RESEARCH ARTICLE

Population genetics of the wolverine in Finland: the road to recovery?

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Abstract

After decades, even centuries of persecution, large carnivore populations are widely recovering in Europe. Considering the recent recovery of the wolverine (*Gulo gulo*) in Finland, our aim was to evaluate genetic variation using 14 microsatellites and mtDNA control region (579 bp) in order (1) to determine whether the species is represented by a single genetic population within Finland, (2) to quantify the genetic diversity, and (3) to estimate the effective population size. We found two major genetic clusters divided between eastern and northern Finland based on microsatellites ($F_{ST} = 0.100$) but also a significant pattern of isolation by distance. Wolverines in western Finland had a genetic signature similar to the northern cluster, which can be explained by former translocations of wolverines from northern to western Finland. For both main clusters, most estimates of the effective population size N_e were below 50. Nevertheless, the genetic diversity was higher in the eastern cluster ($H_E = 0.57$, $A_R = 4.0$, $A_P = 0.3$) than in the northern cluster ($H_E = 0.49$, $A_R = 3.7$, $A_P = 0.1$). Migration between the clusters was low. Two mtDNA haplotypes were found: one common and identical to Scandinavian wolverines; the other rare and not previously detected. The rare haplotype was more prominent in the eastern genetic cluster. Combining all available data, we infer that the genetic population structure within Finland is shaped by a recent bottleneck, isolation by distance, human-aided translocations and postglacial recolonization routes.

Keywords Conservation genetics · *Gulo gulo* · Microsatellites · MtDNA · Genetic structure · Genetic diversity

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Introduction

Conservation of large carnivores represents a challenging issue especially due to the conflicts arising from their interaction with human activities. Large carnivores have suffered from considerable declines globally (Prugh et al. 2009; Ripple et al. 2014), but recent conservation efforts have aided the recovery of several populations in Europe (Chapron et al. 2014). Nevertheless, with extensive home range requirements and natural low densities, large carnivores are particularly sensitive to habitat fragmentation and limited connectivity between the patches (Crooks 2002; Crooks et al. 2011). Isolation can result in a fragmented genetic population structure, a decline of genetic diversity and increased inbreeding, followed by a decrease in reproductive potential and survival of the population (Frankham et al. 2010). Thus, assessing the genetic status is an important objective in predicting the sustainability of large carnivore populations (Frankham 2005).

To that end, non-invasive genetic sampling (e.g. using scats, hair or urine samples as a source of DNA) has proven

an excellent method to measure the genetic status in wild populations of large carnivores (Lamb et al. 2019). The wolverine (*Gulo gulo*), one of the four large carnivores in northern Europe, is a characteristically scarce and elusive species (May et al. 2006, 2012; Persson et al. 2010; Inman et al. 2012; Fisher et al. 2013; Mykrä and Pohja-Mykrä 2015). Studies on wolverines in the wild are difficult to carry out due to their low population densities and the remoteness and harshness of their habitats (May et al. 2006; Persson et al. 2010). Therefore, development of non-invasive DNA-techniques provides an efficient way to learn more about this species (Hedmark et al. 2004, 2007; Brøseth et al. 2010; Magoun et al. 2011; Bischof et al. 2016; Gervasi et al. 2016).

Wolverines are found throughout the northern Holarctic with the European range in northwest Russia, Finland, Sweden and Norway (Fig. 1). Typical wolverine habitats in Europe include alpine heaths and meadows, boreal forests, and mires (Landa et al. 2000; May et al. 2010; Koskela et al. 2013; Aronsson and Persson 2017; Hyvärinen et al. 2019). Approximately 300 to 500 wolverines were roaming in Finland at the beginning of the 1900s (Mykrä and Pohja-Mykrä 2015). During the twentieth century, their numbers severely declined due to human persecution. The decline continued through the 1970 and 1980s, when the breeding population was been decimated and wolverines were rarely observed except around the eastern and northern border of Finland (Fig. 2). Although at least 22 den sites were active in north-eastern Finland during the 1960s (Pulliainen 1968), the fatal combination of governmental bounties for killing and the increased use of snowmobiles in the hunt culminated in only two active dens in 1973 being located (Pulliainen and Nyström 1974). However,

Fig. 1 The sampling sites of wolverine individuals used in this study ($N = 247$) and current wolverine distribution in Finland and Europe. The black dots represent the locations of individuals. Individuals from the same location are displaced around a central point that corresponds to original location. The yellow dashed line represents the division of individuals into predefined populations *East* and *North*, as based on the approximated past population division (see Fig. 2). The extent of the Finnish reindeer husbandry area is depicted with small dots. The main study regions are labelled. The inset shows the current range of wolverines in northern Europe (adopted from Abramov 2016; Danilov et al. 2018; Flagstad et al. 2018; Natural Resources Institute Finland 2018) including the three population strongholds (Boitani et al. 2015)
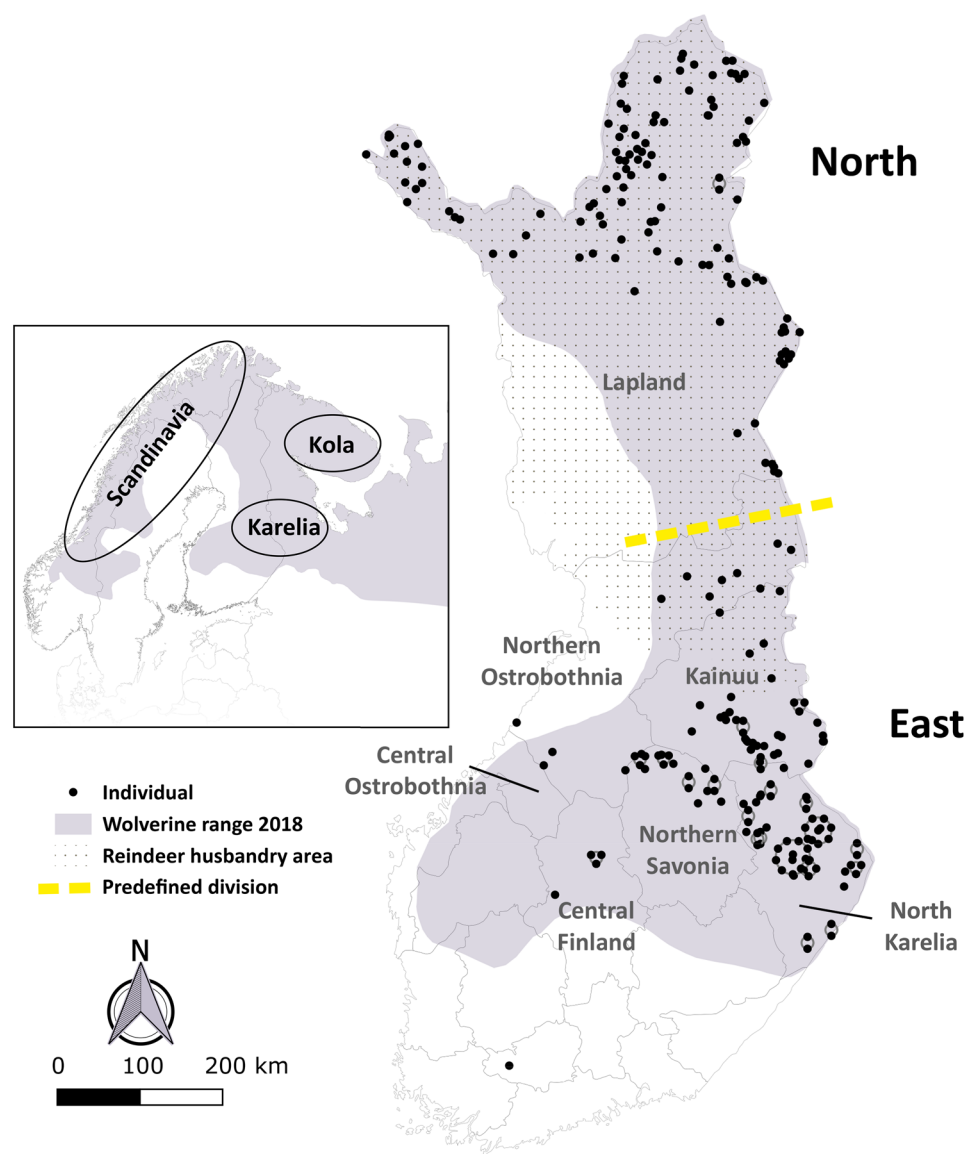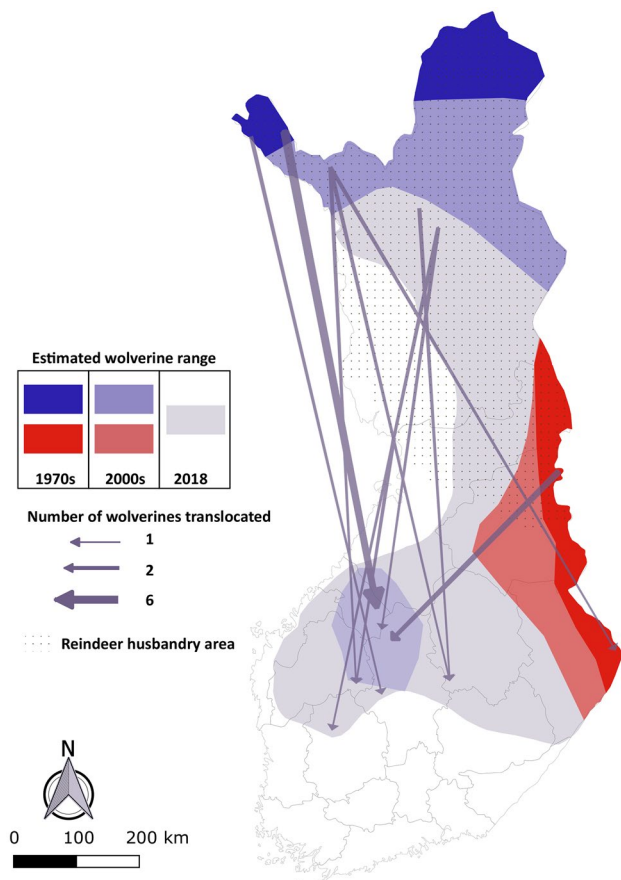
Fig. 2 Approximated increase of the breeding range of wolverines in Finland from 1970s to 2018. Hypothetical genetic clusters due to the bottleneck represented with blue (*North* and *West*) and red (*East*). The arrows represent 11 translocation events of 16 wolverines (10 males and 6 females) from 1979 to 1998 (Pohja-Mykrä and Kurki 2008). The two individuals translocated from eastern Finland were females. The thickest arrow represents two translocation events of three individuals each. The extent of the Finnish reindeer husbandry area is depicted with small dots. The historical Finnish wolverine ranges were based on Pulliainen and Nyström (1974), Landa et al. (2000) and Natural Resources Institute Finland (2018), while the current wolverine range was adopted from Abramov (2016) and Natural Resources Institute Finland (2018)

after wolverines became protected south of the reindeer husbandry area in 1978 and in the whole country in 1982, the population started to recover (Pohja-Mykrä and Kurki 2008). The reindeer husbandry area (Fig. 1), that covers 33% of Finland, is a region where people are practicing reindeer herding, and where the wolverine is considered the most harmful of all large carnivores to reindeer survival (Jernsletten and Klokov 2002). To promote the establishment of a larger breeding population outside the reindeer husbandry area (Pohja-Mykrä and Kurki 2008), 16 wolverines from northern Finland were translocated to 11 locations in western and central Finland during the 1980 and 1990s (Fig. 2).

During the last two decades, wolverine numbers have increased, especially outside the reindeer husbandry area in the boreal forests of Finland (Fig. 2) as likewise in Sweden (Aronsson and Persson 2017). Nevertheless, the recovery in Finland was initially slow, likely due to continued legal culling and poaching (Ermala 2003; Pohja-Mykrä and Kurki 2008). At present, the western periphery of the Eurasian wolverine range displays three population strongholds within northern Europe: one in Scandinavia, including areas of Norway, Sweden and northernmost Finland; one in Karelia, including Eastern Finland and neighbouring parts of Russian Karelia; and one on the Russian Kola Peninsula (Fig. 1) (Boitani et al. 2015). Using a combination of a count method (i.e. count of reproductive dens) and capture–recapture models (i.e. long-term non-invasive genetic sampling) (Gervasi et al. 2016), 300–350 individuals have been identified in Norway, 400–650 in Sweden and about 130 in northern Finland (Persson and Brøseth 2011; Boitani et al. 2015; Kojola 2018; Flagstad et al. 2018). Using only count-based methods (e.g. snow track data, sightings, droppings and carrions killed by wolverine), about 155 and 160 individuals have been estimated in Finnish and Russian Karelia, respectively (Danilov et al. 2018; Kojola 2018). A census population size estimate for the Kola Peninsula varies between 300 and 440 individuals (Boitani et al. 2015; Danilov et al. 2018).

Although genetic population structure of Scandinavian wolverines has been studied (Walker et al. 2001; Flagstad et al. 2004; Hedmark and Ellegren 2007; Ekblom et al. 2018), it has not been assessed in Finland. Based on both microsatellites and SNPs, the extant Scandinavian population appears to be subdivided into two clusters: south-western Norway and the rest of Scandinavia (Walker et al. 2001; Flagstad et al. 2004; Ekblom et al. 2018). On the other hand, no variation was found across Scandinavia in the mitochondrial whole genome (Ekblom et al. 2014) or in the control region of the mtDNA (Walker et al. 2001). The Scandinavian mtDNA haplotype (Arnason et al. 2007; Ekblom et al. 2014) was additionally present in north-east Russia and in Alberta, Canada (Tomasik and Cook 2005; Zigouris et al. 2013) (termed haplotype 15 sensu Zigouris et al. 2013). A recent genomic analysis of Scandinavian wolverines revealed low genetic diversity and an effective population size of below 500 individuals (Ekblom et al. 2018), which may be too low to retain evolutionary potential (Frankham et al. 2014).

Currently, the wolverine is globally listed by IUCN as *Least Concern* because of high population numbers in North America and Russia (Abramov 2016), while on a national scale, they are categorized as vulnerable in Sweden and Russian Karelia, and as endangered in Finland and Norway (Boitani et al. 2015; Henriksen and Hilmo 2015; Hyvärinen et al. 2019). Wolverines are, however, managed by quota-based hunting in Norway, whereas Sweden and Finland belong to the European Union and therefore, legal hunting

is restricted (Gervasi et al. 2016). In Russian Karelia, the species is fully protected by law but illegally hunted for fur (Danilov et al. 2018).

In this study, we examine putatively neutral genetic variation (14 microsatellites and 579 bp of control region mtDNA) of wolverines in Finland in order (1) to determine whether the species reflects a single genetic population within Finland, (2) to quantify genetic diversity, and (3) to estimate the effective population size. We expected to observe weak genetic population structure, as the species is well-known for its capability of long distance dispersal (Gardner et al. 1986; Vangen et al. 2001; Flagstad et al. 2004; Inman et al. 2012). On the other hand, genetic differentiation between eastern and northern Finland might have occurred during the documented bottleneck (Fig. 2), resulting in northern Finland being part of the Scandinavian population and eastern Finland as part of the Karelian population (Fig. 1) (Chapron et al. 2014; Boitani et al. 2015). In this scenario, due to the translocations, wolverines in western Finland would possibly be more similar to their northern conspecifics. Population subdivision due to a recent bottleneck has been proposed to explain population structure in brown bears (*Ursus arctos*) in Finland (Kopatz et al. 2014) and wolverines in Scandinavia (Walker et al. 2001; Flagstad et al. 2004). Genetic diversity and effective population size estimates of wolverine population(s) in south-eastern Finland are expected to be higher than on the edge of the Eurasian range (i.e. northern Finland as part of the Scandinavian populations) due to the assumed connectivity of the Karelian population with larger populations in north-western Russia.

Material and methods

Sampling and molecular analyses

We collected 1281 wolverine samples between the years 1983 and 2018 from northern, central and eastern Finland. Scat samples ($N = 936$) were collected as part of the Scandinavian population from Finnish Lapland and as part of the Karelian population from Northern Ostrobothnia, Kainuu and North Karelia since 2003. Hair samples ($N = 296$) were collected from 33 non-invasive snag sites in the regions of North Karelia, Northern Savonia, Central Finland, Kainuu and Northern Ostrobothnia (all from the Karelian population) since late 2015 (Detailed description in Supplement 1). We obtained an additional 43 tissue samples from wolverine individuals found dead or legally culled (during the 2017–2018 winter season), independent of our research. Most of the tissue samples were from 1999 onwards, except for three museum samples (1983 $N = 01$; 1991 $N = 02$). Additional museum samples ($N = 05$) consisted of blood on

FTA cards (GE Healthcare, UK) (2006–2007) and a tooth (1995).

Microsatellites, PCR amplification and quality control

Details covering the laboratory protocols of DNA extraction and microsatellite genotyping are provided in Supplement 1. Briefly, 14 microsatellite markers were grouped in 2 multiplex sets for amplification (7 in each; Table S1). In addition, two mustelid-specific Y-chromosome-linked loci were included to the first multiplex group for sex determination (Hedmark et al. 2004). Non-invasive samples were replicated at least three times and tissue samples twice to check for consistency between scores. A single locus was scored as a heterozygote, when a clear heterozygous profile among replicates could be identified while other replicates showed up as homozygotes (i.e. allelic dropout). If scoring of a single locus varied inconsistently (i.e. shifting), it was marked as missing.

To verify the uniqueness of each genotype, an identity analysis implemented in the program CERVUS v.3.0.7 (Kalinowski et al. 2007) was used. Genotypes were considered to belong to the same individual if at least 12 out of 14 loci were matching (i.e. 2 mismatches between genotypes were allowed to account for possible genotyping errors) and were of the same sex. Probability of identity for unrelated individuals (PI) and probability of identity for siblings (PI_{sibs}) among all individuals, estimated with CERVUS, were 3.69×10^{-9} and 1.45×10^{-4} (Waits et al. 2001), respectively. To check for genotyping error, program MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al. 2004) was used with a Bonferroni-adjusted 95% confidence interval and 1000 iterations. Individuals from the predefined Karelian and Scandinavian populations (Boitani et al. 2015), hereafter called *East* and *North*, were analysed both separately and by grouping them together. Additionally, rates of allelic dropout and false alleles across PCRs were estimated following Broquet and Petit (2004).

Mitochondrial DNA

A 579 bp fragment of the mitochondrial DNA (mtDNA) control region was amplified from 129 individuals using primers GuloF (Schwartz et al. 2007) and GuloR (5′-CACCTT ATGGTTGTGCGATG-3′; this study). Successfully amplified PCR products were purified using the EXOI/FastAP-method (Thermo Scientific, Lithuania) and sequenced using BigDye Terminator v3.1 (Applied Biosystems, Foster City, California, USA). The haplotype sequences are available in GenBank with Accession Numbers MN854422-MN854423. More details covering the mtDNA sequencing are provided in Supplement 1.

Population genetic analyses

GENEPOP v.4.2 (Rousset 2008) was used to test for deviations from Hardy–Weinberg equilibrium across all loci in both predefined populations and to detect potential linkage disequilibrium between pairs of loci within populations. It is well-known that variation within populations can be explained by isolation-by-distance (IBD; Wright 1943), which was tested in SPAGEDI v. 1.5 (Hardy and Vekemans 2002), correlating spatial distances and pairwise relatedness among all individuals. We used the kinship coefficient estimator described in Loiselle et al. (1995), which is suitable in cases when low frequency alleles are present in the data (Hardy and Vekemans 2002). A distance interval was set using the equal frequency method to 10 spatial distance classes. The kinship coefficient for each distance class and the overall regression slope were run with 10,000 permutations. Jackknifing over loci was applied to estimate standard errors to multilocus average estimates. Additionally, IBD was tested with a Mantel test (Mantel 1967; Diniz-Filho et al. 2013) on genetic and geographical distances of all individuals using GENALEX 6.5 (Peakall and Smouse 2012).

The population structure of Finnish wolverines was examined with the Bayesian clustering method implemented in STRUCTURE v.2.3.4 (Pritchard et al. 2000). The program estimates the likelihood for a given number of genetic clusters (K) in the data and assigns the individuals to the defined clusters. A number of K from 1 to 8 (10 iterations each) was tested under the admixture model with correlated allele frequencies (Falush et al. 2003) and run settings varying from 100,000 to 250,000 as burn-in and 250,000 to 500,000 Markov Chain Monte Carlo (MCMC) replication steps. Alpha, the parameter that implies admixture, was inferred from the data, initially set to 1.0. No prior information was used about sampling locations. Optimal K was estimated using the ΔK method (Evanno et al. 2005) and by plotting the likelihood of K for each value of K (Ln Pr[X|K]) (Pritchard et al. 2000) using the POPHELPER web app (Francis 2017). Additionally, we used the median of medians, median of means, maximum of medians, and maximum of means implemented in STRUCTURESELECTOR (Li and Liu 2018) to account for uneven sampling (Puechmaille 2016). Assignment results were visualized with CLUMPAK (Kopelman et al. 2015). Individuals were assigned to a cluster when the membership coefficient (hereafter q) > 0.8. Subsequent rounds of analysis followed identical settings but using a location prior based on the predefined regions (*East* and *North*), a hierarchical STRUCTURE analysis (Vähä et al. 2007) or sex-specific population structure. In the hierarchical analysis, individuals assigned to a cluster with $q < 0.5$ were discarded after each round of the analysis. Further, to infer if genetic structure could be explained by an underlying family structure, a maximum likelihood model in COLONY (Jones and Wang 2010) was used.

Genetic population structure was additionally examined with GENEPLOT (McMillan and Fewster 2017). GENEPLOT applies the saddlepoint method (McMillan and Fewster 2017), which allows plotting genetic structure with quantile lines for each population. The analysis, using the a priori information of the predefined regions (*East* and *North*), was applied with the "leave-one-out" method (McMillan and Fewster 2017) and the prior as defined Rannala and Mountain (1997). Individuals were subsequently classified based on the GENEPLOT quantiles as *East*, *North*, Admixed or Outgroup.

An alternative approach to Bayesian clustering methods in the form of a discriminant analysis of principal components (DAPC) was used to assess the presence of major patterns in the multivariate data implemented in the package ADEGENET 2.1.1. (Jombart 2008; Jombart and Ahmed 2011) in R (R Core Team 2017). All principal components (PCs, 40) were used to determine the number of clusters maximizing the variation between clusters using the predefined populations. The *find.clusters* function was used to select the optimal number of clusters based on BIC scores with 10^6 iterations. We used cross validation to calculate the number of retained PCs (20) with the *xvalDapc* function.

Individuals were spatially projected with QGIS 2.18.16 (QGIS Development Team 2018). Current core population areas (CCPA; e.g. Tammeleht et al. 2010; Silva et al. 2018) were produced by computing minimum convex hulls around individuals with high membership values ($q > 0.8$) based on most likely population clustering while excluding migrants. If CCPAs were overlapping, the presence of males with very high membership values ($q > 0.95$) or females with high membership values ($q > 0.8$) was assumed as sufficient to include the area. Eight individuals were included twice (six in CCPA *East*; two in CCPA *North*). If CCPAs were bordering at a location where individuals were assigned to different clusters, males with very high membership values ($q > 0.95$) or females with high membership values ($q > 0.8$) were grouped into a cluster of assignment. A total of three individuals did not assign to any CCPA and were discarded from the following genetic analysis.

For every CCPA, we estimated the mean membership values and the proportion of assigned individuals versus admixed individuals for $K = 2$, the family group structure and signs of recent migration. Every individual that was assigned by STRUCTURE, GENEPLOT or GENECLASS 2 (Piry et al. 2004) to another CCPA than the CCPA where the individual was originally found in, except where CCPAs overlapped, was considered as a recent migrant. GENECLASS 2 was used with the partially Bayesian assignment criteria of Rannala and Mountain (1997) and the Monte-Carlo resampling algorithm of Paetkau et al. (2004) (10,000 replicates;

significance level at $P \leq 0.01$) to assess the probability of being a first-generation migrant. These three programs were used to account for the different assumptions used in each of them (Berry et al. 2004). The distance of possible migrants to the cluster of assignment was measured (km, Euclidean distance to border) to reveal dispersal distances from their cluster of assignment.

We tested if stepwise mutations contributed to genetic differentiation by using 10,000 allele size permutations in SPAGEDI. As the allele size permutation test showed no significant contribution of stepwise mutations ($P = 0.82$) to the genetic structure, population differentiation was measured by allelic identity (F_{ST}). Differentiation between CCPAs was measured for microsatellites by F_{ST} with a locus-by-locus analysis of molecular variance (AMOVA). F_{ST} values were analysed for significance with 10,000 permutations in ARLEQUIN v.3.5.2.2 (Excoffier and Lischer 2010).

Genetic diversity was measured for each CCPA in ARLEQUIN by estimating the number of alleles (A), the observed (H_O) and expected heterozygosities (H_E) for all loci. Genetic diversity estimates were compared between the CCPAs using FSTAT v2.9.4. (Goudet 2003) with 10,000 permutations. Inbreeding coefficients (F_{IS}) were estimated using allele identity in GENEPOP. Population-specific F_{IS} indices were tested for significance using 10,000 permutations in ARLEQUIN. Allelic richness (A_R) and private allelic richness (A_P) were estimated for all loci using HP-RARE v. 6-2006 (Kalinowski 2005) with an adjusted sample size according to lowest number of genes. Private alleles were identified using GENALEX.

To disentangle the effect of population admixture and IBD on population structure, we divided the CCPAs by equal distance (150 km) from the supposed contact zone (Fig. S2). A similar analysis of genetic diversity as before was applied on individuals in each buffer, including the number of linked markers. CCPA *North* was divided in three buffer zones, whereas CCPA *East* was divided in four zones. CCPA *West* was excluded from this analysis.

Contemporary effective population size (N_e) was estimated for each CCPA using the linkage equilibrium (Hill 1981; Waples 2006; Waples and Do 2010), molecular coancestry (Nomura 2008) and temporal methods (Nei and Tajima 1981; Pollak 1983; Jorde and Ryman 2007) in NeESTIMATOR v.2.1 (Do et al. 2014). These several independent methods were used, as N_e estimates seem to be sensitive to assumptions of different models (Wang et al. 2016). Notice that estimates given by these methods reflect actually the effective number of breeders N_{eb}, which do not always accurately resemble the N_e (Nomura 2008). The N_e estimate represents the number of breeders in the entire population. Rare alleles, with a frequency less than 2%, were excluded ($P_{Crit} = 0.02$; see Waples and Do 2010), except for the molecular coancestry method, where rare

alleles should not bias the results (Do et al. 2014). For the linkage disequilibrium model, the mean from the estimates of monogamous and random mating was calculated. This was done because wolverines in Fennoscandia exhibit a polygamous mating structure, where male territories overlap several female territories (Hedmark et al. 2007), and thus neither of the mating systems implemented in NeESTIMATOR fits perfectly. For the temporal model, each CCPA was divided into temporal clusters: the CCPA *North-West* in two clusters [2007–2012 ($N = 47$), 2013–2018 ($N = 63$)] and the CCPA *East* in three clusters [2001–2006 ($N = 19$), 2007–2012 ($N = 23$), 2013–2018 ($N = 26$)]. Generation time was approximated as 6 years (Rauset et al. 2015). To equalize the sample size for CCPA *East*, six individuals were randomly chosen to represent the years 2016–2017. Temporal methods, which were chosen because most of the samples were non-invasively collected (Do et al. 2014), were used with a conservative starting census size N_c of 800 individuals for CCPA *North-West* and 1500 individuals for CCPA *East*, assuming connectedness towards the entire population of wolverines in the Russian part of Europe (Landa et al. 2000; Boitani et al. 2015).

We tested for recent genetic bottlenecks for the whole population and for each CCPA separately using the heterozygosity excess method implemented in BOTTLENECK (Piry et al. 1999). We applied the two-phase model (TPM; Di Rienzo et al. 1994) with variance in TPM = 30 and proportion of stepwise mutations in TPM = 70% and used 10,000 iterations. In addition, we calculated the ratios of numbers of microsatellite alleles to their size range (i.e. Garza–Williamson indices 2001) implemented in ARLEQUIN.

Mitochondrial sequences were manually edited and aligned with available GenBank wolverine mtDNA sequences (Wilson et al. 2000; Walker et al. 2001; Tomasik and Cook 2005; Arnason et al. 2007; Schwartz et al. 2007; Frances 2008; Ekblom et al. 2014; Zigouris et al. 2013; Malyarchuk et al. 2015) using the ClustalW algorithm (Thompson et al. 1994) in MEGA 7 (Kumar et al. 2016). Haplotype ($\hat{h}$) and nucleotide (π) diversities were calculated with DnaSP 5 (Librado and Rozas 2009). A median-joining haplotype network was drawn using NETWORK v.4.6.1.6 (Bandelt et al. 1999) with a shorter sequence of 317 bp ($N = 1033$) to allow most of the previously published data to be compared with our data.

Results

Out of a total of 1281 putative wolverine samples, 56.5% ($N = 724$) contained sufficient amounts of DNA for PCR amplification. We successfully genotyped 49.7% ($N = 465$) of the scat and 70.9% ($N = 210$) of the hair samples. A total of 247 wolverine individuals were identified from the

predefined populations with 118 individuals from *North* and 129 from *East* (Fig. 1). The number of males and females in *North* was 51 and 67 and in *East* it was 71 and 53 (5 unknown), respectively. The longest time difference between recaptures was 8 years.

Evidence of null alleles was detected in five loci (Gg454, Gg234, Gg14, Gg465 and Tt4) and stuttering in three loci (Gg234, Gg14 and Tt4). However, these results were not consistent, when predefined populations were analysed separately (Table S2), which suggested the excess of homozygotes did not result from genotyping artefacts. Therefore, all loci were included in further analyses. The overall replicate error was small, 4.1% across all loci, largely caused by allelic dropout (60.8%). We found a non-random allele association between one pair of microsatellite loci in both populations separately using a sequential Bonferroni correction: Gg454-Mvis057 (P < 0.001). The non-random association was likely caused by chance due to small population size and/or recent admixture and therefore both loci were retained. A significant pattern of IBD was found among Finnish wolverines ($b_{loiselle} = -0.043$, $r^2 = 0.137$, $P < 0.001$, Fig. S3; Mantel test: $r^2 = 0.0673$, $P < 0.001$).

Genetic structure

Bayesian clustering assignment in STRUCTURE without prior information of sampling sites suggested two genetic clusters in the Finnish wolverine population based on ΔK, but the posterior likelihood values were increasing until five clusters (Fig. S4). The four estimators suggested by Puechmaille (2016) supported two to four clusters (Fig. S5). Wolverines were divided geographically from eastern to northern Finland, except for the individuals from western Finland (main translocation site), which were assigned together with the northern individuals (Fig. 3a, b). The proportion of individuals assigned to clusters ($q > 0.8$) was high for $K = 2$ (88.7%) but less for $K = 3$ (74.9%), $K = 4$ (63.2%) and $K = 5$ (64.8%). A temporal division of the optimal clusters did not reveal major cluster changes through time (Fig. S6). The addition of location information and performing a hierarchical analysis accentuated the same population structure with minimal differences from the general analysis (Figs. S7, S8). Also, the male and female population structure analysis showed a clear split of the population from east to north (Figs. S9, S10).
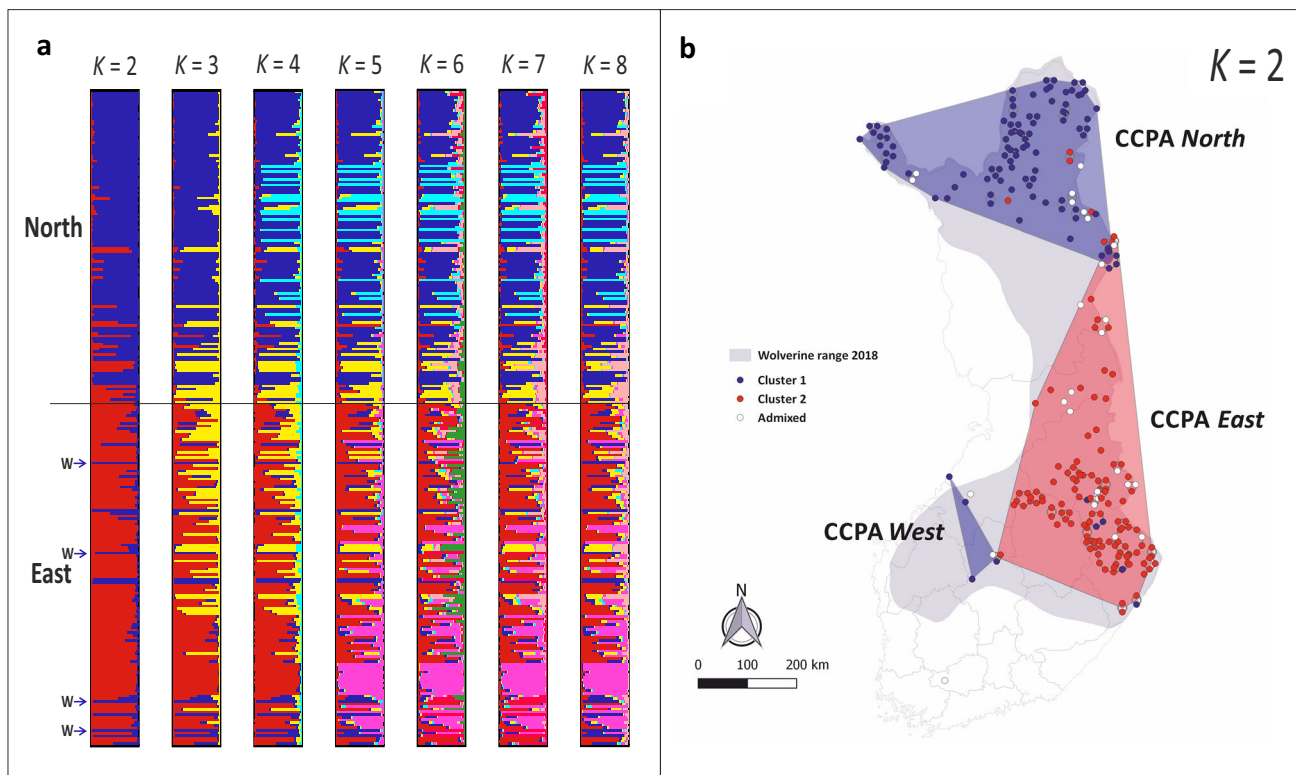
Fig. 3 Wolverine cluster assignment based on 14 microsatellites with STRUCTURE. **a** STRUCTURE assignment plots for runs until $K = 8$ sorted by predefined populations *North* and *East*. Samples assigned to CCPA *West* are marked with a blue arrow and the letter *W*. **b** Spatial distribution of STRUCTURE results for $K = 2$. Colours on the map correspond to individuals with membership value $q > 0.8$. Admixed individuals with $q < 0.8$ are depicted in white. Minimum convex hulls represent current core population areas (CCPAs)

GENEPLOT showed a similar geographic division of population clusters as the general analysis in STRUCTURE, including the assignment of individuals from western Finland to the northern cluster (Fig. S11). Besides, a large proportion of the individuals were included to both populations. A few individuals, mainly from the most northern areas of the predefined *East* population, were not grouped to any cluster ($N = 10$). Interestingly, these outgroup individuals all belong to the third cluster recognized by STRUCTURE at $K = 3$ in the general analysis.

The values of Bayesian Information Criterion (BIC) in DAPC showed a decrease until reaching minimum values between 8 and 12 clusters, though showing a geographic separation from east to north on the first principal component axis (Fig. S12). The geographic clusters were, however, partially overlapping.

Analysis of family groups in COLONY revealed 25 groups of wolverines in Finland. Mean size of one family group was 6.56 ($SD \pm 5.84$; *range* 1–28). Two family groups consisted exactly of those individuals that were assigned by STRUCTURE to the two fine-scale clusters at $K = 5$. Increasing K values after reaching $K = 3$ was thus likely explained by family structure in the data.

Based on the above results, we divided the wolverines in three CCPAs: *East* ($N = 138$), *North* ($N = 110$) and *West* ($N = 04$) (Fig. 3). Although the sample size of CCPA *West* was small, we considered it as a separate cluster due to the geographical disconnectivity with CCPA *North* and the presence of females. CCPA *East* stretched along the eastern border of Finland around the core regions of North Karelia,

Northern Savonia, Kainuu and Northern Ostrobothnia into south-eastern Lapland. The CCPA *North* extended over northern Finnish Lapland. The CCPA *West* included regions of Central Finland, Central Ostrobothnia and southern parts of Northern Ostrobothnia.

The mean membership value for CCPA *East* was $q = 0.84$. Within CCPA *East* 81.2% of the individuals were assigned to the eastern cluster, 7.2% to the northern cluster and 11.6% were admixed. CCPA *North* had a mean membership value of $q = 0.88$ with 85.4% of the assignments to the northern cluster, 5.5% to the eastern cluster and 9.1% of admixed individuals. The mean membership value for CCPA *West* was $q = 0.96$ with all individuals assigned to the northern cluster.

When family groups were compared between CCPAs, a clear distinction was found in their composition, which was reflected also when sexes were analysed separately (Fig. S13). In CCPA *East*, half of the individuals belonged to 4 family groups (*total* = 21 family groups; *average per group* = 7 individuals). In CCPA *North*, nearly three-quarters of the individuals belonged to only 6 family groups (*total* = 16 family groups; *average per group* = 7 individuals). In CCPA *West*, all four individuals belonged to a single-family group.

Four recent migrants were found in CCPA *North* with strong assignments to CCPA *East* (STRUCTURE $K = 2$; Table 1). Comparably, using GENEPLOT one of these individuals was assigned as a recent migrant and GENECLASS 2 assigned two of these individuals as potential first generation migrants. Mean distance of all migrants from the border of CCPA *East* was 127 km. Five recent migrants were

Table 1 Recent migrants detected with STRUCTURE ($q > 0.8$ marked in bold), GENEPLOT and GENECLASS 2 in CCPA *North* and *East*, except where CCPAs overlapped

Individual	Location	Distance to border (km)	Sex	Year	q (*North-West*)	q (*East*)	GENEPLOT	GENECLASS
North								
Gg060	Inari	144	M	2011	0.1981	**0.8019**	Admixed	
Gg061	Inari	144	M	2011	0.1353	**0.8647**	Admixed	
Gg089	Sodankylä	165	F	2009	0.0252	**0.9748**	Admixed	F_0 Migrant
Gg093	Savukoski	55	F	2014	0.1545	**0.8455**	East	F_0 Migrant
East								
Gg113	Salla	76/362	M	1999	0.6929	0.3071	North	
Gg134	Kuhmo	335/267	F	2017	0.7805	0.2195	North	
Gg159	Sotkamo	382/201	F	2004	**0.9890**	0.0110	North	F_0 Migrant
Gg185	Nurmes	424/203	F	2007	**0.9657**	0.0343	Admixed	
Gg186	Nurmes	424/203	M	2007	**0.9745**	0.0255	Admixed	F_0 Migrant
Gg236	Joensuu	510/233	M	2017	**0.9566**	0.0434	Admixed	
Gg244	Tohmajärvi	579/272	M	2017	**0.9565**	0.0435	Admixed	

Euclidean distance to border is a distance of northern individuals to the border of CCPA *East*, and for eastern individuals to the border of CCPA *North* and CCPA *West*, respectively. The q values are from STRUCTURE $K = 2$. For the GENEPLOT assignments, see "Material and methods". The individuals detected with GENECLASS 2 are potential first generation migrants

found in CCPA *East* with strong assignments to CCPA *North* (STRUCTURE $K = 2$; Table 1). Using GENEPLOT, three migrants were assigned, one of these was the same as with STRUCTURE. GENECLASS 2 detected two potential first generation migrants in CCPA *East*, both were detected with STRUCTURE as well. The mean distance of migrants from the border of CCPA *North* was 390 km and from the border of CCPA *West* 249 km.

The AMOVA analysis (CCPA *West* combined with *North*) showed that 9.97% of the variation was explained by variance among CCPAs ($F_{ST} = 0.100$, 95% CI = 0.046–0.160, P < 0.001), which was comparable but not as strong as the pairwise F_{ST} of clusters from STRUCTURE at $K = 2$ ($F_{ST} = 0.152$, 95% CI = 0.075–0.239, P < 0.001).

Genetic diversity

Estimates of genetic diversity for the CCPAs are summarized in Tables 2 and S3. The mean expected heterozygosity (H_E) was higher in CCPA *East* than in CCPA *North-West*. Besides, CCPA *East* had a higher mean allelic richness (A_R) and mean private allelic richness (A_P) than CCPA *North-West*. Nevertheless, none of the differences in estimates of genetic diversity between the clusters were significant (P > 0.345). Also, the mean inbreeding coefficients were low and not significant at the population level (P > 0.387). There was no difference in genetic diversity within the CCPAs and within the clusters from STRUCTURE ($K = 2$; Table S4). Four private alleles were exclusively found in CCPA *East*, though with low frequencies (< 0.011). Yet, private alleles were abundant considering the STRUCTURE based clusters at $K = 2$. Five private alleles were found in cluster 1 (i.e. northern; *mean allele frequency* = 0.068), whereas 10 were unique to cluster 2 (i.e. eastern; *mean allele frequency* = 0.141). There were two private alleles characteristic (*mean allele frequency* > 0.1) for cluster 1 (Mvis075: allele 142; Gg465: allele 183) and three (*mean allele frequency* > 0.3) for cluster 2 (Gg443: allele 90; Gg454: allele 135; Gg234: allele 96). No private alleles were found in CCPA *West* when tested apart from CCPA *North*. Increased

allelic richness was observed in both CCPAs, closer to the contact zone, where the assignment probability was lower (Table 3). However, no clinal patterns were detected for H_E, F_{IS} or for the number of linked markers.

Effective population size

Very low and similar estimates of current N_e were found for both CCPA *East* and CCPA *North-West* using several methods (Table 4). The molecular coancestry method resulted in lower N_e estimates [*North-West*: $N_e = 8.9$ (95% CI 3.7–16.2); *East*: $N_e = 14.0$ (95% CI 7.2–23.0)] than the linkage disequilibrium method [*North-West*: $N_e = 34.9$ (95% CI 25.4–48.1); *East*: $N_e = 36.1$ (95% CI 29.1–44.6)]. Imprecise N_e estimates (i.e. large confidence intervals) were produced for CCPA *East* using several temporal methods.

A recent bottleneck was detected in Finnish wolverines, as the Wilcoxon's sign-rank test showed a significant excess of heterozygotes (P < 0.001). Furthermore, when tested separately on both CCPAs, CCPA *East* had a significant excess of heterozygotes (P < 0.001) but not CCPA *North-West* (P = 0.059). Additionally, Garza–Williamson modified indices suggested a bottleneck for both CCPAs [$M_{North-West} = 0.49$ (± 0.12), $M_{East} = 0.52$ (± 0.12)].

mtDNA

The analysis of mtDNA sequences demonstrated the presence of two haplotypes in Finland (Fig. 4). A common haplotype was found in 80.5% of individuals in Finland (in CCPA *North*, 90.3% and in CCPA *East* 80.0%) (Fig. S14). A rarer haplotype (19.5%), differentiating by five substitutions on 579 bp, was more prevalent in CCPA *East* (20.0%) than in CCPA *North* (9.7%). In CCPA *North*, the occurrence of the rare haplotype was restricted to the southernmost parts. All sequenced individuals from CCPA *West* ($N = 03$) carried the rare haplotype. The common haplotype was identical to haplotype 15 (Zigouris et al. 2013), which was found in wolverine populations in Sweden (Arnason et al. 2007; Ekblom et al. 2014), Far East Russia (Tomasik and Cook 2005) and North America (Zigouris et al. 2013). The rare haplotype

Table 2 Estimated genetic diversity for both CCPAs given by number of alleles (A), allelic richness (A_R), private allelic richness (A_P), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficients (F_{IS})

	North-West (n = 114)						East (n = 138)					
	A	A_R	A_P	H_E	H_O	F_{IS}	A	A_R	A_P	H_E	H_O	F_{IS}
Mean	3.8	3.7	0.06	0.489	0.470	0.01	4.07	4.0	0.28	0.568	0.560	− 0.02
SD	1.4	1.4	0.12	0.178	0.158		1.54	1.5	0.40	0.104	0.113	

A_R and A_P were set according to the lowest number of 184 genes

Table 3 Mean estimated genetic diversity given by number of alleles (A), allelic richness (A_R), private allelic richness (A_P), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficients (F_{IS}), the number of linked markers (LD) and the assignment probability (q) for both CCPAs divided by buffers with increasing distance from the contact zone. Each buffer has a width of 150 km (Fig. S2). A_R and A_P were set according to the lowest number of genes. Significant F_{IS}-values are marked with asterisk

|	North									East								
	N	A	A_R	A_P	H_E	H_O	F_{IS}	LD	q	N	A	A_R	A_P	H_E	H_O	F_{IS}	LD	q
Buffer 1																		
Mean	22	3.5	3.1	0.03	0.527	0.548	−0.0394	0/91	0.80	11	3.4	3.2	0.04	0.552	0.533	0.0383	0/91	0.78
SD		1.1			0.178	0.188			0.25		1.2			0.177	0.225			0.24
Buffer 2																		
Mean	69	3.4	2.8	0.06	0.463	0.451	0.0277	3/91	0.92	12	3.2	3.1	0.05	0.544	0.477	0.1236*	0/91	0.86
SD		1.3			0.189	0.177			0.18		1.1			0.164	0.219			0.15
Buffer 3																		
Mean	16	2.8	2.6	0.02	0.521	0.497	0.0478	0/91	0.94	62	3.6	3.0	0.05	0.548	0.570	−0.0417	3/91	0.87
SD		0.9			0.143	0.174			0.08		1.4			0.127	0.145			0.24
Buffer 4																		
Mean										48	3.1	2.7	0.04	0.556	0.573	−0.0350	2/91	0.90
SD											0.9			0.071	0.128			0.19

*P ≤ 0.05

Table 4 Effective population size estimates for CCPA clusters using different methods in NeESTIMATOR (95% confidence intervals jackknifed on loci within brackets)

	Linkage disequilibrium	Molecular coancestry	Temporal			
			Pollak	Nei/Tajima	Jorde/Ryman	
North-West ($N=114$)	34.9 (25.4–48.1)	8.9 (3.7–16.2)				
			22.4 (12.9–46.6)	20.5 (11.8–41.8)	14.2 (9.2–29.9)	2007–2012 vs. 2013–2018
East ($N=138$)	36.1 (29.1–44.6)	14.0 (7.2–23.0)				
			59.5 (11.2–∞)	NA (15.1–∞)	NA (19.9–∞)	2001–2006 vs. 2007–2012
			46.8 (19.1–489.3)	60.2 (20.9–∞)	79.2 (21.8–∞)	2001–2006 vs. 2013–2018
			30.8 (11.3–∞)	35.4 (12.9–∞)	36.4 (14.1–∞)	2007–2012 vs. 2013–2018

Considering the temporal analysis, the CCPA *North-West* had two temporal clusters: 2007–2012 ($N = 47$) and 2013–2018 ($N = 63$); while the CCPA *East* had three temporal clusters: 2001–2006 ($N = 19$), 2007–2012 ($N = 23$) and 2013–2018 ($N = 26$). For two estimates we got unrealistically large or negative values likely due to the method not being accurate for our data, which were marked as "NA" in the table. For more details, see "Material and methods"

has not been found in previous studies. The mean nucleotide diversity (π) of wolverines in Finland was 0.00272 and the haplotype diversity ($\hat{h}$) was 0.315. The nucleotide and haplotype diversities were higher in CCPA East ($\pi=0.00279$, $\hat{h} = 0.323$) than in CCPA North ($\pi=0.00156$, $\hat{h} = 0.181$).
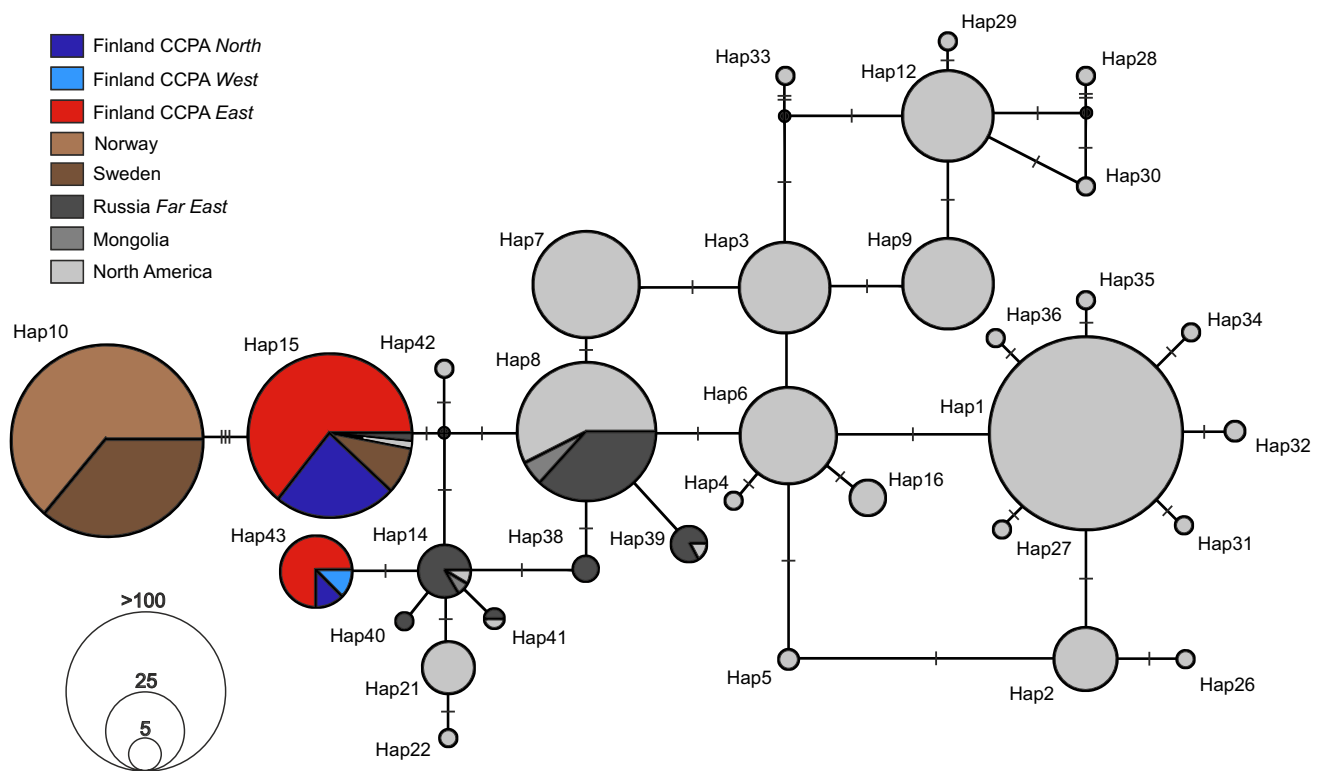
Fig. 4 Median-joining haplotype network reconstructed from a short fragment (317 bp) of the mtDNA control region for wolverines (*N* = 1033) using Network. Node sizes are proportional to haplotype frequencies. The proportion of sampling localities is shown within each node. Each black perpendicular line represents a point mutation. Median vectors are represented with black nodes. The haplotype numbers are named after Zigouris et al. (2013), except for haplotype 43 (new from this study). All Finnish sequences (*N* = 129) are from this study. Some earlier recognized haplotypes merged with other haplotypes due to using a short fragment [as in Zigouris et al. 2013: Hap11 merges with Hap1, Hap13 with Hap6, Hap23 (North America) with Hap14 (Russia and Mongolia)]. Note that haplotype 10 is likely the same as haplotype 15 (see "Discussion"). The other sequences were obtained via GenBank from: Wilson et al. (2000), Tomasik and Cook (2005), Arnason et al. (2007), Schwartz et al. (2007), Frances (2008), Ekblom et al. (2014), Zigouris et al. (2013) and Malyarchuk et al. (2015)

Discussion

Genetic structure

In this study, we assessed putatively neutral genetic variation (microsatellites and mtDNA) in a population of wolverines that is recovering from a recent population bottleneck. Based on microsatellites, we identified two major genetic clusters of wolverines in Finland, a northern (CCPA *North-West*) and eastern cluster (CCPA *East*). Both Bayesian and non-Bayesian clustering methods supported the same two genetic clusters, however, varying in the strength of subdivision. The spatial scale of the clusters is consistent with the genetic structure of other wolverine populations that experienced range contractions in Scandinavia (Walker et al. 2001; Flagstad et al. 2004; Ekblom et al. 2018), at the peripheral part of the wolverine distribution in eastern Canada (Zigouris et al. 2012) and in the north-western US (Kyle and Strobeck 2001, 2002; Cegelski et al. 2006). Interestingly, similar clusters with contact zones in northern Finland have been found in

other species co-occurring in the area with, at present, a continuous distribution (Carlsson et al. 2004; Kopatz et al. 2014; Honnen et al. 2015; Kangas et al. 2015),

We found a significant pattern of IBD across the whole study area. A strong pattern of IBD can cause an overestimation of clusters, leading to incorrect interpretation of population structure (Frantz et al. 2009). If the appearance of major population clusters could be explained by IBD, we would expect that the population structure would be consistent with a clinal change, however, without affecting the spatial genetic diversity. Contrastingly, when two divergent clusters meet each other, the population at the contact zone is expected to be more admixed and have a higher genetic diversity and inbreeding coefficient (Wahlund's effect 1928), as well as increased linkage disequilibrium (Slatkin 2008), compared to sites further from the contact zone. We observed stronger admixture and higher allelic richness towards the contact zone, but no increase in expected heterozygosity, the inbreeding coefficient and the number of linked markers. Especially in the CCPA *East*, the

mixed genetic ancestry due to former translocations might have diluted the expected signs of admixture. Based on these results, we suggest that IBD plays an important role in shaping the contemporary genetic population structure of wolverines, consistent with previous studies in other wolverine populations (e.g. Kyle and Strobeck 2002; Rico et al. 2015; Ekblom et al. 2018). To rule out that the two clusters are an artefact of discontinuous sampling in a population with IBD (Tucker et al. 2014), sampling has to be extended to Russian Karelia.

We found that CCPA *East* and CCPA *North-West* are connected but significantly differentiated ($F_{ST} = 0.100$). The genetic differentiation within Finland is stronger than what was found previously for wolverines between northern Scandinavia and southern Norway ($F_{ST} = 0.045$–0.088), also based on microsatellites (Walker et al. 2001; Flagstad et al. 2004). When compared to North American wolverine populations (Kyle and Strobeck 2002; Cegelski et al. 2006; Zigouris et al. 2012), an F_{ST} estimate of the same magnitude as between the Finnish clusters seems to be typical among peripheral populations. A microsatellite study on coexisting brown bears (Kopatz et al. 2014) found a similar division of genetic clusters between north and southeast Finland. Nevertheless, low population differentiation was detected ($F_{ST} = 0.025$) due to strong recovery after a bottleneck, fading out temporary population subdivision (Kopatz et al. 2014; Hagen et al. 2015). Indeed, wolverine numbers in Finland have increased during the last decades, however, not as much as the number of bears (Hagen et al. 2015). The slower recovery of wolverines compared to bears can be ascribed to the intensity of the bottleneck. Whereas the minimum of bears was estimated to be 150 individuals in 1970 (Pulliainen 1990), wolverines were at the same time nearly extirpated from Finland (Pulliainen 1982). Therefore, clusters detected in this study might very well represent the subdivision of the wolverine population during the bottleneck. There are no evident geographical barriers limiting movements of individuals between the clusters. The reindeer husbandry area of Finland (Fig. 1) does not seem to constitute a barrier between the clusters either, as the supposed contact zone is situated 300 km north from the southernmost reindeer husbandry areas. Finally, genetic clusters based on microsatellites do not necessary reflect that wolverines are locally adapted (Rico et al. 2015).

We found genetic evidence of the translocation history in Finnish wolverines. The high genetic similarity between northern Finnish Lapland (CCPA *North*) and western Finland (CCPA *West*) is likely due to the translocations that took place in the end of the twentieth century (Fig. 2). At that time, there were no wolverines in western Finland. This type of high similarity between the source and the translocated populations have previously been described also in other cases of reintroductions to areas where the species

had completely disappeared (Wisely et al. 2003; Mowry et al. 2015). Our results suggest that the translocated wolverines have survived and reproduced for decades in western Finland. Indeed, translocations have the ability to alter the genetic make-up of populations and the genetic signature of translocations can remain detectable for a long time (Puckett et al. 2014; Grauer et al. 2017; Hapeman et al. 2017). However, we did not detect signs of expansion from the western cluster towards the east but rather an expansion of the natural eastern cluster to the west. Although, we did not observe any temporal change of clusters through time, we assume that admixture will increase considering the dispersal capacity of wolverines (Gardner et al. 1986; Vangen et al. 2001; Flagstad et al. 2004; Inman et al. 2012) and the recent population growth (Frosch et al. 2014; Hagen et al. 2015; Pigneur et al. 2019).

In our study, population structure based on microsatellites and mtDNA did not match perfectly. We found two mtDNA haplotypes within Finland. The common haplotype is the same as the only haplotype found in Scandinavia (haplotype 15) by Arnason et al. (2007) and Ekblom et al. (2014), and likely the same as the haplotype described by Walker et al. (2001) (Fig. 4; Zigouris et al. 2013) for Scandinavia (haplotype 10). This haplotype 10 was found in 169 individuals by SSCP, but sequenced only from 2 (Walker et al. 2001). These sequences differ from haplotype 15 by 3 substitutions, but most likely represent the same haplotype because none of the previous studies has detected more than 1 haplotype from Scandinavia. The rarer Finnish haplotype (hereafter haplotype 43) has not been detected previously in wolverines and is the closest to a haplotype found previously in Far East Russia and Mongolia (haplotype 14). Haplotype 43 was more abundant in CCPA *East* (20.0%) than in CCPA *North* (9.7%). The low occurrence ($N=03$) of haplotype 43 in CCPA *North* could be explained by for example a single female dispersal event from the east. Similarly, the occurrence of haplotype 43 in all samples of CCPA *West* ($N=03$), could be explained by the coincidence that some of the translocated females (2 out of 6 with an eastern origin) happened to carry this haplotype. Alternatively, it could be due to secondary contact after the recovery of CCPA *East* or a combination of these scenarios. This remains speculative, however, due to the low number of samples from western Finland.

In contrast to the microsatellite analyses, mtDNA haplotypes were not clearly spatially distributed within Finland. Haplotype 43 was, nevertheless, mostly present throughout eastern and western Finland but with no clear clustering pattern. In studies on the genetic structure of wolverines in North America, differences between these markers were linked to female philopatry, male-biased dispersal, long term population fragmentation and current variation in gene flow between regions (Chappell et al. 2004; Tomasik and Cook 2005; Cegelski et al. 2006; Schwartz et al. 2007; Zigouris

et al. 2012). Nuclear markers showed no population structure in large continuous wolverine populations, whereas mtDNA did (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004). Importantly though, nuclear marker-based population structure is becoming more apparent towards the periphery of wolverine's distribution (Kyle and Strobeck 2001, 2002; Cegelski et al. 2003, 2006; Zigouris et al. 2012). Our results on microsatellites fit well with the common assumption that peripheral wolverine populations exhibit significant population structure and points towards limited gene flow throughout Finland.

The fact that only two distinct mtDNA haplotypes are found in Fennoscandia so far could imply that Fennoscandian wolverine populations have lost haplotypes due to a recent bottleneck or a post-glacial founder effect. Walker et al. (2001) suggested that the cause of a single mtDNA haplotype in Scandinavia lies in a post-glacial founder effect, as the authors did not find any other haplotypes in their 10 pre-bottleneck (1922–1968) samples. The existence of haplotype 43 in Finland, especially in CCPA *East*, could have arisen when the species colonized Finland via a north-eastern route (Jaarola et al. 1999). On the other hand, European archaeological material from the Late Glacial Period shows that wolverines were roaming far south of their current range (Sommer and Benecke 2004), suggesting that after the LGM a bidirectional re-colonization of Fennoscandia is possible. Colonization via both routes were described for brown bears using ancient mtDNA sequences (Bray et al. 2013) as well as for other boreal mammals in Fennoscandia (Ruiz-González et al. 2013; Kangas et al. 2015; Wallén et al. 2018). The widespread haplotype 15 in Fennoscandia, although found in Far East Russia and Mongolia, could have a European origin, whereas haplotype 43 could have an eastern origin. Alternatively, the severe recent bottleneck might have impacted the genetic make-up of the Fennoscandian wolverine population by removing other haplotypes (Nei et al. 1975). Haplotype 43 might then relate to a relict population, which was more common in Finland before the bottleneck. It was present already in our oldest (1983–1991) samples of CCPA *East* ($N = 02$), but a wider temporal and geographical sampling scheme is needed to resolve the origin of the two haplotypes.

Altogether, we suggest that the genetic population structure of wolverines in Finland is due to the combined effects of a genetic cline from east to north caused by IBD, past demographic events and translocation history. The two applied markers provided different insights into how population history has shaped the genetic structure. Further transboundary studies are needed to study possible additional effects of isolation-by-resistance (IBR; McRae 2006) and putative barriers to gene flow. The amount of dispersing wolverines between the clusters appears low considering the innate travel capacity (Gardner et al. 1986; Vangen

et al. 2001; Flagstad et al. 2004; Inman et al. 2012). During the last two decades, only a few migrants were detected between the clusters. As we did not sample the Russian part of Karelia, we might have missed migration events due to wolverines using forest corridors in Russian Karelia (Lindén et al. 2000).

Genetic diversity

Genetic diversity was in general higher in CCPA *East* ($H_E = 0.57$, $A_R = 4.0$, $A_P = 0.3$) than in CCPA *North-West* ($H_E = 0.49$, $A_R = 3.7$, $A_P = 0.1$). As each study uses a partially different set of microsatellites, comparison of diversity measures should be interpreted with caution. Nevertheless, the heterozygosity estimate of CCPA *North-West* ($H_E = 0.49$) is lower than in previous studies reported for Scandinavian wolverines from their southern ($H_E = 0.53$), northern ($H_E = 0.51$) (Flagstad et al. 2004) and central range ($H_E = 0.51$) (Hedmark and Ellegren 2007). The lowest heterozygosity estimates within Fennoscandia has been obtained from two newly founded subpopulations that were established in central Sweden ($H_E = 0.41$ and $H_E = 0.39$) (Hedmark and Ellegren 2007). Generally, the allelic richness in Finland ($A_{R\ East} = 4.0$, $A_{R\ North-West} = 3.7$) was higher than in central Scandinavia ($A_R = 3.0$) (Hedmark and Ellegren 2007). Especially CCPA *East* has been expanding during the last decade, which might explain the higher estimates of genetic diversity. Likewise, a minor increase in genetic variability has been observed in peripheral, expanding North American wolverine populations (Zigouris et al. 2012). In addition, proximity to larger Russian populations might elevate genetic diversity by bringing in new alleles. Lower variation in CCPA *North-West* suggests that this population, as part of the peripheral Scandinavian population (Boitani et al. 2015), might receive only limited gene flow, comparable to peripheral wolverine populations of the north-western US (Cegelski et al. 2006).

Effective population size

The two wolverine clusters in Finland have considerably low effective population sizes based on our microsatellite data, with several methods resulting in current $N_e < 50$. Alarmingly, low estimates were detected for both Finnish clusters, even though they are assumingly part of larger transboundary wolverine populations. CCPA *North-West* belongs likely to the Scandinavian population (Boitani et al. 2015), where a current N_e of 87 individuals was estimated using neutral SNP markers (Ekblom et al. 2018). The difference between the estimates might be caused by sampling bias and number of loci used (Antao et al. 2011; Luikart et al. 2010). In our study using 14 multi-allelic microsatellites, the assumption of random sampling was violated by including only northern

Finnish samples for estimation of N_e for the Scandinavian population, whereas a wider sampling scheme throughout Sweden and Norway was applied in Ekblom et al. (2018) using 384 bi-allelic SNPs.

Effective population sizes are expected to decrease from core populations to edge populations (Vucetich and Waite 2003). Therefore, we would have expected that N_e of CCPA *East* could have been higher than in CCPA *North-West*. As N_e estimates were low, it is possible that CCPA *East* is not a part of a larger Eurasian core population. The main corridor to connect taiga species of Fennoscandia with larger taiga complexes in Russia is located between the White Sea and Lake Onega (Lindén et al. 2000). As wolverines are currently not found south of Lake Onega (Danilov et al. 2018), restricted connectivity through this isthmus might limit gene flow and separate the Karelian population from the Arkhangelsk population. Furthermore, low N_e suggest that both Finnish clusters are still recovering from the recent bottleneck and/or gene flow is limited. Comparable N_e estimates have been found in other large mammals, if a population has gone through a bottleneck and/or are isolated (e.g. Saimaa ringed seal *Phoca hispida saimensis*: $N_e = 11–113$, Valtonen et al. 2014; Finnish wolves (*Canis lupus*): $N_e = 38–43$ Aspi et al. 2006; Iberian lynxes *Lynx pardinus*: $N_e = 8–23$ Casas-Marce et al. 2013; insular Canada lynx *Lynx canadensis*: $N_e = 7–8$ Prentice et al. 2017). However, similar estimates have been obtained also for stable populations with ongoing migration (e.g. brown bears: $N_e = 9–37$ Schregel et al. 2012; cougar *Puma concolor*: $N_e = 38–112$ Juarez et al. 2016).

Implications to conservation management

For the conservation of wolverines in northern Europe, connectivity throughout the range is of utmost importance to retain genetic variation. Our study shows that wolverines in Finland are divided in two genetic clusters, which reflect well the subdivided population at the time of the bottleneck. Furthermore, the eastern cluster contains an mtDNA haplotype not present in the rest of Scandinavia and especially the northern cluster had low genetic diversity. To sustain gene flow from Karelia towards Scandinavia, connectivity between the Finnish eastern and northern clusters is crucial and additionally, persistence of the western cluster should be monitored. The Finnish wolverine population has endured times of persecution but is making a comeback to its former range. Importantly, the recent recolonization of wolverines into the boreal forest of Sweden shows that the species is flexible, when circumstances are beneficial (Aronsson and Persson 2017). The positive attitude towards wolverines outside the reindeer husbandry area also facilitates the establishment of wolverines into less optimal habitat (Pohja-Mykrä and Kurki 2008). In addition, local accumulations of snow are often sufficient to provide den sites (Pulliainen

1968; Magoun et al. 2017), even in regions where the average snow depth is low.

Due to climate change, wolverine habitat is expected to decrease and become more fragmented (McKelvey et al. 2011). Although, wolverine numbers in Finland are increasing, the bottleneck has altered the genetic make-up. Furthermore, temporary population growth does not necessarily secure a long-term, genetically diverse population (Jansson et al. 2012). Therefore, the level of connectivity with other extant population clusters in Eurasia needs to be resolved in order to propose management decisions, which are relevant for the conservation of wolverines throughout the range.

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