i.e., parents). We calculated 95% confidence intervals around migration rates using the binomial distribution (R base package function 'binom.test').

To study inbreeding, we calculated Multilocus Heterozygosity. MLH estimates based on 10,000 or more SNPs correlates near to perfect with whole genome MLH estimations ($r^2 \approx 0.99$ from a ROH study on wolves (*Canis lupus*), by Kardos et al., 2018). In addition to MLH, we detected Runs of Homozygosity (ROHs). The fraction of the genome that contains ROHs (F_{ROH}) has been shown to be the best estimator for inbreeding (Keller et al., 2011; Kardos et al., 2015), especially when sample size is small (Gazal et al., 2014). Moreover, in contrast to other inbreeding estimators, this metric is comparable among different populations and species, provided that similar segment sizes and variation because of recombination rate are considered. Kardos et al. (2018) reported a high correlation between F_{ROH} estimates based on whole-genome sequences and estimates based on 10,000 SNP markers. Nevertheless, SNP estimations of F_{ROH} tend to be

biased upwards. Specifically, for whole genome sequence F_{ROH} values of 0.0625, SNP based F_{ROH} estimates ranged from 0.05 to 0.10; for whole genome sequences F_{ROH} values of 0.125, SNP based F_{ROH} estimates ranged from 0.10 to 0.20. Following recommendations of Howrigan et al. (2011) we defined the number and length of ROHs with PLINK1.9 as a tract of at least 50 completely homozygous, moderately pruned SNPs (i.e., LD $r^2 > 0.5$, heterozygote loci not allowed); 50 such SNPs is equivalent to approximately 5 Mb. We restricted ourselves to quantification of ROHs longer than 5Mb, so as to minimize the chance of false positives (Howrigan et al., 2011; Kardos et al., 2018). Following McQuillan et al. (2008) and Keller et al. (2011) we distinguished ROHs of > 20 Mb, 10–20 Mb, and 5–10 Mb. Given that the length of ROHs is related to the number of generations since the shared ancestor as $1/2 g M$, with g and M representing generations and Morgan, respectively (Howrigan et al., 2011), and assuming that 1 Morgan ≈ 1 Mb, these classes roughly correspond with a shared ancestor < 2 , 2–5, and 5–10 generations ago (Ceballos et al., 2018).

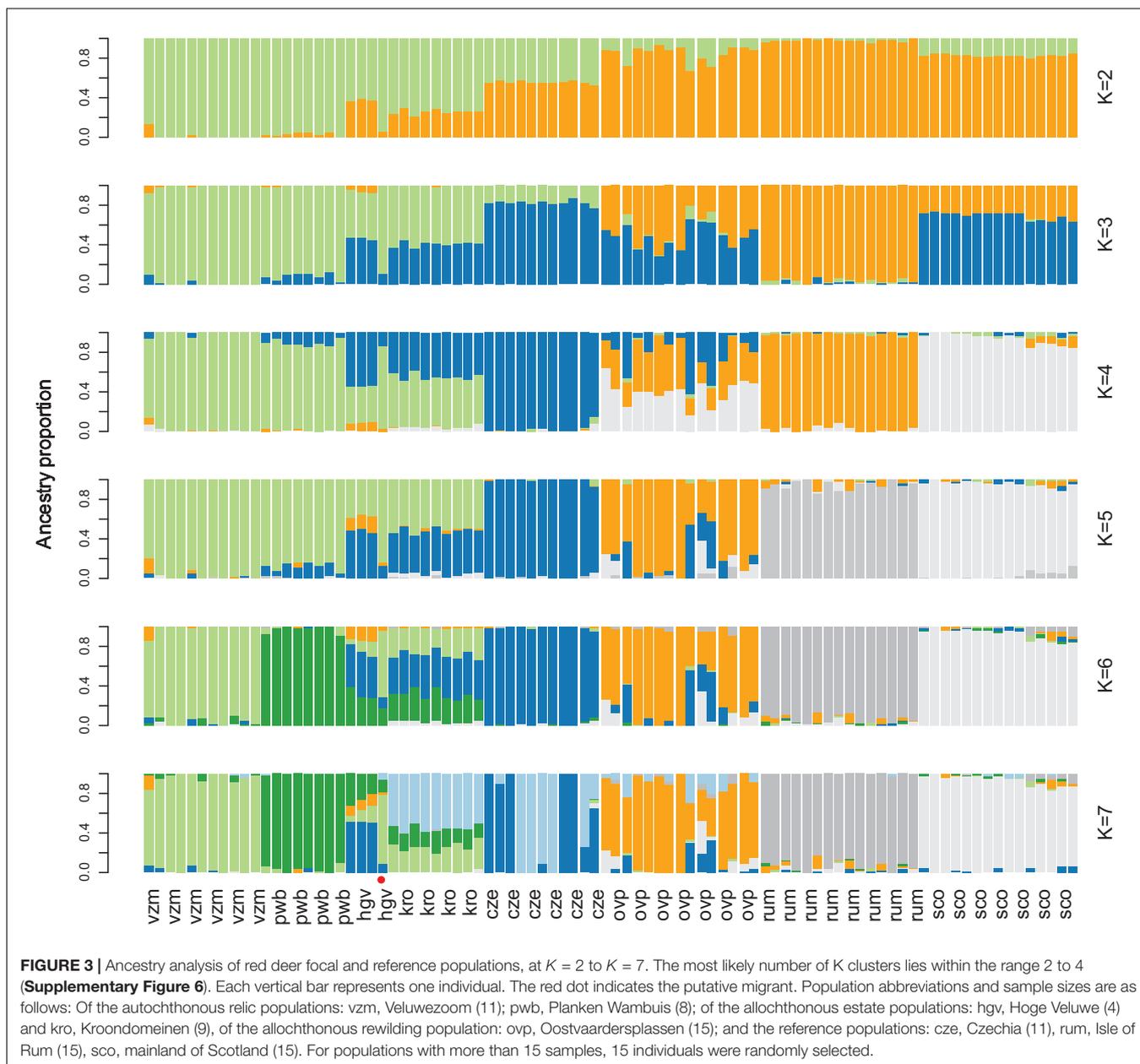
RESULTS

Genetic Structure

PCoA and ancestry analyses showed that the Dutch red deer populations formed discrete genetic clusters, with little to no gene flow (Figures 2B,D,3). Deer of the same source populations had distinct ordination scores and ancestry coefficients. For all population comparisons, F_{ST} -values were significantly different from zero, and ranged from 0.04 to 0.15, with the highest values observed in the Veluwe estate populations ($P = 0.001$; Supplementary Table 2).

Nevertheless, an apparent scattered (rather than a clumped) ordination for deer of the rewilding Oostvaardersplassen population as well as the relic Veluwezoom population occurred (Figure 2B). Oostvaardersplassen deer had relatively high within-population genetic distances, as well as strong variation in genetic distances to other populations (Supplementary Figure 3A) – the latter causing the scatter (Supplementary Figure 3A). In the relic Veluwezoom population, in contrast, deer were genetically similar to each other (low within-population genetic distances), and strongly dissimilar to deer of other populations (high among-population genetic distances) (Supplementary Figure 3B). Here, the scatter was caused by variation in both the within-population genetic distances (low vs. very low) as well as in the among-population genetic distances (high vs. very high) – as illustrated in Supplementary Figure 3D. Furthermore, whereas genomes of deer of Oostvaardersplassen were characterized by a high proportion of heterozygote genotypes, genomes of deer of Veluwezoom had high proportion of genotypes that were homozygous for the major or minor allele (Supplementary Figure 3E). Hence, the scattered ordination of the Oostvaardersplassen and Veluwezoom population were caused by contrasting genetic properties.

Despite the existence of genetic clusters within the Veluwe area, there was a single animal with a dispersal history among



the neighboring deer populations of the Veluwe: this animal was sampled within the estate Hoge Veluwe, but had a genetic signature in between its source population and the relic population Veluwezoom (based on the first two axes of PCoA of Veluwe deer, Figure 2D, and the ancestry analysis, Figure 3). Most probably, this individual was the first generation offspring of a disperser. None of the other 34 Veluwe samples indicated dispersal events. Considering zero dispersal events of 35 sampled deer, and one dispersal events of 70 parents, the estimated migration rate was 0.009 (95% CI: 0.000 – 0.052, Binomial exact calculation).

PCoA and ancestry analysis showed that the genetic clusters of Dutch red deer had diverse ancestry. First, the allochthonous Oostvaardersplassen population had higher genetic similarity

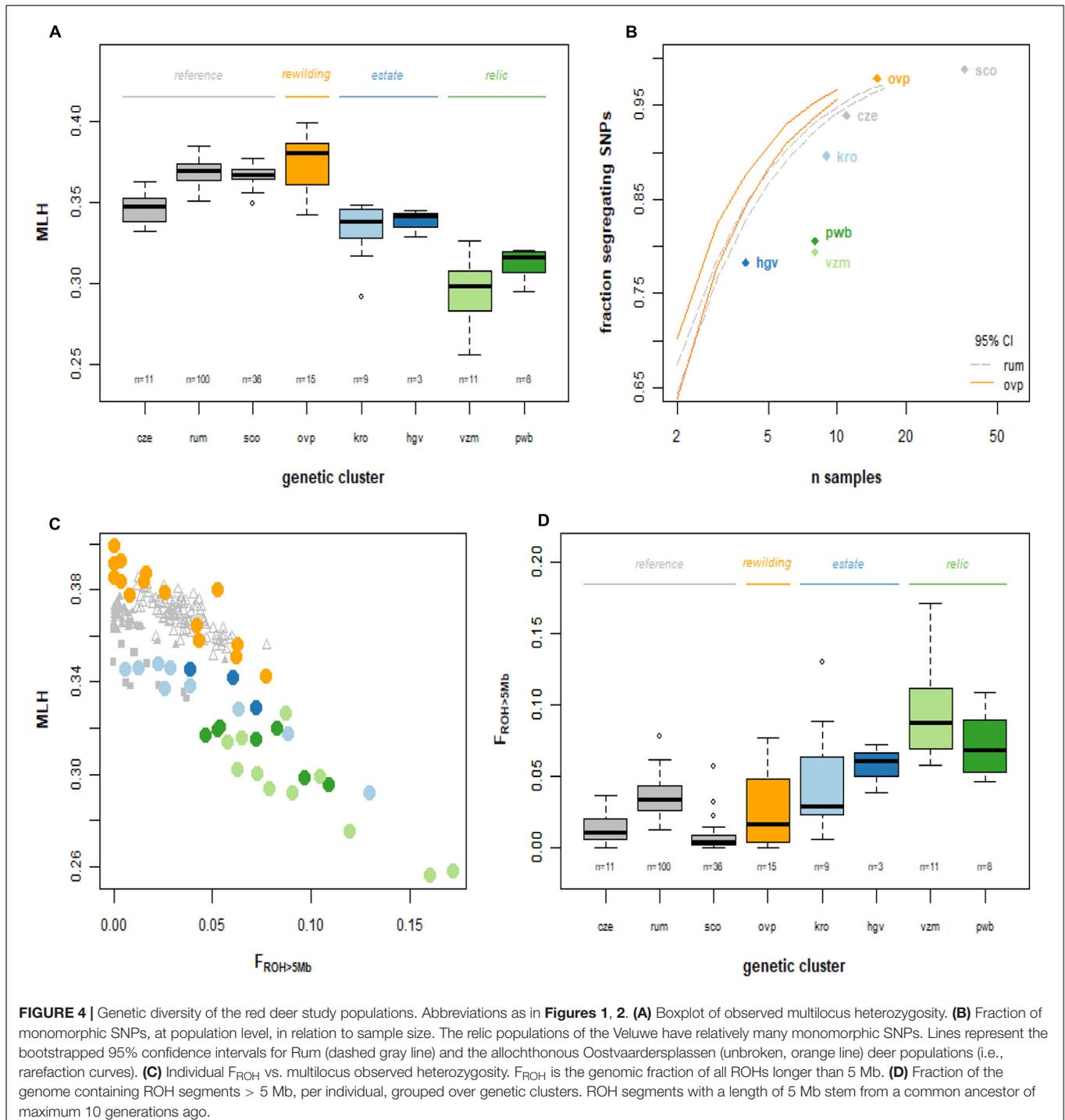
to Scottish and Czechian reference populations than to other Dutch populations (Figures 2B, 3). The Oostvaardersplassen population additionally showed a high degree of within-population heterogeneity (high within-population Hamming genetic distance, Supplementary Figures 2, 3; and scattered ordination, Figure 2B). Indeed, some individuals were most similar to deer from other populations (Supplementary Figure 4). Second, the autochthonous and allochthonous deer of the Veluwe had a partially shared genetic ancestry (Figure 3; green cluster). A signal of allochthonous ancestry also occurred—the allochthonous estate populations were genetically more similar to each other than to surrounding autochthonous relic populations (PCoA; Figures 2B,D). The allochthonous estate populations had high ancestry scores for the genetic

cluster allocated to the Czechia reference population (**Figure 3**; blue cluster). This genetic signature was small or absent in the autochthonous relic populations Planken Wambuis and Veluwezoom, respectively.

Inbreeding

We observed strong variation in the Multilocus heterozygosity (MLH) of red deer of Netherlands. The most heterozygous

individual (maximum MLH = 0.40, from the allochthonous rewilding population) had a 54% higher MLH than the least heterozygous individual (minimum MLH = 0.26, from the autochthonous relic population). Median multilocus heterozygosity differed significantly among the Dutch red deer populations (**Figure 4A**; Kruskal Wallis $\chi^2 = 100.4$, d.f. = 7, $P < 0.001$). Deer from the autochthonous relic populations had a lower median MLH than deer from both the allochthonous



rewilding and estate populations, and also lower than deer from European reference populations (Wilcoxon rank pairwise comparison test, $P < 0.001$, **Supplementary Table 3**). Among the allochthonous populations, deer from the estate populations had lower median MLH than the rewilding population, and also lower than the two Scottish populations. Furthermore, the autochthonous relic populations, and to a lesser degree also the allochthonous estate populations, had a high proportion of non-segregating SNPs (**Figure 3B**).

Multilocus heterozygosity was strongly and positively correlated with the fraction of the genome containing ROH segments larger than 5Mb ($F_{ROH > 5Mb}$; **Figure 3C**; Spearman rank correlation, $r_s = -0.54$, d.f. = 256, $P < 0.001$). Among the red deer populations of Netherlands, there were significant differences in $F_{ROH > 5Mb}$ (Kruskal Wallis $\chi^2 = 106.1$, d.f. = 7, $P < 0.001$; **Figure 3D**). Notwithstanding substantial individual variation (with maximum $F_{ROH > 5Mb}$ of 0.08), the rewilding population had the lowest median $F_{ROH > 5Mb}$ values. Conversely, red deer of the autochthonous relic populations had a significantly higher median $F_{ROH > 5Mb}$ than the allochthonous rewilding population as well as European reference populations (Wilcoxon rank pairwise comparison test, $P < 0.05$; **Supplementary Table 4** and **Supplementary Figure 5**). Red deer of these two relic populations had a minimum $F_{ROH > 5Mb}$ of 0.05, and a median $F_{ROH > 5Mb}$ of 0.07 (Planken Wambuis) and 0.09 (Veluwezoom). Most of the detected FROH segments had a length of 5–10 Mb, corresponding to a shared ancestor 5–10 generations back in time (**Supplementary Figure 5**). Despite having few individuals with high $F_{ROH > 5Mb}$, the median $F_{ROH > 5Mb}$ of the allochthonous rewilding and estate deer populations did not differ from reference populations.

DISCUSSION

Using genome-wide SNP analysis we showed that because of historic and contemporary human impacts, red deer populations of Netherlands differ greatly, and sometimes unexpectedly, in genetic composition. Population isolation (because of anthropogenic barriers) and translocation resulted in adjacent red deer populations having little gene flow and, consequently, discrete genetic clusters with diverse ancestry. Concurrently, red deer populations were shown to vary widely in genetic diversity. In particular, autochthonous relic populations were substantially inbred, despite their relatively large contemporary population size. Altogether, the genetic landscape of red deer of Netherlands can be characterized as a complex mosaic of patches with distinct, uncorrelated properties.

The findings illustrate the substantial distortion that humans can cause to the natural spatial pattern of genetic variation of red deer specifically and ungulates in general. Earlier microsatellite studies indicated that historic and contemporary anthropogenic interventions affect the genetic diversity of red deer populations in, inter alia, Scotland, Iberia, Belgium and Germany (Nussey et al., 2006; Pérez-Espona et al., 2013; Queiros et al., 2014; Hoffmann et al., 2016; Frantz et al., 2017). Recently, there has been a growing attention on the effects of translocation for

genetic variation in ungulates (e.g., Iacolina et al., 2018; Gille et al., 2019; Jahner et al., 2019). Our SNP study on the main extant red deer populations of the human-dominated and fine-grained landscape of Netherlands, improves the resolution of our understanding of the human-caused alteration of the genetic landscape. We demonstrate that the genetic differences among deer populations come in the form of sharp discontinuities, and at a spatial scale much smaller than typical male red deer dispersal distances (around 10 km and up to 50 km, Pérez-Espona et al., 2008). This is in contrast to a recent study on red deer and wild boar in the human-dominated landscape of Belgium (Frantz et al., 2017). The study of Frantz et al. (2017) was based on many (thousands) individuals, but few (tens) of markers – the opposite of the study design of our research. Next to showing fine-scale genetic structure, we are the first to apply Runs of Homozygosity as a means to assess genetic effects of human interventions on red deer, revealing hitherto unnoticed substantial inbreeding. Given that in a preceding microsatellite study (Groot et al., 2016) other red deer populations of Northwestern Europe (not included in this research) had much lower levels of genetic diversity than Dutch populations, we presume that SNP genotyping of other red deer populations will reveal similar or even higher levels of inbreeding.

The observed substantial distortion of the genetic landscape of the Dutch red deer populations highlights the limitations of landscape genetics null models of panmixia (gene flow is ubiquitous), Isolation by Distance (gene flow decreases over geographic distance), and even Isolation by Resistance (gene flow is impeded by barriers) (Manel et al., 2003) for managed ungulate populations. None of these models captures the gene flow patterns of populations that are fragmented, because of human interventions, but also supplemented through translocation, practices common for the management of many ungulates (Seddon et al., 2012), prompting the need for the development of specific approaches.

The relevance of historical human interventions was highlighted by our finding that alterations of the genetic landscape may be long-lasting. In the Veluwe the height of the anthropogenic impact is therefore long past. Bottleneck and translocation events date back to beginning of the 20th century, which, assuming a generation time of 7 years (Coulson et al., 1998) is 14 deer generations in the past. Before this study we therefore assumed that gene flow between the putative Veluwe autochthonous and allochthonous Veluwe populations would have resulted in homogenization. This would predict that presently there should be little genetic structure, and that levels of genetic variation would be similar between subpopulations by now. The detected migrant offspring provided evidence that gene flow is not absent. The allochthonous estate populations, however, were shown to still have low genetic similarity to adjacent autochthonous populations. The partial shared ancestry indicates that there has been some admixture between the estate and relic populations (possibly because of few occasional breakouts, for example during World War II, Rijk and Pelzers (1991)), but that at large fences around estates have prevented gene flow and admixture, and hence have maintained the segregation of allochthonous estate and

autochthonous relic populations. The significance of this finding is that anthropogenic barriers do not merely cause genetic differentiation – they also may preserve the signatures of historical anthropogenic interventions. Hence, in landscapes with anthropogenic barriers, historic anthropogenic effects on ungulates may be maintained much longer than anticipated.

The implication of our study is that present-day abundance and distribution is a poor predictor of the genetic status of ungulates. The autochthonous relic Veluwezoom population is a good case in point. This population is currently the largest of all the Veluwe subpopulations sampled (spring census since 2000: more than 700 animals; Spek, 2014). Moreover, because of highway overpasses this area should be well connected to the other Veluwe subpopulations. Nevertheless, all diversity indicators show this population to be the most isolated and most inbred. We thus posit that the poor genetic status is a legacy of the historic bottleneck combined with an effective absence of genetic exchange. When we shared our findings about inbreeding with them, managers of the Veluwezoom red deer population were surprised. After all, the main bottleneck is thought to have occurred approximately one century ago, though is poorly documented (Rijk and Pelzers, 1991). Even recent demographic events may quickly be forgotten, as we realized when we contacted a retired employee to discover the origin of the founders of the Oostvaardersplassen red deer population (established early 1990s). Furthermore, the management of the autochthonous Veluwe populations was attempting to follow best conservation genetic practices. The management was not aware that conservation geneticists have recently altered the recommendation of minimum inbreeding effective population size to 100 (Frankham et al., 2014). Second, the management was advised an optimistic N_e/N_c -ratio of 0.33 - a ratio universally adopted by ungulate managers in Netherlands (Groot Bruinderink et al., 2000; Spek, 2014), but one that does not account for historic bottlenecks (Vucetich et al., 1997).

A complicating factor is that genetic consequences of historic anthropogenic impacts may be strong yet difficult to detect. In the autochthonous relic populations Veluwezoom and Planken Wambuis the median observed values of $F_{ROH} > 5$ Mb were larger than 0.0625. Using medium-density SNP data, we may have slightly overestimated $F_{ROH} > 5$ Mb (see section “MATERIALS AND METHODS”). The F_{ROH} values are above levels expected for offspring of third order relatives, and among the highest reported for a non-insular ungulate population (Hasselgren and Norén, 2019). Such a level of inbreeding was associated with lowered survival and reproductive performance (lowered calf survival on the Isle of Rum, reduced ibex population growth; Walling et al., 2011; Huisman et al., 2016; Bozzuto et al., 2019). In red deer, inbreeding also may be expressed through the occurrence of overbites (shortened lower jaw, or brachygnathia; Zachos et al., 2007). Typically however, inbreeding depression effects (if any) are subtle, and conditional on stressful environments (Keller and Waller, 2002; Pemberton et al., 2016). Phenotypically, deer of the relic inbred Veluwezoom population do not show signs of inbreeding depression. The population has a high growth rate, deer appear to be in good condition, and health issues are not reported (Spek, 2014). Furthermore,

even the preceding microsatellite study, involving nine molecular markers, had not detected inbreeding (Groot et al., 2016).

Our study also pinpointed the dilemma between inbreeding and outbreeding. In the introduced deer of the rewilding area Oostvaardersplassen, we detected inbreeding as well as admixture within the same population, and even in genomes of the same individual. In addition, the finding that autochthonous and allochthonous populations are still differentiated and have different genetic ancestry, shows that in areas with a population translocation history, the option of alleviating inbreeding through facilitation of gene flow would lead to admixture. We agree with the current consensus among conservation geneticists that inbreeding should be treated with more concern than outbreeding (Bell et al., 2019). Nonetheless, for ungulates the natural spatial genetic structure is substantially affected by fragmentation and translocation, and may, peculiarly, be further altered by defragmentation measures. In the Veluwe, where interpopulation phenotypic differences are allegedly persistent (Figure 1), it is not unlikely that such admixture will affect antler size and shape of autochthonous populations, and thus have more conspicuous phenotypic effects than inbreeding (though not negative). A potential scenario is swamping of allochthonous genetic variation into the gene pool of autochthonous deer (Bell et al., 2019). Irrespective of whether such phenotypic variation is adaptive in many ungulates, managers may influence the genetic integrity and phenotypic traits such as the shape of horns and antlers and coat coloration. The recent rewilding movement has embraced the concept of ecological substitutes, thereby implicitly ignoring intra- and interspecific phenotypic variation (Lorimer et al., 2015). Yet, many translocations of ungulates have been and are being dictated by aesthetic considerations (Seddon et al., 2012), thereby deliberately modifying local phenotypic variation. The consequence is potential disruption of local adaptation, and homogenization of geographic variation (Gippoliti et al., 2018; Kolodny et al., 2019).

The multitude and diversity of anthropogenic impacts and the consequential genetic complex of the genetic landscape pose a challenge for ungulate managers to effectively account for inbreeding and outbreeding consequences. We argue that for ungulate populations historically and presently subject to anthropogenic impact (i) genetic variation cannot be inferred from contemporary distribution and abundance, and (ii) assessment of genetic status is necessary to enable appropriate management. Given that genetic variation may bear the ‘marks’ of unknown or underestimated anthropogenic effects in the past, we recommend managers of ungulate populations to start with a baseline-assessment of standing genetic variation using modern genomic approaches. Ungulate managers may imagine they can slow down loss of genetic variation sufficiently in a population by ensuring high population census size (with little fluctuation, equal sex ratio, etc.) and by connecting subpopulations in the landscape. Historical impacts on population demography, however, may have had such adverse effects that these positive interventions do not suffice and the poor genetic status of a population is not alleviated (at least not on a time scale of decades). Conversely, managers may be unaware of the presence and extent of allochthonous genetic ancestry,

and hence of the potential admixture and homogenization effects of their interventions. For genetic monitoring purposes, we recommend the use of genome-wide SNP data or, if affordable, whole genome sequences. The relatively high costs may, at the present, still constrain sample size and cause a trade-off between population coverage and individual width. Yet, as illustrated by our study, SNP data enable the determination of fine-scale genetic structure, the direct estimation of migration rates, and the precise assessment of genetic status. In particular, genomic approaches facilitate the use of accurate, individual-based measures (e.g., MLH, F_{ROH}), rather than indirect measures that are equilibrium-based and averaged over populations (e.g., population-level heterozygosity with a few loci, allelic richness). The high levels of inbreeding in the autochthonous red deer populations underline the argument that continuation to ignore genetic factors may severely hamper conservation efforts. We advocate that genetic monitoring has to be made integral to ungulate management and policy making.

In our study we illustrate that genetic variation of present-day ungulates is much affected by ongoing and historic human interventions, and put forward that this likely effects ecological interactions as well. Whereas fragmentation and the associated loss of genetic variation may reduce adaptive potential to future environmental change, translocation possibly causes distortion of ongoing local adaptation processes. A first step towards understanding the ecology of present-day ungulate populations of human-dominated landscapes is therefore a genetic investigation of the often unknown and complex demographic history.

DATA AVAILABILITY STATEMENT

The data are located on the Dryad repository: <https://doi.org/10.5061/dryad.hqbkzh1dp>.

ETHICS STATEMENT

Samples of deer of Isle of Rum were collected and used following approval of the Animal Welfare and Ethical Review Body of the University of Edinburgh, and under appropriate United Kingdom Home Office licenses (Johnston et al., 2017). For the other deer samples, an ethical review process was not required, because samples were collected from deer already

culled as part of population management programs. The samples of mainland Scotland were collected by hunters of the Forestry Commission Scotland. Red deer samples of Netherlands were collected from animals culled as part of population control programs regulated by Faunabeheereenheid Gelderland and Faunabeheereenheid Flevoland. In Czechia, samples were collected from deer culled according to the law No. 449/2001 Sb. Hence, in Czechia, Netherlands, and Mainland Scotland no animal was specifically killed for this research.

AUTHOR CONTRIBUTIONS

JJ, PH, H-JM, LI, RY, SW, and HP contributed to the conception and design of the study. GG, LB, JH, and JP provided samples. RC coordinated the laboratory genetic analysis. JJ performed the statistical analysis, in consultation with PH, H-JM, JH, JP, RY, SW, and HP. JJ wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.535715/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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