



DEPARTMENT OF BIOLOGICAL AND
ENVIRONMENTAL SCIENCES

THE GENETIC LANDSCAPE OF FENNOSCANDIAN WOLVES

Exploring founder lineages and shared inbreeding events



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Index (table of contents)

Abstract	2
Sammanfattning	3
Introduction	4
<i>Background</i>	4
Aim and hypothesis	7
Methods	8
<i>Platform</i>	9
<i>Data manipulation</i>	9
<i>Admixture</i>	9
<i>PCA</i>	10
<i>Runs of homozygosity</i>	11
Results	11
<i>Admixture</i>	11
<i>PCA</i>	13
<i>ROH analysis</i>	16
Discussion	22
<i>Conclusions</i>	23
Acknowledgements	23
References	25
Appendix 1. Popular Science Summary	29
<i>The Genetic Landscape of Fennoscandian Wolves (Canis Lupus)</i>	29
Appendix 2. Data	31
Appendix 3. Supplementary material	31

Abstract

This thesis investigates the genetic structure and admixture of Scandinavian wolves, focusing on the origins of the founders and their connections to Finland and Russia, utilizing high-resolution genomic data. The Scandinavian wolf population was extinct in the wild in 1970, with an immigrating pair recolonizing the area in 1983, followed by inbreeding and an additional 4 founders to date. We aimed to explore the overlap of runs of homozygosity (ROH) segments between Scandinavian and Finnish wolves while employing admixture and PCA analyses on published samples from the named locations. The Admixture and PCA results indicate that early founders predominantly originated from Russian and Finnish populations, confirming previous findings regarding their lineage. In contrast, later founders exhibited a mixed genetic origin from a subgroup in Finland that was difficult to geographically pinpoint, but clustered around central, southern and western Finland. Additionally, shared inbreeding events were identified between contemporary Scandinavian wolves and Finnish wolves, both following the arrival of the early founders in 1983 & 1991 *and* late founders in 2008 & 2013. Shared inbreeding events across clusters and temporal classes strengthen this. Notably, late founders bear signs of inbreeding events occurring in Scandinavia *before* their arrival, evident of gene flow to Finland early enough to affect subsequent gene flow back to Scandinavia. This also refutes assumptions of unrelated founders and immigrants to Scandinavia in pedigree studies that lay the groundwork for wildlife management decisions of the species. However, the small sample size from South-western Finland remains a limitation of this study, highlighting potential for improvement in future studies. Overall, our results contribute to the specific knowledge gaps of the founders' origins and shed light on previous suspicions of gene flow between the neighboring populations which is of importance for the genetics-focused management of the Scandinavian wolf population.

Sammanfattning

Detta masterarbete har undersökt den genetiska strukturen hos skandinaviska vargar, med särskilt fokus på grundarnas ursprung och deras kopplingar till Finland och Ryssland, baserat på helgenom data. Den historiska skandinaviska populationen utrotades i det vilda 1970 och en återkolonisering skedde 1983 av ett immigrerande par till centrala Sverige, med efterföljande inavel och ytterligare 4 immigranter med grundarstatus. Syftet var att undersöka inavelshändelser genom överlapp av segment med runs of homozygosity (ROH) mellan skandinaviska och finska vargar samt ursprung för grundarna genom att använda Admixture- och PCA-analyser på publicerade prover från dessa områden. Admixture- och PCA-analyserna visar att de tidiga grundarna huvudsakligen härstammar från en finsk-rysk population, vilket bekräftar tidigare fynd om deras släktskap. I kontrast till detta uppvisar de senare grundarna ett blandat genetiskt ursprung från en grupp i Finland, vars geografiska hemvist är svår att lokalisera exakt men med klustrar kring centrala, södra och västra Finland. Dessutom identifierades delade inavelshändelser mellan nutida skandinaviska och finska vargar, både efter ankomsten av de tidiga grundarna 1983 och 1991, och senare grundare 2008 och 2013. Gemensamma inavelshändelser över kluster och tidsklasser stärker dessa resultat. Det är särskilt anmärkningsvärt att de senare grundarna bär tecken på inavelshändelser som skett i Skandinavien innan deras ankomst, vilket tyder på ett tidigt genflöde till Finland som senare har påverkat flödet av gener tillbaka till Skandinavien. Detta motsäger även antaganden om obesläktade grundare och immigranter till Skandinavien i pedigree-studier som ligger till grund för beslut inom viltförvaltningen av arten. Emellertid kvarstår den lilla provstorleken från sydvästra Finland som en begränsning i denna studie, med möjligheter till förbättring i framtida undersökningar. Sammantaget bidrar våra resultat till att fylla specifika kunskapsluckor om grundarnas ursprung och tidigare misstankar om genflöde mellan de närliggande populationerna, vilket är av betydelse för den genetikfokuserade förvaltningen av den skandinaviska vargpopulationen.

Introduction

Background

Large carnivores in Europe, after facing dwindling numbers due to various anthropogenic pressures, have been exhibiting a promising trend of recolonizing their historical distributions (Chapron et al. 2014). As a challenge for biodiversity conservation and the current stark decline of 73% of monitored wildlife populations over just 50 years (McRae et al., 2024), it is vital to take into account the conservation of top predators: A recent meta-analysis verified the efficacy of often controversial top predators as biodiversity indicators and functional candidates for directing conservation actions for broader ecosystem benefits (Natsukawa & Sergio, 2022). Their capacity to survive in human-dominated landscapes has been described as an underappreciated conservation success, underlining that often too small European protected areas can be combined with areas with higher human densities to facilitate large carnivore populations- if allowed to persist) (Chapron et al., 2014). Such coexistence, however, hinges off adaptations for coexistence that have been lost (livestock-guarding dogs, shepherds) and is vulnerable to social acceptance for poaching in areas such as Norway and Scandinavia (Chapron et al., 2014). This is exemplified by the grey wolf (*Canis lupus*) in Scandinavia; In regard to directing conservation actions, its population dynamics and habitat preferences significantly influence local ecosystems and biodiversity (Milleret., 2016); In regards to coexistence, its recovery is limited by poaching and legal culling (Chapron et al., 2014).

The Scandinavian wolf population is currently a subject of interest for both conservation and contentious socioeconomic issues: A meta-analysis has surveyed declining positive attitudes after the return of the species to Scandinavia due to its growing presence and associated policies, media attention and encounters (Dressel et al., 2015). Among these reasons are conflicts with hunters (wolves taking game, but also risks for hunting dogs) and livestock owners (e.g wolves killing sheep). Recently, the Swedish Government has decided to lower the wolf population to initially 270 individuals and finally 170 individuals further down the line (Swedish Government, 2024). To date, the population in Scandinavia can be estimated to be in between 414 and 470 individuals (Milleret et al., 2024). When it comes to the effective population size and what the populations size should be, SKANDULV (the Scandinavian Wolf Research Project) made assessments where experts differed in opinions (Liberg et al., 2015): One group took social discontent into account, proposing to set the short-term genetic minimum viable population (MVP) at an effective population size (N_e) of 50, given at least one effective immigrant per generation. This equates to about 170 wolves, with a recommendation to double this (340) for a favorable reference population (FRP) above MVP. They view the Swedish wolf population as ecologically sustainable across southern Sweden and favor natural immigration, but accept human-assisted translocation if migration halts, limited to wolves that have naturally migrated into northern Sweden. 2 of the authors suggest that Sweden's current wolf population (~400) meets the FRP threshold under the Article 17 Guidelines, but they recommend allowing it to grow to an ecologically effective 600,

provided immigration continues and social tolerance improves, with controlled hunting as part of the approach. One author, however, advocates a strict interpretation of the Habitats Directive, requiring FCS to be achieved within Sweden alone at 1,700 wolves or at carrying capacity (~1,200), unless substantial connectivity with Finland justifies a lower threshold (around 600 wolves). 3 of the authors emphasize that restrictive policies in northern Sweden to protect reindeer herding hinder reaching FCS. In contrast to the latest governmental decision, other researchers looking at an effective management and conservation of Scandinavian wolves outlined the following needs (Laikre et al., 2016): To maintain robust genetic health and connectivity, approximately 3-5 genetically effective (distant) migrants per generation should move between subpopulations in Fennoscandia (Figure 1.) and the combined effective population size (number of individuals that effectively participates in producing the next generation) of the metapopulation (Scandinavia, Finland and neighboring parts of Russia) should strive to approach 500. The report that the latest governmental decision was based on highlighted the importance of the study for a sustainable wolf population and that a FRV will not be reached with a census size as small as 170 (Miller & Dussex., 2024). As such, the conservation of wolves in Scandinavia is a complex issue due to wildlife politics, but the importance of genetic monitoring remains clear.

Another issue is that inbreeding depression has been documented in the Scandinavian wolf population, impacting morphological (Räikkönen et al., 2006, 2013) and other fitness-related traits (Bensch et al., 2006; Liberg et al., 2005). Studies have also recently showed how the Scandinavian wolf population can, on average, have more inbred individuals than what is visible on pedigree information alone and show the importance of taking the immigrant's genetic status (degree of inbreeding and relatedness) into account (Kardos et al. 2018; Åkesson et al. 2023). A study looking at the genetic relatedness of immigrants saw that 15 of 20 were closely related (half sibs, full-sibs or parent-offspring) with at least one of the other immigrants (Åkesson et al., 2022). Another recent study states that the assumption of unrelated founders in pedigree studies is refuted by genomic findings of significant haplotype sharing between the founders and signifies the importance of whole-genome analysis (Viluma et al. 2022). Whole-genome analysis allowed Kardos et al. in 2018 to observe patterns of full or near-full homozygosity of entire chromosomes in samples of inbred Scandinavian wolves. In other words, runs of homozygosity (ROH) as a proxy for inbreeding allowed researchers to better assess the occurrence of inbreeding and subsequently the risks of inbreeding depression, which can manifest in the named ways of fitness-decreasing traits.

The history of the wolf in Scandinavia consists of a historical population and the current one founded after recolonization. Historical bag statistics from the onset of the 1800s indicate a substantial wolf population in Sweden, estimated between 1,000 and 2,000 individuals but intense hunting pressure, fueled by state-sanctioned bounties, significantly diminished this number throughout the century (Aronson & Sand. 2004). The Hunting Act of 1966 sought to remedy the deteriorating situation that continued, but by 1970 the population was deemed functionally and genetically extinct (Huayi et al., 2021). It took until 1983 for a founder event by an immigrating pair to occur in central Sweden, but this was followed by a sequence of incestuous breeding within the established pack (Vila et al., 2003, Liberg et al., 2005,

Wabaken et al., 2011). As outlined by Vila et al., 2003, the population stayed static in growth from 1983 to 1990 until the arrival of an immigrant 1991 with subsequent exponential population growth and increase in genetic variation, saving it from inbreeding depression. By 2002, the population was considered to be founded by only three individuals from the Eastern wolf population with a distribution in south-central Scandinavia, 900 km from the larger Finnish-Russian source population and consisting of approximately 100 wolves (Liberg et al. 2005; Vila et al. 2003; Wabakken et al. 2001). At this point, indications of genetic differentiation between the eastern (Finnish-Russian) population and the Scandinavian population were found due to genetic drift, studied by limited autosomal genetic markers (Vila et al., 2003). This study used microsatellite data (small pieces of DNA that repeat) to trace the origin of the founders to the eastern neighbors in Finland and Russia. This would help debunk controversies and near-folklore conspiracy theories of wolves having been imported, let out of zoos and outplanted in secret (Linnel et al., 2005). Additionally, Flagstad et al. 2003 described the erasure of the historical Scandinavian gene pool after the extinction 1970 and the connection between expansion events near the Finnish-Russian borders and increased immigration to the Scandinavian peninsula. Since then, 2 immigrants in 2008 and 1 in 2013 have been deemed to contribute as founders to the Scandinavian population (Table 1., Svensson et al., 2024). Though there were additional immigrant wolves between 2013 and 2021 (with occasional reproductions), they haven't been classified as founders given the lack of successful reproductions by their offspring (Svensson et al., 2024; Åkesson et al., 2023).

Table 1. Overview of the founders up to date and their respective territory and year of establishing the territory in Scandinavia.

Name	Territory and year	Sex
D-85-01	Nyskoga 1983	Female
G1-83/FM1	Nyskoga 1983	Male
G1-91/FM1	Gillhov 1991	Male
M-09-03	Prästskogen/Galven 2008	Male
M-10-10	Kynna 2008	Male
G31-13	Tiveden 2013	Female

Recent studies using whole genomic data have addressed the origin of the Scandinavian wolf population, with support for an origin in the Finnish-Russian Karelian population given the clustering of the first female founder, and offspring to the two first male founders, with Finnish-Russian wolves (compared to Russian wolves further away) (Smeds et al., 2021). The same study also confirmed the lack of dog admixture with Fennoscandian wolves, important to dispel early fears of dogs and wolves hybridizing. However, it has also been stated that comparisons with genomic data of other nearby European populations is needed to exclude other sources of origin (Smeds et al., 2019), for example wolves from the Baltics (Figure 1; Hindrikson et al., 2017). A western-Finnish wolf population has recently been established after an absence of more than 100 years in the area (possibly due to a population

expansion from eastern Finland and Karelian Russia) and might be a possible source of origin for more recent immigrant wolves in Scandinavia (Kaartinen., 2011). An undertaking to approach these scenarios was outlined by Stenøien et al in 2021: With a sample size of 1309 wolves and dogs from various regions, their analysis showed the closest genetic relatedness to be with contemporary wolves in South-Western Finland, while stating that the historical and extirpated Scandinavian wolf population's genetic signature may only be found in captive wolves still held in zoos. Moreover, some of the lowest signs of dog ancestry and highest signs of inbreeding are found among the Scandinavian wolves, compared to wolves globally. All this stated background information left the following knowledge gaps for this study to try answering: A founder-focused study akin to Vila et al. 2003 has not been done with recent whole genome data, given newly inferred data for the missing genotypes of the first two male founders (Smeds & Ellegren 2023) and the need to investigate the origin of later founders from 2008 and onward. Moreover, an examination of the inbreeding events in common between the Scandinavian wolves and Finnish wolves is warranted to discern gene-flow patterns between the regions.

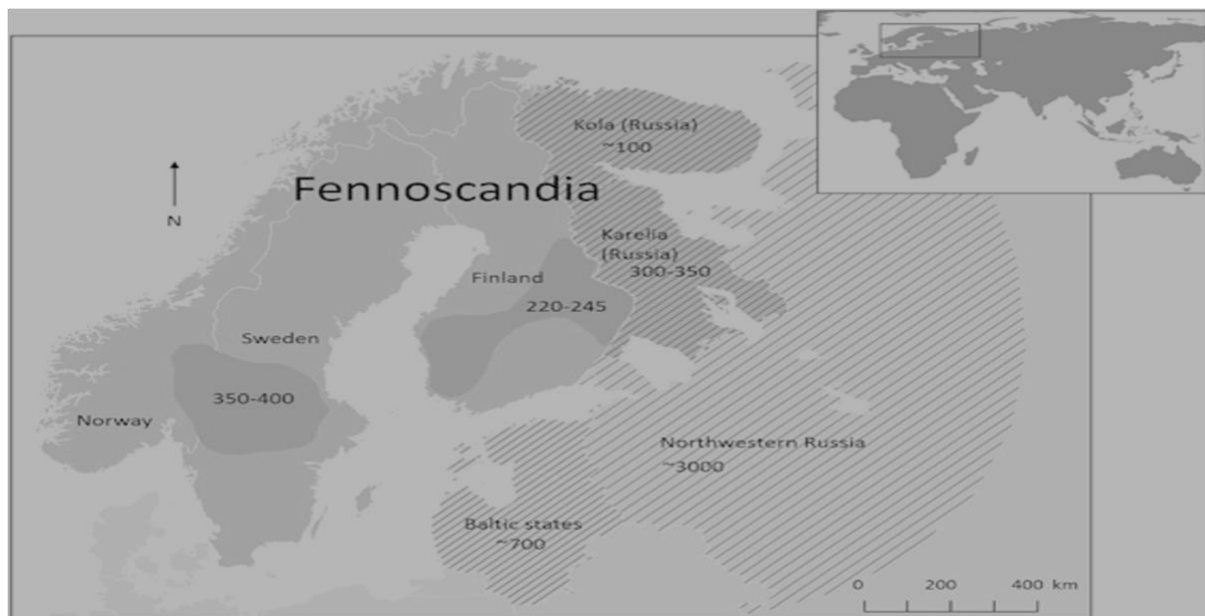


Figure 1. Map of wolf distributions and census sizes from 2016 (Laikre et al., 2016). The area of Fennoscandia (medium gray) comprises Norway and Sweden (Scandinavia), Finland and the Russian regions of Kola and Karelia. Detailed wolf distribution data are available for Finland and Scandinavia, and the dark gray areas indicate current wolf occurrence. Such information is missing for Karelia and Kola and it is thus unclear where in those areas wolves occur (marked with stripes).

Aim and hypothesis

This study aimed to address important knowledge gaps in the genetic history of the Scandinavian wolf population by investigating the origins of more recent founders, particularly those arriving after 2008, using high-resolution whole-genome data, including the recently reconstructed genotypes of the two original male founders. Previous research focused on early founders but had not fully explored the genetic connections between

contemporary Scandinavian founders and neighboring populations, such as the recently established Western Finnish wolves, which did not exist until recently. Additionally, the degree to which Scandinavian wolves had contributed back to Finnish populations remained unclear. This study examined these dynamics, focusing on shared inbreeding events and admixture analysis. Unlike previous studies that relied on limited genetic markers, this utilizes higher-resolution methods directly to founders, building on approaches like Flagstad et al. 2003 and Vila et al. 2003. The hypothesis was that the more recent Scandinavian founders and offsprings showed closer genetic ties to the contemporary South-Western Finnish wolves and that recent bi-directional gene flow may have played a role in establishing the Western Finnish population, given the broader population-level insights from Stenøien et al. 2021.

Methods

Data

Table 2. *Count of individuals in each sample group. Total of 296 samples.*

Sample area/class	Count of samples
Scandinavian	86
post-2008-E	57
pre-2008-E	22
post-2008-W	16
Rus	15
Late-Founder	3
Founder	3
pre-2008-W	2
Total sum	296

Extracted from the works of Smeds & Ellegren (2023), the following samples were used (Table 2): The latest Scandinavian samples, with all 6 founders (including reconstructed genotypes for the first male founders lacking whole-genome sequencing), and a selection of 86 offsprings. The genotypes of the two unsampled male founders were inferred by matching haplotype combinations in their offspring to genotypes observed in sequenced individuals, associating each haplotype with specific alleles at variant sites, and then adding these inferred genotypes to the VCF file (Smeds & Ellegren, 2023; Viluma et al., 2022). Moreover, 15 Russian and 92 Finnish wolf samples were used, with the latter group being further divided into 22 Eastern and 2 Western Finnish wolves collected before 2008 and 57 Eastern and 16 Western Finnish wolves collected after 2008. The year 2008 was used as a breaking point due to the subsequent arrival of the later founders in the Scandinavian population. These were in a VCF-format (Variant Call Format) mapped to the dog genome assembly [CanFam.3.1](#). The

VCF contained 10,622,231 autosomal SNPs (single nucleotide polymorphisms) for chromosomes 1 to 38.

Platform

The data treatment carried out required high-performance computing capabilities, available through the Swedish national infrastructure SUPR ([the Swedish User and Project Repository](#)) and NAISS ([National Academic Infrastructure for Supercomputing in Sweden](#)).

Data manipulation

To manipulate whole-genomic datasets, bcftools (Danecek et al., 2021) has been used. This allows for retrieval of metrics from the already existing VCF-files. Contents that can be displayed vary from quality control scores to number of variants and regions involved in the dataset. Every merge and subsetting of the dataset require the creation of a new VCF-file, which in turn requires an indexing. Also done through bcftools, this creates a .tbi file that allows for efficient retrieval of data from the VCF without needing to read the whole file. The position of each variant in the VCF becomes known through the .tbi file, which speeds up any data manipulations and reduces the amount of memory needed.

Admixture

To address the aim of the study, an admixture analysis was undertaken to look at ancestral proportions from different populations for each Scandinavian founder in our sample group. Admixture analysis helps delineate population structure, by identifying the ancestral contributions of different populations to the genetic makeup of a sample group. This analysis is particularly valuable for identifying the contributions of different ancestral groups to the genetic composition of individuals, especially in cases where populations have experienced recent admixture or migration events. By employing software like ADMIXTURE, which uses algorithms grounded in population genetics theories such as the Hardy-Weinberg equilibrium (HWE), researchers can assess the extent to which genetic variation deviates from expected frequencies. Indicating phenomena such as recent admixture, non-random mating and underlying genetic structure.

Focusing on only the 6 founders from the Scandinavian population, the 97 Finnish and 15 Russian wolves, the ADMIXTURE software (DH. Alexander et al., 2009) was used: The software helped run 10 iterations of different values of K (number of populations) ranging from 2 to 10, with each run being conducted 10 times to ensure robustness of the results. By testing a range of K, several distinct populations present in the dataset can be discerned and a lower K tests the broadest separation while higher K would explore more detailed substructures. Necessary to this, an optimal K identification needs to be assessed, done with a cross-validation (CV) test through the same software. The statistical method evaluates the performance of a model by partitioning the data into subsets, training the model on some subsets and validating it on others. Subsequently, a range of CV error rates are given across

different values of K , for each iteration. A mean of the CV error rate of all runs for a given k is taken to compare the error rate between the values of K to see the best fit: The lower the rate, the better the fit (usually). In addition, the admixture analysis also shows the number of matching runs per K . If a specific K yields the same result across multiple runs (the same proportions for the same set of individuals), it indicates a consistency and confidence in the inferred population structure. Lastly, the fixation index (F_{ST}) for the inferred populations was extracted to add to the analysis, which is a measure of the genetic structuring and population differentiation (ranging from 0, indicating no differentiation, to 1, indicating complete differentiation).

The admixture results were then visualized through the software Pong (Behr et al., 2016): First by generating a filemap to associate the admixture coefficients with the sample identifiers and then use an ind2pop.txt file to map individuals to their respective sampling location. Pong then creates interactive plots of the ancestry proportions for everyone, through different iterations and clusters, even allowing highlighting of specific clusters or results of interest.

PCA

To aid the analysis of the genetic variation within the wolf populations, a Principal Component Analysis (PCA) clustering was done. The principal components are the directions along which the data vary the most, with the first component capturing the greatest variance, the second the second greatest variance and so on until we understand the structure of the data. By reducing high-dimensional data to a few principal components, visualization is helped, and outliers can be easily identified as well as the relationships between different groupings (in this case the founders with *and* without the rest of the Scandinavian samples, the Finnish subgroups and the Russian wolves).

Firstly, the VCF-files were prepared with the software VCF2PCAcluster (He et al., 2024), which converts the files to a suitable format for PCA. Genotype information from the VCF was extracted and structured into a numeric matrix where rows represent individuals and columns represent genetic variants. Among the required inputs to the programme is the number of clusters (K), which was inferred from the admixture analysis. Akin to the admixture software, VCF2PCAcluster tries to find the best fitting grouping for the samples while combining it with a 3DPCA. The output was a file that can be easily loaded into R for visualization, where the package scatterplot3d (Ligges & Mächler., 2003) was used to plot the three first principal components at once. Moreover, the cluster assignments for the individuals and the coordinates of the Finnish wolves (SM. Table 1) were merged with the package dplyr (Wickham et al., 2020) and further mapped out with the help of sf (Pebesma., 2018) to match clusters and coordinates with the map of Finland. A 3D PCA was produced

with Founders (Early and Late), post/pre 2008 Western/Eastern Finnish wolves and Russian wolves.

Runs of homozygosity

To delve deeper into specific gene flows and shared relationship between Finnish wolves, more specifically from after 2008, and the arrival of the late founders to Scandinavia, an aspect to examine was the shared inbreeding events. A method for this is to look at runs of homozygosity (ROH), which has a high relationship with true inbreeding, and assess shared segments of ROH between Scandinavian wolves, Western Finnish and Eastern Finnish wolves. The threshold size of 8000 kb for detecting ROH was chosen because longer segments are more indicative of recent inbreeding events, as they suggest that the individuals share a common ancestor more recently than shorter segments (Cockerill et al., 2022). These segments indicate shared inbreeding events that, if found across groups, could give a clue about migration patterns. Finnish wolf samples before and after 2008 brought a temporal dimension to the analysis. PLINK (Purcell et al., 2007) was used to convert the VCF file into PED/MAP format, which is a format needed to run the ROH analysis on PLINK. In turn .hom files (short for homozygosity) are produced and read into R together with the population information. The state of ROH lengths among Scandinavian, Finnish and Russian wolves were plotted with ggplot (Wickham, 2016) as well as the overlaps in ROH segments between the Scandinavian and Finnish wolves.

Results

Admixture

With a visualization of the cross-validation error rate, the lowest rate found is at $K=5$, with an increase after suggesting adding more populations does not improve the model fit (Fig.3). This is a prelude for choosing the best fitting K -value to analyze the admixture results and inputting K for the VCF2PCA command. The admixture results are presented (Fig 2.) with all K from 1 to 10, showing the representability of the runs as well under the K value (e.g. for $K=5$, 9/10 iterations showed the same results). The green cluster is predominantly found among Russian samples, with a spread of occurrences among Finnish and Scandinavian founder samples with a value of $K<5$. However, that is stratified even further in $K=5$, into an orange cluster that dominates the late founders' ancestral proportions while covering around half of the early founders' proportions. The early founders, as seen in the rightmost 3 bars, bear the second largest proportion from the green cluster and smaller proportions of pink, purple and brown clusters. This mix of clusters is represented in the Russian samples but also a selection of Finnish samples. The most recent founders, unequivocally belonging to the orange cluster, need a focus on $K=5$ to see matches with other sample groups. Finland also sticks out by having all five lineages represented among the samples there.

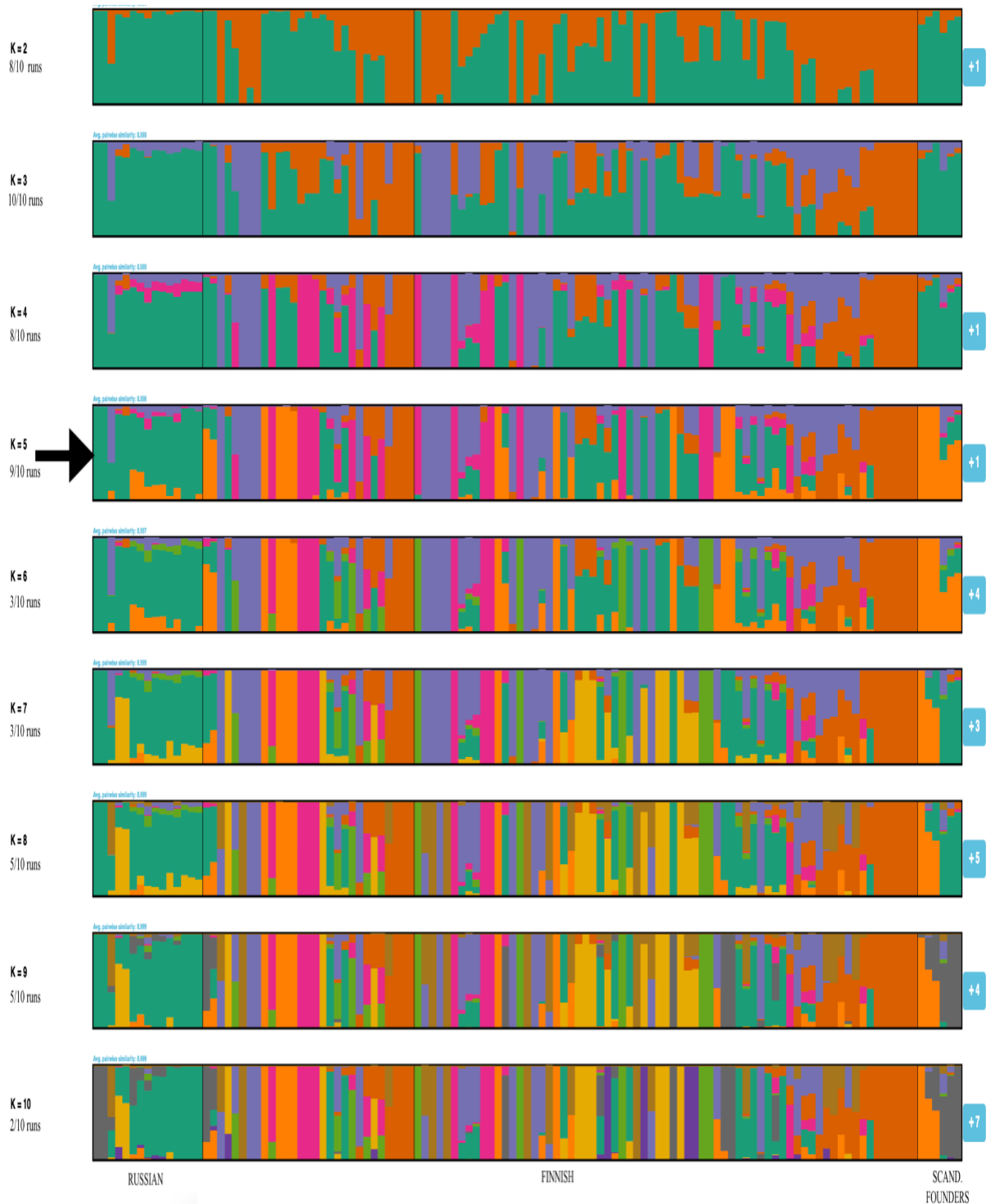


Figure 2. Admixture results for $K=2$ to $K=10$, with an arrow highlighting $K=5$. From left to right: Russian, Finnish and Scandinavian (founders) sample groups ($N=119$). Rightmost 3 founders are from before 2008 and leftmost 3 founders are from 2008 and onwards. The number of different modes (variation of results) available in each cluster is seen at the rightmost margins (e.g +7 for $K=10$, meaning a total of 8 different modes).

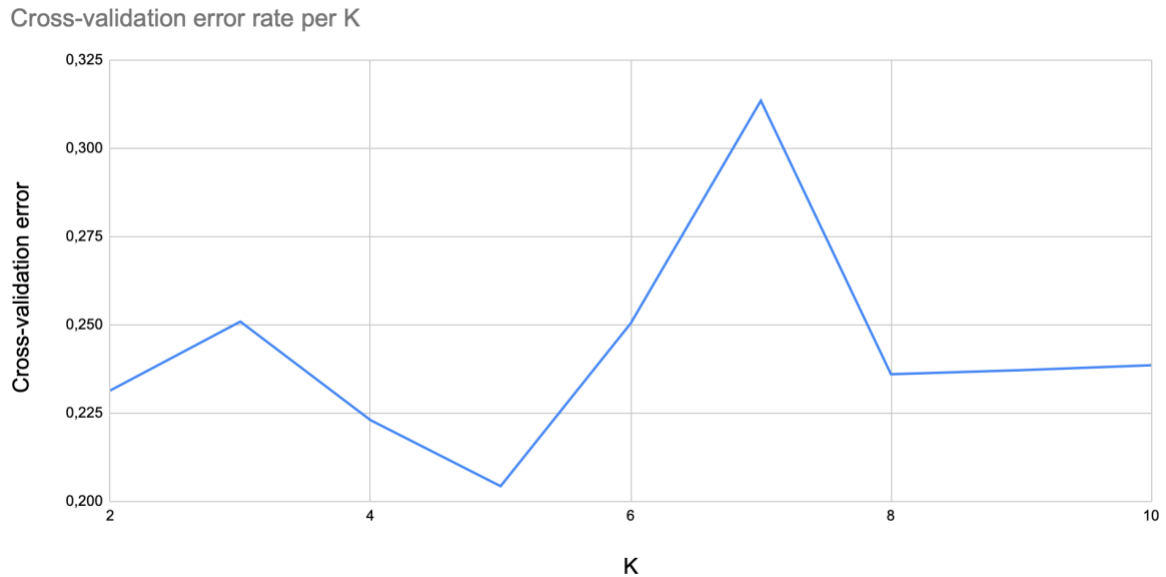


Figure 3. A plot of the cross-validation error rate per cluster k . The plot shows the average error rate for different numbers of clusters used in the admixture analysis.

A closer look at $K=5$ (Fig 4.) reveals the match in orange-colored individuals between late founders and Finnish wolves. Note that individuals sharing the near-full ancestral proportions from the orange cluster are of interest here. While samples in Russia have smaller proportions of orange ancestry, it is the fully orange bars in Finland that seem to best match with the later founders.

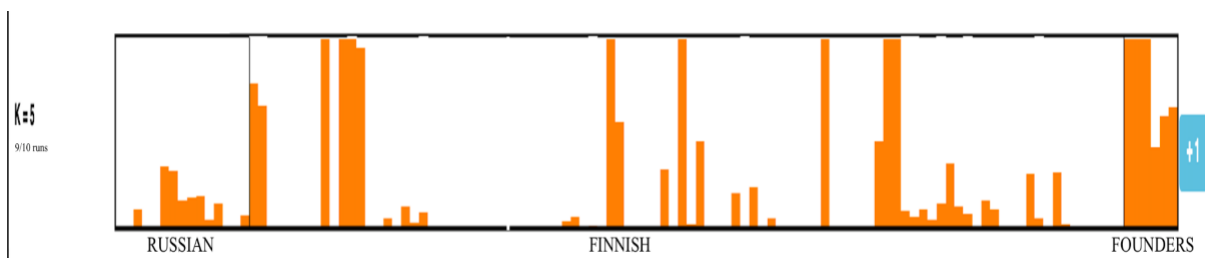


Fig 4. Admixture results for $K=5$, highlighting the orange cluster that the late founders have the highest ancestral proportion of. Each bar represents an individual. Delineated by straight lines from left to right: Russian & Finnish samples and Scandinavian (founders). Rightmost 3 founders are from before 2008, and the leftmost 3 founders are from 2008 and onwards.

PCA

Moving on, further analysis of the genetic variation in and between the sample groups was done with the PCA and mapping of clusters in Finland. All samples further away from the Finnish-Russian border are assigned to the category Western Finnish wolves and the rest to Eastern, as seen with less distortion of the map in Supplementary. Fig 1. However, not all clusters align along those categories: E.g the orange cluster of interest from the admixture

analysis is found both in the Western and Eastern group, having the biggest spread of points in a cluster on the map (Fig. 5). Note that these colors do not necessarily match with the admixture cluster colors but are generated separately through another clustering method with VCF2PCACluster. The orange cluster in this map however does overlap with the individuals of the admixture analysis. Moreover, the clusters in the map also overlap geographically, especially in central Finland and near the Russian border. The cluster points are mapped out over Finland due to the available coordinates of these samples, the high diversity noted in the admixture analysis and the aim of investigating the genetic landscape in relation to the founders.

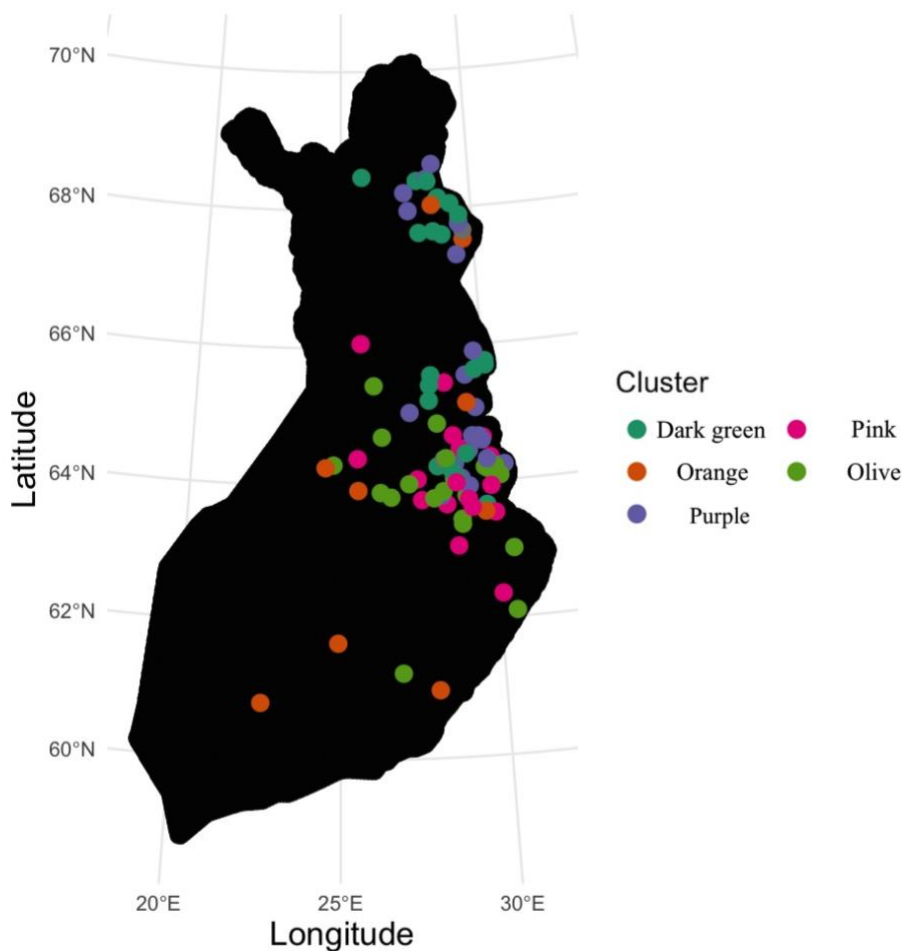


Figure 5. A map of the samples, their dominant cluster from the VCF2PCACluster analysis (corresponding to Figure 6) and their geographical point in Finland. Reservation for some distortion of the map, see Supplementary Fig.1 for reference.

A temporal dimension is analyzed by categorizing Finnish wolves into subgroups of pre- and post-2008 sampled wolves and 3D plotting the PCA (Fig. 6) to capture most of the variation. All early founders (D-85-01, FM1, FM2) are found in the Dark Green cluster, with most of the Russian samples and a predominately Eastern Finnish group, 19 samples after 2008 and 2

before 2008. One post-2008 Western Finnish sample is found in the cluster as well, with a location in North-Western Finland (see Fig. 5). The late founders are found in the Orange cluster, separating along PC3 from the previous founders, together with 4 Eastern (W10, W35r, W72r, W98r) and 4 Western (W32, W37r, W68r, W70r) samples from after 2008 and 1 Eastern (W59) wolf sampled before 2008. This cluster is also the smallest one and reminiscent of the number of few fully orange individuals from the admixture plot (Fig 4.). Along the same axis is the Olive cluster, adjacent to the Orange, with the largest representation of Western Finnish wolves sampled after 2008 (8) and 12 Eastern post-2008 and 4 pre-2008 (also a single Russian sample). These are, with the Orange points, among the most western and southern samples we can find on the map, but the Olive ones are more concentrated to central Finland. Further down PC2 and PC3, the Purple cluster separates from the rest with 2 Russian samples and a predominantly Eastern Finnish selection (14 post-2008, 9 pre-2008) and 1 Western Finnish wolf sampled after 2008 (W22). Their distribution is also concentrated two-fold near the Russian border, both in central-east and north-east Finland. At the lower PC1 end and across the middle portion of PC2 and 3, the Pink cluster encompasses both Eastern and Western samples, from before and after 2008. The Pink samples are more centrally laid in Finland. See Supplementary Table 3 for the total counts and tab 2 in the metadata for more details.

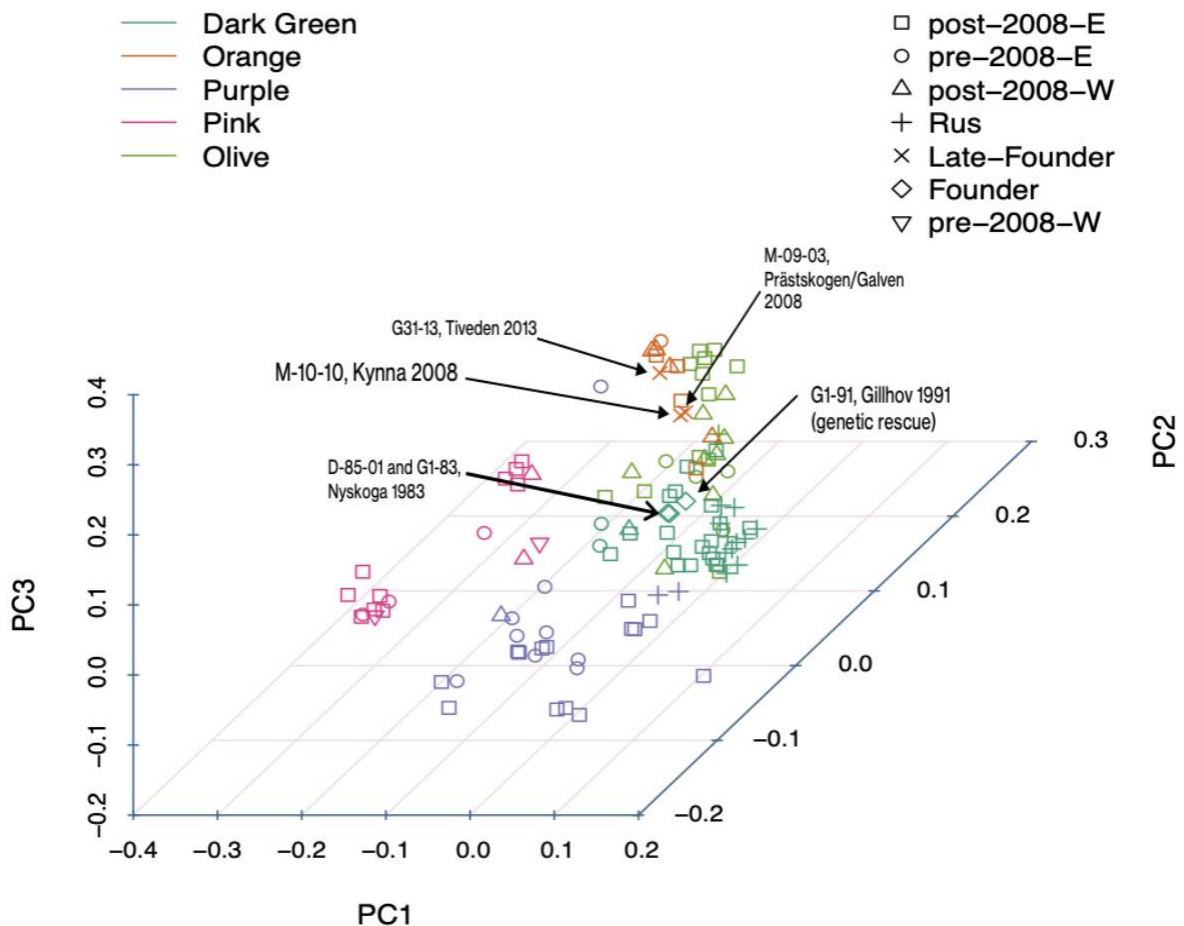


Figure 6. A 3D PCA plot with late founders & early founders (marked and named with arrows), Western and Eastern Finnish wolves sampled before and after 2008 and Russian wolves. $k=5$. $N=117$. These cluster points do not show, as in the admixture plot, the mix of ancestral proportions present in the samples. Rather, the predominant cluster assigned to the sample is shown.

ROH analysis

The ROH-analysis showcases the Scandinavian populations greater lengths of ROH compared to Finnish and Russian samples, with the latter having similarly lower ROH lengths, but also a presence of more outliers in the Finnish population nearing the average ROH length of the Scandinavian population (Fig. 8).

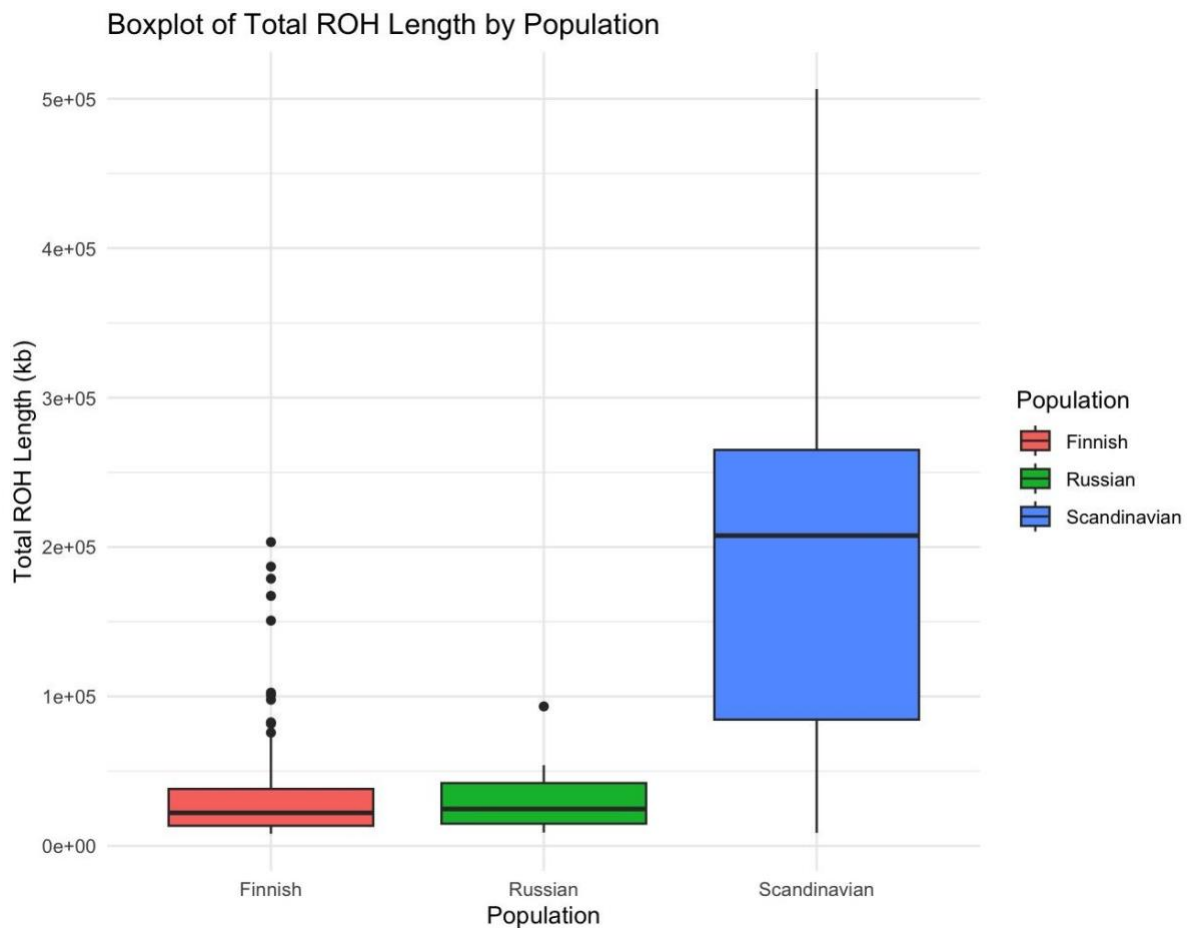


Figure 8. Box plot of the total ROH length by population. Finnish, Russian and Scandinavian samples included. Outliers marked with black dots.

Another angle of showcasing the significantly higher number of ROH length among Scandinavian wolves is looking at how the majority of the longest ROH segments are present in Scandinavian samples, with some Finnish occurrences sticking out as previously seen (Figure 9.). The shortest segments are also mostly seen among Finnish wolves, with some Russian and Scandinavian samples among them. However, given the cutoff at 8,000 kb, they all indicate inbreeding events occurring in the most recent generations.

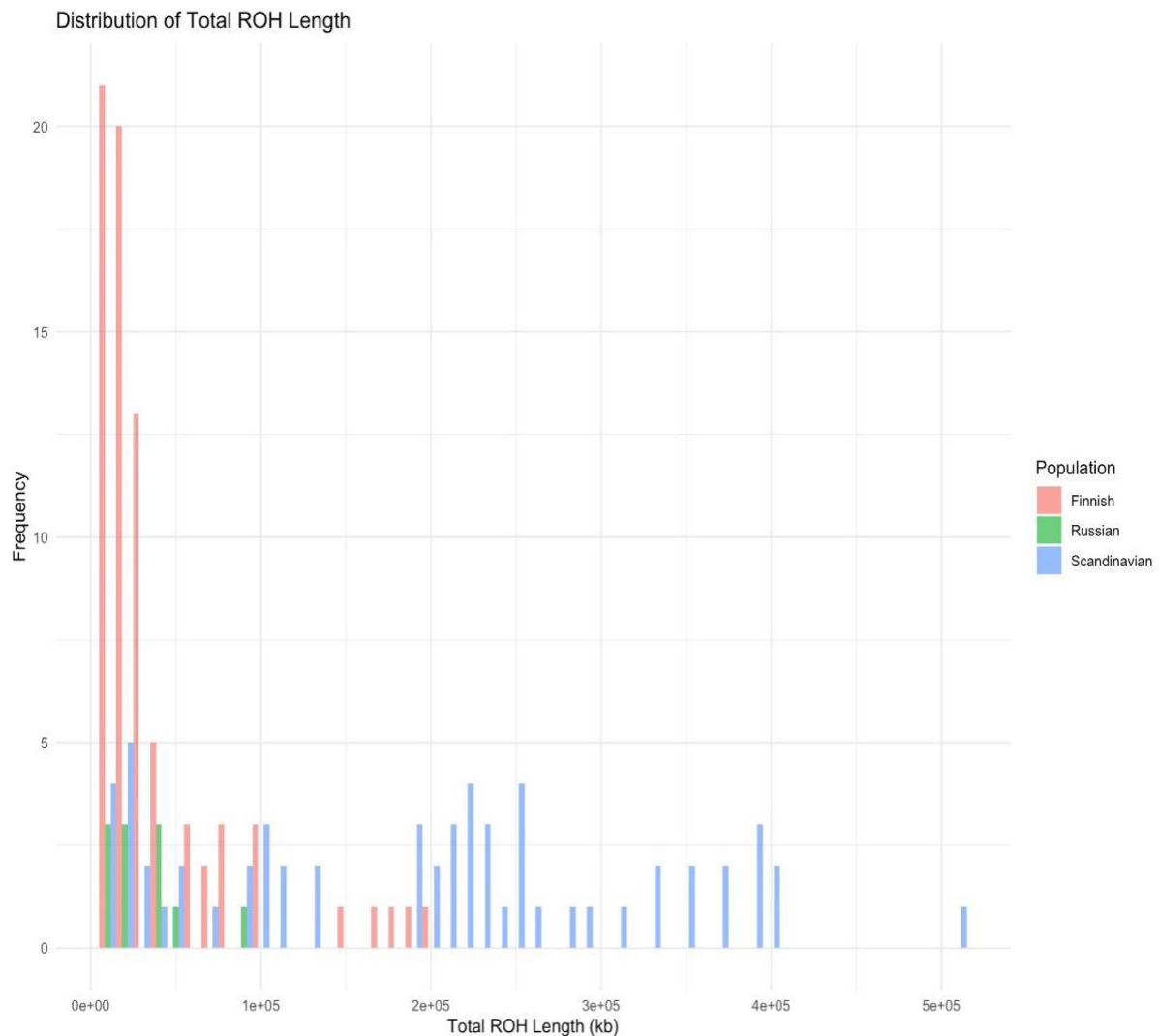


Figure 9. Histogram of total ROH length and the frequency of ROH segments of various lengths among Finnish, Russian and Scandinavian samples.

A total overview of the ROH segments found among Finnish and Scandinavian samples depicts the prevalence of longer ROH segments among Scandinavian individuals (Fig. 10), but also allows for further visualization of overlaps in ROH segments between Finnish and Scandinavian individuals (Fig. 11). Overlaps in ROH segments between the different sample groups were found across 18 chromosomes. Examples (Supplementary Table 2.): The outlier

W58, a pre-2008 (more precisely, 2001-sampled) Eastern Finnish wolf, shares 3 segments with Scandinavian wolves: 2 females (offsprings) from 1989 (the first Scandinavian territory after recolonization), a male from 2007 and a male from 2006. The sample belongs to the Purple cluster and can be observed sharing one segment with post-2008 sampled Western wolves as well (W93r). W65r, a post-2008 (2016-sampled) Eastern Finnish wolf, shares 2 segments with Scandinavian wolves: A female from 1998, 3 samples from 2007, another from 2010, a male from 2011, one from 2013, lastly one from 2015. The sample shares in turn a segment with other Eastern and Western Finnish wolves that do not necessarily overlap with any Scandinavian samples and is found in the Olive cluster. W99r, an Eastern Finnish wolf sampled 2014, shares 3 segments with Scandinavian wolves, in company with only post-2008 sampled Finnish wolves sharing those segments too. The Scandinavian samples in conjunction with W99r range from a couple offsprings from the first territory in 1989 (first territory) to both older and more recent samples until 2015. W99r also occurs in the Dark Green cluster, together with the earlier founders. W4, also an Eastern wolf sampled later (2015) shares 4 segments with Scandinavian wolves, among which 1 is shared with a late founder (M-09-03, 2008) and its offspring. Moreover, samples ranging from 1998 to more recent years share segments with it, as well as both Eastern and Western Finnish wolves. W4 is clustered with the Olive group. Finally, W82r, an Eastern Finnish wolf sampled 2015, shares one long ROH segment with Scandinavian wolves from 2000 and 2007, together with other Eastern Finnish wolves sampled after 2008. The sample is found in the Purple cluster.

ROH Segments on Chromosomes

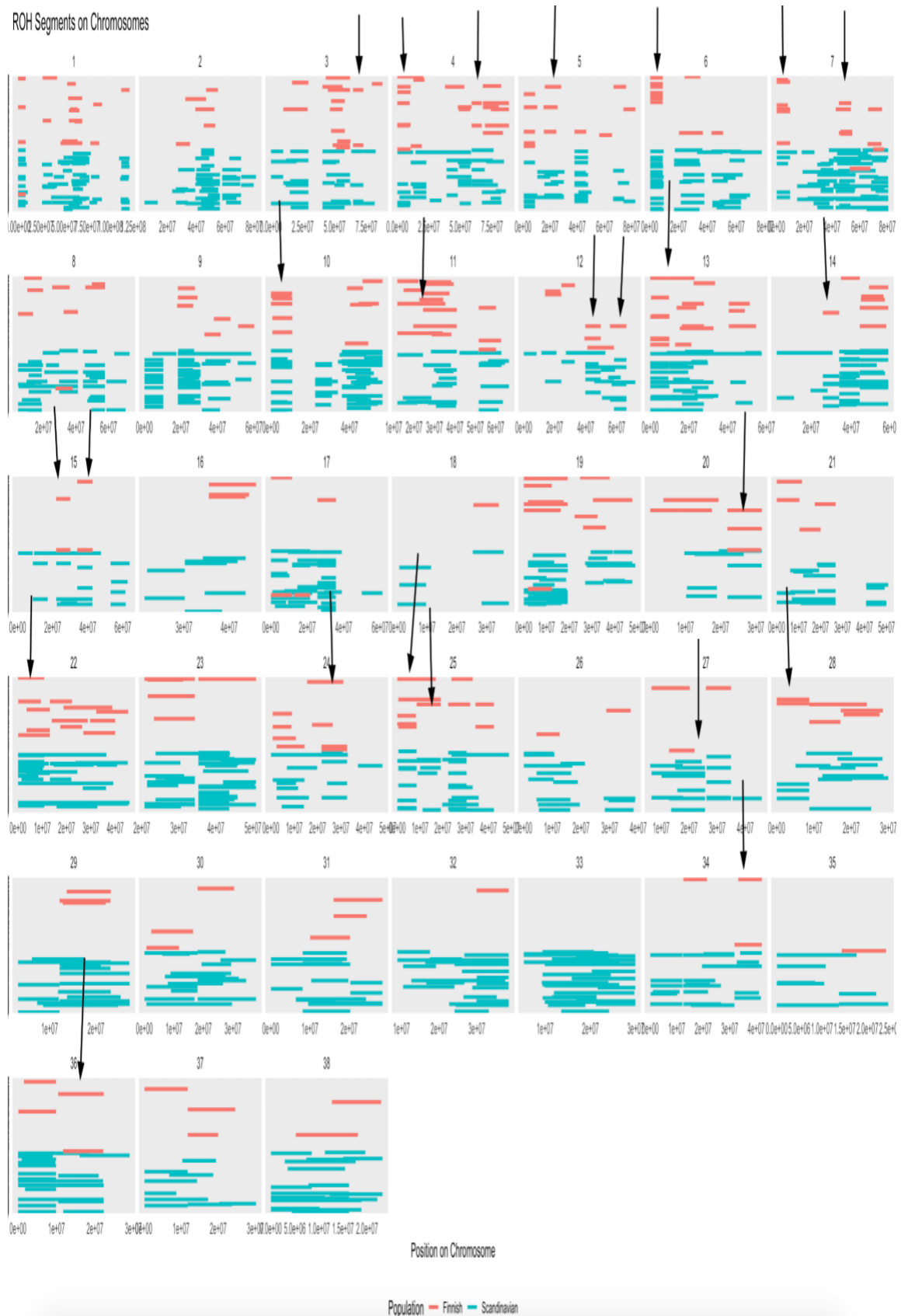


Figure 10. The ROH Segments present from chromosome 1 to 38 among Finnish and Scandinavian samples. The individuals are on the y-axis while the segment lengths are depicted horizontally. Arrows point at segments with overlap between sample groups.

Shared ROH Segments by Chromosome

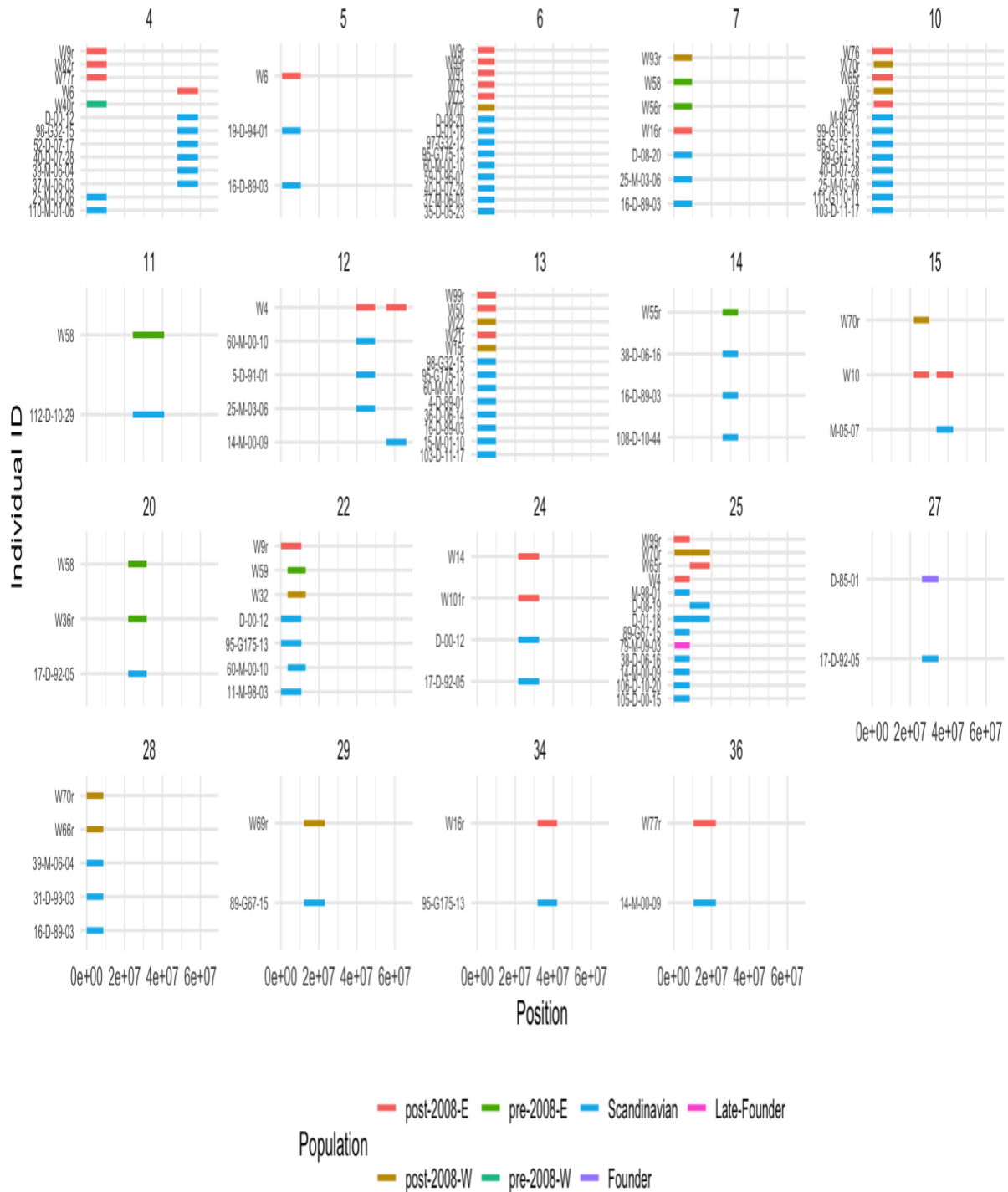


Figure. 11. The shared ROH segments between Finnish and Scandinavian samples (arrows in Figure 10). The figure highlights segments where there are overlaps between individuals from early founders, late founders, Scandinavian born and pre/post-2008 sampled Western and Eastern Finnish wolves.

Overviewing the individual samples, their group category and overlaps in ROH samples shows a general prevalence of post-2008 Finnish samples among those overlapping with Scandinavian samples. (Figure 12). Western Finnish samples after 2008 were of interest and

thus dot plotted to connect overlapping samples and easier visualize whether an inbreeding event can be thought to have occurred in Scandinavia and then observed in Finland. Not only does that seem the case in the plot, but there are also wide representation from founder-connected samples, whether it is the late founder of Prästgalven in 2008 and seen shared segments in recent Western Finnish samples or the Tiveden founder's offspring (2015) seen sharing a segment with a later occurring West Finnish sample (2016). Even the earliest founders' offspring in the first recolonized territory (Nyskoga) before the arrival of the 1991 founder are seen leaving traces of their inbreeding in Finnish samples.

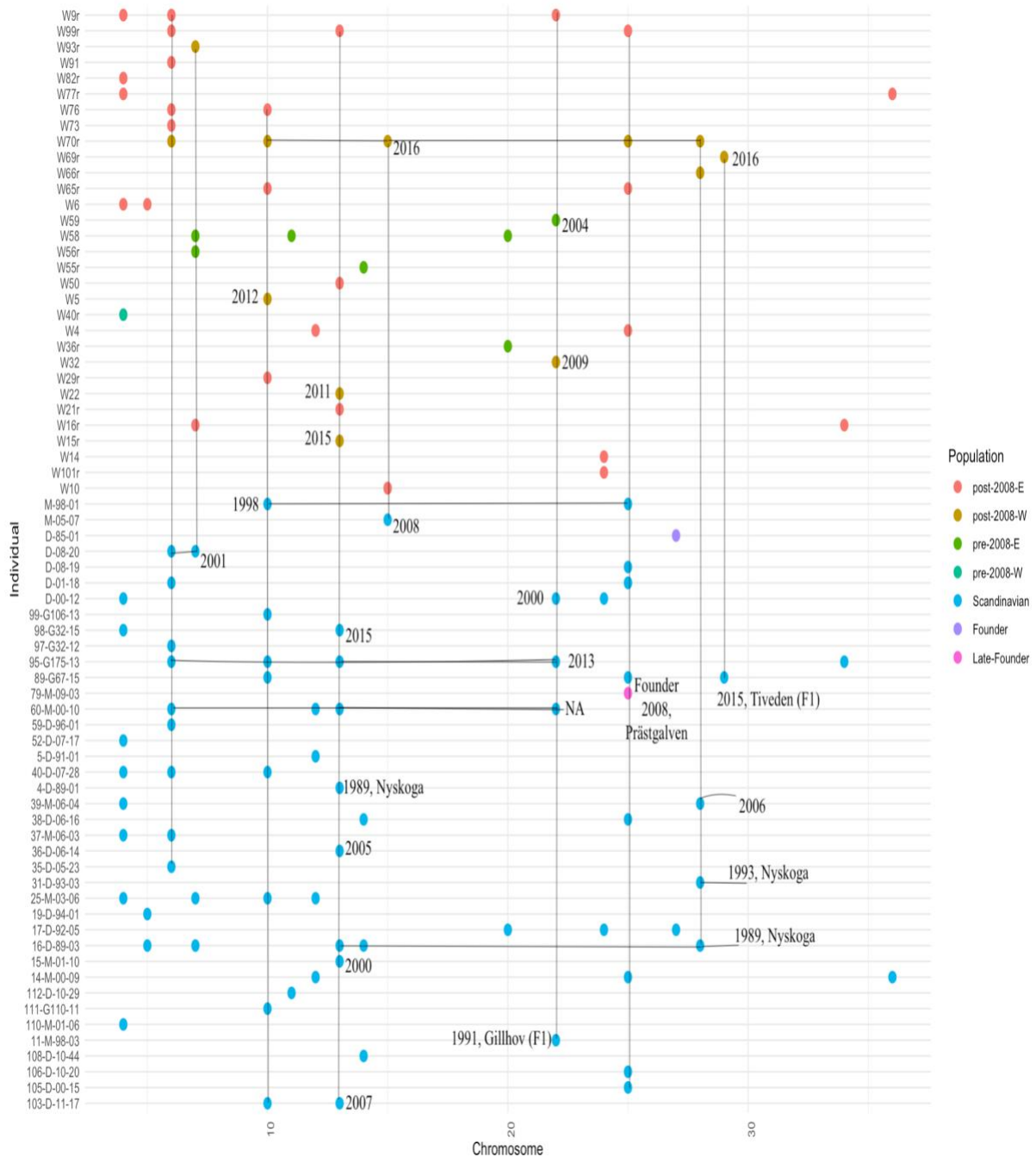


Figure 12. Dot plot of the individuals sharing segments and on which chromosome across groups of Founders, Scandinavian born wolves and pre/post-2008 sampled Western and Eastern Finnish wolves. Lines are drawn between post-2008-W samples overlapping with Scandinavian samples, with years and territorial information

highlighted to see temporal trends. Lines are also drawn horizontally to highlight a repeated individual's occurrence.

Discussion

The results of this study offer compelling insights into the genetic structure of wolves across Scandinavia and Finland. Several indications suggest that inbreeding events that began in Scandinavia may be reflected in Finnish wolf populations. This is particularly evident from overlapping ROH segments, which indicate shared ancestry and past migration events between regions. The clustering and PCA analysis suggest that more recent founders in Scandinavia likely came from elsewhere in Finland, though it remains unclear precisely which region. The evidence does seem to point at a cluster with presence in South-Western to central Finland, but even then, our analysis showed a spread throughout the country for this cluster. The previous findings (Vila et al., 2003; Smeds et al., 2021) about a Finnish-Russian origin for the original founders are also strengthened by the clustering with a mix of Russian and Finnish ancestry proportions. The high genetic diversity seen in Finland, as illustrated by the clustering at $k=5$, indicates a more nuanced genetic background than previously thought.

The $K=5$ structure provides significant insight into genetic differentiation. Notably, the PCA highlights clusters of wolves with varying levels of genetic diversity. For example, the dark green cluster contains the early founders and shows the highest genetic relatedness to Russian and Finnish wolves. Meanwhile, the orange cluster, which includes the late founders, stands apart, indicating a possible genetic source from a different part of Finland or an admixture of wolves not fully explored in prior studies. The presence of these distinct clusters across geographic regions, particularly between Eastern and Western Finnish wolves, suggests that some genetic isolation may have occurred historically (possibly during the last glaciation), followed by more recent admixture. In the light of this, F_{ST} values (Supplementary table 4.) for the Admixture clusters show some genetic structure among them, in accordance with Whitlock's and McCauley's (1999) threshold of 0.2 corresponding to approximately 1 migrant per generation. This strengthens the results of having $K=5$ as optimal K . The presence of Scandinavian-sourced inbreeding events across Finnish groups, even in the East, could mean several complex paths of migrations: Scandinavian input to eastern Finland where groups later migrated to south-western Finland or direct Scandinavian input to south-western Finland that has spread to the rest of the country.

The shared ROH segments provide an interesting layer of complexity, indicating shared ancestry across Finnish and Scandinavian wolves. These segments, particularly those found in both the early founders' descendants but also later samples, suggest that inbreeding events have not only persisted but also crossed geographic borders into Finland since the recolonization of Scandinavia took place. The visualization of ROH overlap between all Finnish sample groups and their Scandinavian counterparts supports the hypothesis of shared inbreeding events, suggesting that wolves may be traveling and reproducing across these regions. Even a late founder shown to share inbreeding events with earlier Scandinavian

samples influenced by the inbreeding sequences during the re-colonization refutes the assumptions of unrelated founders. The shared segments also add weight to the hypothesis of two-way gene flow between Finland and Scandinavia, with Western Finland emerging as a potential source of new genetic input into the Scandinavian wolf population or vice versa. However, the precise role of Western Finland in this gene flow remains uncertain due to the skewed sample sizes and geographic gaps, particularly from southwestern Finland, which was underrepresented in the dataset. Despite this underrepresentation, 1 out of 2 pre-2008 W wolves are shown to share a ROH-segment with Scandinavian and other Finnish samples.

One of the key limitations of this study is the uneven sample distribution across Finland, particularly in the southwestern region. Future research should focus on acquiring a more comprehensive dataset that includes additional samples from this area. Research groups have shown the power of an extensive whole-genome dataset and the potential to improve on this study should be near at hand (Stenoien et al., 2021). Increasing the sample size, particularly from underrepresented regions and subpopulations, will provide a more accurate understanding of gene flow and inbreeding events. Specific assignment tests for each individual worth treating in this study would also have deepened the knowledge, however they proved difficult to perform due to the sheer size of the WGS-data and the limitations of the local computer.

In addition to expanding the sample size, further research should investigate the role of gene flow between Russia and Scandinavia. While the study identified strong genetic connections between Scandinavian and Finnish wolves, the influence of Russian wolves, particularly those from the Kola Peninsula, remains underexplored (Laikre et al., 2016).

Conclusions

This study provides insights into the genetic origins and population structure of Fennoscandian wolves, in particular how Scandinavian founders relate to neighboring populations and how their lineages might have looked like. While there is no definitive conclusion regarding the gene flow from Western Finland to Scandinavia, the shared ROH segments and the genetic diversity seen in clusters suggest complex patterns of migration and inbreeding between these populations. The results call for a more detailed investigation into the sources of recent Scandinavian founders and their Finnish connections, with focus on overcoming current limitations in the dataset.

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Appendix 1. Popular Science Summary

Zakaria Hachicho

The Genetic Landscape of Fennoscandian Wolves (*Canis Lupus*)

Imagine the story of the Scandinavian wolf—a tale of survival, disappearance, and an unexpected return. Wolves in Scandinavia faced extinction in the wild in the 1970s, only to re-establish thanks to a single immigrant pair in 1983. Over the years, new wolves have occasionally joined the population, some even managing to contribute enough to the genetic pool to be considered a *founder*. My research delves into the genetics behind these wolves, focusing on their origins and connections with nearby Finnish and Russian populations.

Founders and Their Legacy

The first three founders arrived in 1983 & 1991 while the later three ones arrived in 2008 & 2013, each bringing a mix of genes that have carried through generations. By analyzing the DNA of these founders and their descendants, I traced how genetic material from wolves with Finnish and Russian roots has mixed within the population. Early founders mostly connected with Eastern Finnish and Russian wolves, while later ones show links to Western & Central Finland. This cross-border blending has influenced the genetic landscape of Scandinavian wolves, showing a mix of genetic origins within the population.

The Detective Work on Inbreeding

While the founders have helped create the population, the limited number (especially in the beginning) has led to inbreeding over time. In a small population like this, wolves often share the same ancestors, leading to what we call “shared inbreeding events”. These events result in the same genetic traits being passed down repeatedly, increasing risks of health issues. This research uses these events as genetic markers to identify how genes have flowed between the regions: If inbreeding events occurring in Scandinavia can be seen in Finland, that would signify a *gene flow* to Finland from Scandinavia.

My findings show that inbreeding among Scandinavian wolves is not just a local issue; there are shared inbreeding events between these wolves and certain groups in Finland. This genetic overlap suggests that wolves have traveled between regions. Interestingly, even a late founder bears evidence of a shared inbreeding event *before* arriving in Scandinavia. This means that even among immigrants to the region that we hope are contributing fresh genetic diversity, we have signs of relatedness and influence from earlier Scandinavian wolves. This highlights the challenges of maintaining a healthy gene pool and the importance of

monitoring the status of immigrants that hold potential of offsetting the inbreeding in Scandinavia.

What Does It Mean for Conservation?

Understanding the genetics of these wolves helps conservationists make informed decisions to support a healthy population. Management in Scandinavia builds on the genetic studies that are available. The better the information is on the origins of these wolves and their patterns over the borders, the better the decisions can be. By confirming that Finnish wolves contribute to the Scandinavian genetic pool and vice versa, my study provides evidence that maintaining and even increasing these cross-border connections is beneficial. For the Scandinavian wolf, every new gene helps in the fight against genetic isolation and the risks of inbreeding, adding to their chances of survival.

Appendix 2. Data

[Genotypes](#)

[Metadata](#)

Appendix 3. Supplementary material

Supplementary table 1. [Finnish wolves-Dates and coordinates](#)

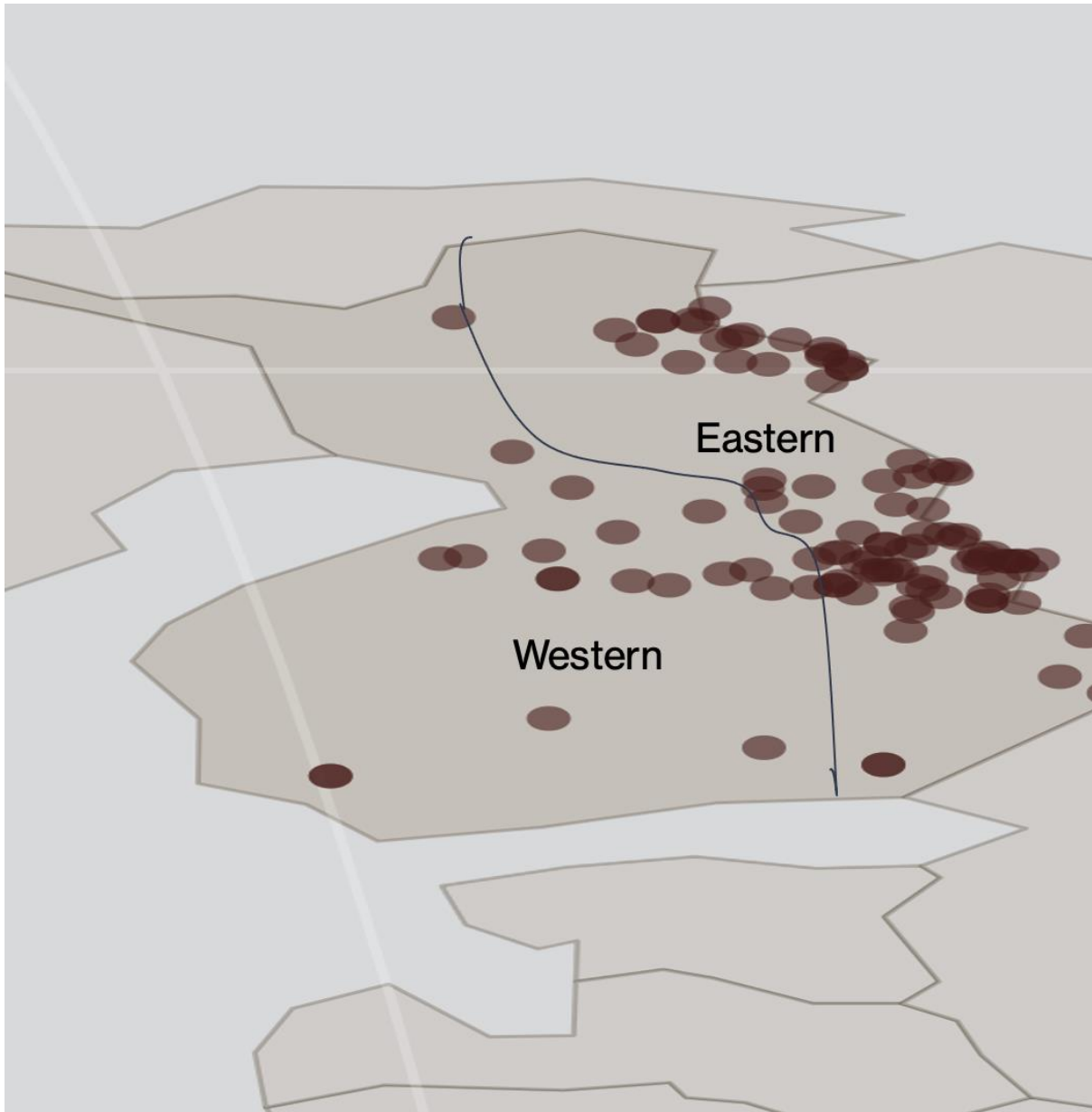
Supplementary table 2. [Shared ROH data](#)

Supplementary table 3. *Count of individuals from sample groups Founders, Finnish wolves and Russian wolves assigned to different clusters via VCF2PCACluster.*

Group/Cluster	Dark Green	Orange	Purple	Pink	Olive
Founder	3	0	0	0	0
Late-Founder	0	3	0	0	0
post-2008-E	19	4	14	10	12
post-2008-W	1	4	1	2	8
pre-2008-E	2	1	9	3	4
pre-2008-W	0	0	0	2	0
Rus	11	0	2	0	1

Supplementary table 4. *Pairwise F_{ST} values among clusters named after their colors from the admixture analysis, $K=5$. The F_{ST} values presented show moderate genetic differentiation and some genetic structure, with some values even being above 0.15, suggesting more restricted gene flow between certain clusters (e.g. between Orange and the rest) compared to others, but overall, a structure that shows a level of interconnectedness between the clusters.*

F_{ST}	Brown	Orange	Green	Pink	Purple
Brown	-	0.172	0.145	0.135	0.108
Orange	0.172	-	0.166	0.171	0.153
Green	0.145	0.166	-	0.141	0.109
Pink	0.135	0.171	0.141	-	0.108
Purple	0.108	0.153	0.109	0.108	-



Supplementary Figure 1. *Finnish wolf samples in the dataset and their distribution in Finland.* Line is drawn where samples got categorized as W for Western or E for Eastern, to retain Finnish-Russian Karelian wolves in the east and more mobile and western wolves to central and western part of Finland.