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Y chromosome haplotype distribution of brown bears (*Ursus arctos*) in Northern Europe provides insight into population history and recovery

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Abstract

High-resolution, male-inherited Y-chromosomal markers are a useful tool for population genetic analyses of wildlife species, but to date have only been applied in this context to relatively few species besides humans. Using nine Y-chromosomal STR and three Y-chromosomal single nucleotide polymorphism markers (Y-SNPs), we studied whether male gene flow was important for the recent recovery of the brown bear (*Ursus arctos*) in Northern Europe, where the species declined dramatically in numbers and geographic distribution during the last centuries but is expanding now. We found 36 haplotypes in 443 male extant brown bears from Sweden, Norway, Finland and Northwestern Russia. In 14 individuals from southern Norway from 1780 to 1920, we found two Y chromosome haplotypes present in the

extant population as well as four Y chromosome haplotypes not present among the modern samples. Our results suggested major differences in genetic connectivity, diversity, and structure between the eastern and the western populations in Northern Europe. In the west, our results indicated that the recovered population originated from only four male lineages, displaying pronounced spatial structuring suggestive of large-scale population size increase under limited male gene flow within the western subpopulation. In the east, we found a contrasting pattern, with high haplotype diversity and admixture. This first population genetic analysis of male brown bears shows conclusively that male gene flow was not the main force of population recovery.

Introduction

The genetic structure and diversity of a population are greatly influenced by gene flow. A large number of studies have shown that lack of immigration, and thus lack of gene flow from outside the population, can lead to a significant reduction of population viability (e.g. Dunn *et al.* 2011; Hedrick *et al.* 2014). The concept of "genetic rescue" is based on the prevention or reversal of this situation and an increase in gene flow has been shown to lead to higher fitness and demographic growth (La Haye *et al.* 2012; Heber *et al.* 2013). Most mammals display male-biased dispersal, i.e. males disperse farther and more frequently from the natal home range than females. Therefore males may display higher levels of gene flow among regions than females (Greenwood 1980), resulting in males generally exhibiting a weaker spatial genetic structure and a stronger contribution to both genetic diversity within and genetic connectivity among populations than the females (Ishibashi *et al.* 2013; Quaglietta *et al.* 2013). Thus, it can be hypothesized that in species with male-biased dispersal, males are responsible for counteracting the effects of genetic drift and population fragmentation, and therefore may play an important role in population recovery and

demographic growth. However, this hypothesis has not been sufficiently evaluated, partly because the male contribution to population genetic connectivity, diversity, and structure is poorly understood in most wildlife species.

Previous studies have mostly assessed male gene flow either indirectly, by contrasting data on autosomal microsatellites (Short-Tandem-Repeats, STRs) and mtDNA (e.g. Baker *et al.* 2013; Hua *et al.* 2013; Ishibashi *et al.* 2013) or by comparing male versus female genetic spatial autocorrelation (Banks & Peakall 2012). However, due to differences in marker systems (in the case of comparisons between mtDNA and autosomal markers) and recombination of bi-parental markers, these approaches only give an imprecise measure of the occurrence and influence of male migration and gene flow. In contrast, the analysis of male-inherited, Y-chromosomal variation enables the direct assessment of male gene flow independently of mtDNA and autosomal DNA variation (Hurles & Jobling 2001; Kayser *et al.* 2005; Roewer *et al.* 2005). In addition, being similar to autosomal STRs in terms of diversity, mutation rates, and methodological unambiguity of allele designation (Hurles & Jobling 2001; Kayser *et al.* 2005; Roewer *et al.* 2005), Y-STRs can be applied to answer similar questions regarding population structure and connectivity at the same temporal and spatial resolution as autosomal STRs. Y-STRs have therefore been increasingly used to investigate population genetic processes both in humans (e.g. Rubicz *et al.* 2010; Salazar-Flores *et al.* 2010; Zupan *et al.* 2013; Tateno *et al.* 2014; Karmin *et al*. 2015) and other primates (Langergraber *et al.* 2007; Schubert *et al.* 2011; Nietlisbach *et al.* 2012; Inoue *et al.* 2013). Y-STRs are often combined with Y-chromosomal single nucleotide polymorphism markers (Y-SNPs), especially in human studies (Larmuseau *et al.* 2014, Roewer *et al.* 2013, Karafet *et al.* 2008). Y-SNPs have a slower mutation rate than Y-STRs and thus can form the phylogenetic backbone onto which the Y-STR haplotypes can be arranged (Jobling 2012). As

such, they can give insight into phylogenetic events that date back in time much longer than Y-STRs (Weiwei *et al.* 2013, Geppert *et al.* 2011, Karafet *et al.* 2008) and represent a higher level of order than Y-STRs, whose phylogenetic relationship with each other can be obscured, due to allele size homoplasy (Hailer & Leonard 2008).

In wildlife species, population genetic studies using Y-STRs are still rare, although a growing number of phylogeographic and phylogenetic studies exist, such as for brown hares (*Lepus europaeus*) in Europe and Anatolia (Mamuris *et al.* 2010), sheep (*Ovis ssp.*) (Meadows *et al.* 2006), colobine monkeys (*Colobinae*) (Roos *et al.* 2011), or snow voles (genus: *Chionomys*) (Yannic *et al.* 2012b). Here, we apply high-resolution Y-chromosomal markers to assess the importance of male gene flow during the recovery of a large terrestrial carnivore from near extinction across its distribution range in Northern Europe. All four large terrestrial carnivores, lynx (*Lynx lynx*), wolves (C*anis lupus*), wolverines (*Gulo gulo*), and brown bears, declined dramatically in abundance and geographic distribution during the last centuries, but are recovering now and expanding into areas of previous extirpation across Europe (Chapron *et al.* 2014). This "carnivore comeback" provides an opportunity to determine the role of genetic connectivity as population recovery proceeds (Hagen et al. 2015). For the study species, we used the brown bear (*Ursus arctos*) in Northern Europe, owing to the pronounced male-biased dispersal and female philopatry that has been documented in the species (McLellan & Hovey 2001; Støen *et al.* 2005; Støen *et al.* 2006; Zedrosser *et al.* 2007a), which suggests that connectivity among populations occurs primarily via male dispersal (Manel *et al.* 2004). Recently, using autosomal STRs, Kopatz *et al.* (2014) documented that the demographic recovery, i.e., the increase of population size after the bottleneck, of the brown bears in Finland was supported by immigration from Russia, whereas in Sweden and Norway, brown bear numbers increased from near extinction without

significant immigration of eastern bears. In addition, genetic connectivity between the eastern (Finland, Russia) and western (Norway, Sweden) subpopulations (Figure 1) of the Northern European brown bear has been found to be low (Schregel *et al.* 2012; Kopatz *et al.* 2014). These differential recovery histories and the low connectivity between the eastern and western parts of the species' range offer an opportunity for applying Y-chromosomal markers to study the impact of male gene flow in the recovering brown bear populations in Northern Europe.

Our expectation was that the different recovery histories, i.e. high vs. low immigration during the recovery process, might explain differences in the Y chromosome haplotype diversity of the post-bottleneck brown bears between the eastern and western subpopulations in Northern Europe. Specifically, we expected that high versus low immigration during the recovery process was associated with high versus low Y chromosome haplotype diversity in the post-bottleneck subpopulations, respectively. Given the evidence that the connectivity among the brown bear subpopulations in Northern Europe occurs primarily by male dispersal (Manel *et al.* 2004; Støen *et al.* 2005; Støen *et al.* 2006; Zedrosser et al. 2007a), we also expected that male gene flow may be a potentially important driver of the range-wide demographic recovery process by increasing the gene flow among regions and thus counteracting negative effects of fragmentation. A significant impact of male gene flow similar to a "genetic rescue effect" that would be relevant to explain the range-wide recovery would assume a high degree of Y chromosome haplotype admixture within the two subpopulations, independent of recovery history. This would then be consistent with the global pattern of male-biased gene flow across continents and in phylogeographic time scales, as recently reported based on nine of the twelve Y-chromosomal markers we have used here (Bidon *et al.* 2014). In contrast, a pronounced spatial structuring of Y chromosome

haplotypes within the two subpopulations would indicate that large-scale demographic population recovery has occurred relatively independently of male gene flow. We also included historical samples from southern Norway between ~1750 and ~1950, i.e. in an area where brown bears became functionally extinct around the 1920s (Swenson *et al.* 1995). This was done to assess any temporal changes in the genetic composition of the post-bottleneck population. Our prediction was that recovery from near extinction without supporting immigration would be associated with a long-lasting reduction in Y-haplotype diversity.

Thus, we tested whether or not male dispersal was a key driver of large-scale genetic connectivity and the recent population recovery among Northern European brown bear subpopulations. In addition, by comparing historic and modern haplotypes and haplotype diversity, we could obtain empirical data about the genetic consequences of the historic population bottleneck for Y-haplotypic diversity.

Material and methods

Demographic history of the brown bears in Northern Europe

Once abundant in Northern Europe, brown bears were persecuted to near extinction during recent centuries (Servheen *et al.* 1999). In Norway, in the west of Northern Europe, the brown bear became functionally extinct (i.e. bears did not inhabit Norway permanently any longer), around the 1920-30s, whereas in Sweden, east of Norway, ~130 individuals survived in three refugee areas (Swenson *et al.* 1995). In Finland, in central Northern Europe, ~150 individuals survived in the northern and eastern parts of the country (Ermala 2003). To the east of Finland, in Northwestern Russia, a similar population decline occurred during the 1930-1940s in the southwestern part of Russian Karelia, as well as on the Karelian Isthmus of the Leningrad region (Danilov 2005). After protective measures had been initiated during the

1960s and 1970s, simultaneously in all four countries, these populations have gradually recovered demographically (Swenson *et al.* 1995; Danilov 2005; Kojola *et al.* 2006; Kindberg *et al.* 2011). The most current estimates for each country are: ~2800 individuals in Sweden in 2013 (Naturvårdsverket 2014), a minimum of 136 in Norway in 2014 (Aarnes *et al.* 2015), and 1,150-1,950 in Finland in 2009 (Wikman 2010). The most recent estimates in western Russia are from 1990, stating that there are ~500 individuals in Murmansk Oblast to the north and ~3 500 in Russian Karelia to the south (Chestin 1992; Danilov 1994).

Sampling

Extant individuals from 2006-2012

We used DNA samples of verified male individuals, analyzed and stored in the course of regional and national monitoring programs conducted in Sweden and Norway, as well as during previous studies conducted in Finland (Schregel *et al.* 2012, Kopatz *et al.* 2012, 2014, Hagen *et al.* 2015). We only used DNA samples that had been identified positively with no less than six autosomal STRs plus one sex identification marker. The laboratory protocols of DNA extraction and analysis are accredited according to the EN ISO/IEC 17025 standard (Norwegian accreditation: test 139). According to these protocols, to verify an identification, the genotyping must have been performed independently three times if a sample was homozygous for the respective marker and twice if heterozygous. Samples that did not meet these requirements were not given a positive identification and hence were not used for this study. Further details of the DNA extraction, PCR amplification, and genotyping protocols are described in Andreassen *et al.* (2012). Following the criteria outlined above, we selected a total of 491 DNA samples of male brown bears, which had been collected in Norway, Sweden, Finland, and northwest Russia during 2006-2012, with the study area spanning from 60°-69°N and 12°-59°E. Of these samples, 236 were extracted from fecal and 93 from hair

samples, obtained noninvasively during monitoring programs (see also Schregel *et al.* 2012). In addition, 162 were extracted from muscle tissue samples obtained from legally shot bears. The distribution of samples is illustrated in Figures 1, 2, 4 and S1. Sampling procedures have been previously described elsewhere (Eiken *et al.* 2009; Andreassen *et al.* 2012; Kopatz *et al.* 2012; Schregel *et al.* 2012; Kopatz *et al.* 2014).

Historical samples from Norway 1750-1956

We sampled a total of 130 historical specimens from museums and private collections in southern Norway. These samples originated from approximately 1750 to 1956 and represented bears that existed in southern Norway before the extinction of the majority of the resident brown bear population. Although the last bear to be legally killed in southern Norway was shot in 1956, functional extinction was reached already by the 1920-30s, with 1931 being the last of at least three consecutive years during which brown bears were killed in southern Norway (Swenson *et al.* 1995). Whenever possible, multiple samples, including various tissues (muscle, brain, nose, and mouth palate), skin, hairs, and tooth powder, were obtained from each specimen. We collected each sample separately to minimize the danger of contamination with other historical bear samples, wrapped the sample in aluminum foil, placed it in a self-sealing plastic bag, and stored it at room temperature.

We removed the tooth powder samples with a drill after scraping the tooth surface with a scalpel and discarding the powder from the beginning of drilling at a speed of around 60 rpm to avoid overheating. We drilled in a clean room and cleaned the tools, as well as surfaces, after each sample, first with 10% chlorine solution, followed by a rinse with water, and then dried with sterile tissue to minimize cross-contamination. The powder was immediately placed in a tube containing 300 µl of extraction buffer from the Qiagen Investigator© kit (Qiagen) and stored at -20°C until DNA extraction.

Genetic analysis

Analysis of Y-chromosomal STRs

We genotyped all samples with 9 Y-STR markers developed specifically for bears (Bidon *et al.* 2014) (Table S1) and validated for brown bears (Aarnes *et al.* 2015). The repeat unit of marker UarY318.1 is a tetra-nucleotiode, the remaining 8 markers all have a di-nucleotide repeat motive. Prior to amplification, we divided the STRs into two multiplex sets, which were optimized so that the fragments did not overlap in length and had even peak sizes (Aarnes *et al.* 2015). The PCR reaction was set up in a 10 µl reaction volume containing 5 µl 2x Multiplex PCR MasterMix (Qiagen), 1xBSA (NEB), and 1 µl template DNA (~1 ng). The primer concentrations we used are listed in Table S1. We used a touchdown PCR program with an initial heating of 95°C for 10 min, followed by 10 cycles of 30 s at 94°C, 30 s of 69°C (reduced by 1°C for each consecutive cycle), and 1 min at 72°C, followed by 20 cycles of 30 s at 94°C, 30 s at 59°C, and 1 min. at 72°C, and then an elongation step of 45 min at 72°C. For the historical DNA samples, the second cycling step was extended from 20 cycles to 30 cycles. The first and the last four samples on every 96-well plate were positive controls, consisting of three reference males and one reference female. The female sample was included to control for primer contamination, as well as male specificity of the markers. Negative controls (without template DNA) were included for every seventh sample.

We analyzed PCR products on an ABI 3730 genetic analyzer and scored the PCR fragments with GeneMapper 4.0 (Applied Biosystems). Fragment lengths of the positive control samples were determined independently prior to the analysis of the sample material to serve as size calling control in the main analyses. Fragment length was identified automatically, but each result was also examined manually. We accepted size calling when the peak height of a fragment was higher than a threshold value of 600 relative fluorescent

units (RFU). All samples were typed and confirmed at least two times. Haplotype names were assigned according to the order in which they were registered during STR genotyping; all registered Y-STR profiles are shown in Table S3. A new haplotype definition was accepted only if it was found more than once or if the discerning allele/s were typed and confirmed at least twice. Samples that failed to amplify at one or more markers were only assigned a haplotype if the PCR amplification results of the remaining markers allowed this to be done unambiguously.

Analysis of Y-chromosomal SNPs

We chose three different bear-specific Y-linked SNPs (UAY579.1B545, UAY318.2C713, UAY318.2C839, see also table S2) for haplogroup analysis, based on the previously confirmed sequence variation among Northern European brown bears for only these 3 SNPs (see Bidon et al. 2014). The SNP primers were designed using the Custom TaqMan® Assay Design Tool (Applied Biosystems) and labeled with FAM and VIC dye (Table S2). PCRs were set up in a 9 µl reaction volume, containing 5 µl TaqMan Genotyping MasterMix (2X), 0.25 µl TaqMan assay mix (40X), 0.045 µl BSA(100X) and 1 µl DNA (~1ug), and then amplified on the ABI 7300 Real-Time PCR System (Applied Biosystems) with a protocol of 2 min at 60°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. To control for amplification success and between-run variability, four positive controls (three males and one female) were included in the first and last row of each plate. To control for contamination, a blank was included for every seventh sample. Before the main analysis, we performed two types of test runs; i) to assess within-run variation, we analyzed one sample 14 times with each of the respective markers and evaluated the consistency of the results manually, ii) to assess amplification success with different template types, we analyzed 2 samples extracted from hair, 4 samples extracted from fecal material, and 4 from muscle

tissue twice with each marker and evaluated results manually. Test run results showed no classification variation according to template type and high within- and between-run consistency (see appendix S3 for examples of the scatterplots of the respective test runs). This, in combination with the haplotypic nature of the Y chromosome, implicates that misclassification of Y-SNP alleles is an unlikely source of error and therefore, for the Y-SNP analysis, the samples were generally amplified only once per marker. Haplogroup names were assigned according to the numbering of Y-SNP genotypes in Table S3.

Genetic analysis of historical brown bear samples

To avoid contamination with DNA from the extant brown bears, the historical samples were stored and handled, i.e. DNA extraction and PCR setup, in a separate laboratory building, in which brown bear samples had never been handled or stored. We extracted DNA using the Qiagen Investigator kit following the manufacturer's instructions for tissue and tooth/bone powder, respectively. To control for contamination during extraction, a blank sample was included for every 11th sample. Extracted DNA was eluted in 40 µl elution buffer and stored at -20°C.

First, all samples were PCR amplified with three sex-specific markers, as previously described, two located on the Y and one on the X chromosome (Bidon *et al.* 2013). Based on the analysis results, only samples that showed all three peaks, with the two Y-specific markers displaying a peak height of ~1500 RFU, were selected for further analysis. Historical samples displaying a lower peak height had been shown to fail or show only partial amplification of the nine Y-STRs in earlier test runs (results not shown). In case more than one sample per individual displayed eligible amplification results, we selected the one with the highest peak heights for the subsequent analyses. We then amplified the selected samples

independently three times with the nine Y-STR multiplex set-up, using the same touchdown PCR program as for extant samples, but with a total of 40 PCR cycles (see above). No positive controls were included in the PCR to avoid contamination, but a negative control was included for every fifth sample. To control for between-run variation during the analysis with the genetic analyzer, we included four independently amplified positive controls with known allele sizes. Allele size calling was confirmed at least twice; haplotype assignment and definition followed the same criteria as for the extant samples, outlined in the respective section. The Y-SNP analysis for the historical DNA samples followed the same procedures as for the modern DNA samples and was performed only for the samples which were assigned a Y-STR haplotype.

Statistical analysis

To assess the degree of haplotype admixture and diversity across the study area, we calculated haplotype frequencies, haplotype diversity, and mean number of pairwise difference (MPD, which describes the mean number of pairwise differences between all pairs of haplotypes in the sample, taking into account the number of mutational steps, the number of haplotypes, and the frequency and number of individuals) for each of the 18 sampling locations, using the program Arlequin 3.5.1.2 (Excoffier & Lischer 2010). To illustrate similarity of haplotypes and possible correlation between geographic proximity and haplotype groups, we used the unweighted combination of number of STR repeats for each allele and locus and the Y SNP sequence substitute data for the respective Y chromosome haplotypes to construct a median-joining network using the software Network v.4.6.1.2. (http://www.fluxus-engineering.com/sharenet.htm).

Next, we assessed and compared the population genetic structure of male lineages within and between the two known subpopulations in the west (i.e., the Scandinavian Peninsula), and the east (i.e., northeastern Norway, Finland and western Russia) (Figure 1). In the following, we use the term subpopulation to refer to the two portions (east and west) of the Northern European brown bear population, the term sampling location describes the respective counties where the samples were collected, and the term group refers to a grouped set of sampling locations. The haplotypic, nonrecombinant nature of Y-STR data renders most frequently used approaches to identify genetic clusters or groups in a continuous population inapplicable. A common way to group samples is therefore to use the sampling location as the group-defining variable. This works well for large-scaled studies, where the sampling area stretches across several continents (e.g. Meadows *et al.* 2006; Bidon *et al.* 2014) and for studies in group-living species (e.g. Langergraber *et al.* 2007; Schubert *et al.* 2011). This approach is sometimes followed by hierarchical analysis of population differentiation, i.e. subsequent pooling or grouping sampling locations which display nonsignificant F_{ST} values among each other (e.g. Kayser *et al.* 2005). Like many other species, however, the brown bear is distributed more-or-less continuously across the study area, with no easily discernable delineations. Therefore, we devised a different strategy to find a reasonable grouping of sampling locations in Y-STR data from continuous populations. This approach consisted of applying a cluster analysis based on haplotype frequencies from all of the sampling locations using the function 'hclust' in the program R (R coreteam 2013). We used the results of this analysis to initially group the data for the subsequent AMOVA analysis, performed with Arlequin (Excoffier & Lischer 2010). To validate the results of the clustering analysis, we performed several runs with different groupings. Our aim with this was to find the grouping that would minimize within-group variation and maximize among-group variation, while taking into account geographic

location of the sampling area. To investigate whether there was a difference in the magnitude of population structure in the two independently recovering Northern European brown bear subpopulations, we also performed an AMOVA for the Scandinavian and Finland/-Russian sampling areas separately. As another means to test our grouping, we used the number of different alleles between haplotypes to estimate genetic distance among sampling locations, which is similar to a weighted F_{ST} (Excoffier & Lischer 2011), with the software Arlequin (Excoffier & Lischer 2010). To view the result of the pairwise F_{ST} estimation, we performed an analysis of principal coordinates (PCoA) using the program GenAlEx 6.5.01 (Peakall & Smouse 2006, 2012). To estimate the genetic differentiation among the groups, we then pooled the data based on the results of the AMOVA analyses and the estimated pairwise F_{ST} among sampling locations, and estimated pairwise F_{ST} among these groups, again using number of different alleles as a measure of distance. To view the result, we again performed a PCoA.

Results

Contemporary Y chromosomal diversity and distribution

Y chromosome haplotypes (i.e. a combination of a 9-locus Y-STR haplotype and 3-locus Y-SNP haplogroup) were determined for 443 male brown bears sampled during 2006 to 2012. None of the samples showed different allele sizes (i.e. false alleles) in the respective runs. The remaining 49 samples could not be assigned to any specific Y chromosome haplotype, due to failed PCR amplification at one or several loci, and were discarded from the study. Y-SNPs analysis showed that two Y-SNPs (UAY579.1B545 and UAY318.2C713) were variable among the 443 male bears. For Y-SNP UAY318.2C713 only 146 samples out of 443 could be determined. However, the detected SNP variation confirmed the haplogroup/haplotype combinations shown in Table S3. Although the third Y-SNP in this

study (UAY318.2C839) had previously been found to be variable among bears in populations further east in Europe (Bidon et al. 2014), it was monomorphic in our samples. Accordingly, we found only three different Y-SNP haplogroups (Figure 1, Table S3), containing five, 20, and 11 Y-STR haplotypes, respectively. The resulting 36 different Y chromosome haplotypes and their total and local frequencies are shown in Table 1. An overview of Y chromosome haplotype distribution in Norway, Sweden, Finland and northwest Russia is shown in Figure 2, with close-up maps in the supplementary files (Figures S2, S3 and S4).

We detected an east-west division according to recovery history both in the number and diversity of Y chromosome haplotypes. All Y chromosome haplotypes in Y chromosome haplogroup 1 were found exclusively in the eastern subpopulation, i.e. in northeast Norway, Finland and northwest Russia, but were absent from the west, i.e. Sweden and the western and southern parts of Norway. Consequently, we found only four Y chromosome haplotypes in the west (among 192 males), compared with 7 to 13 Y chromosome haplotypes at the various sampling locations in the east (Table 2). Accordingly, Y chromosome haplotype diversity (hd) per sampling location ranged from 0.439 to 0.971 (mean = 0.727), with higher values to the east (Table 2). Specifically, the diversity decreased relatively modestly from AOKM in Russia to PA in northeastern Norway, before it showed an abrupt decline from FLL and AN towards NB, TR and further south, indicating the geographic delineation, between the two independently recovering subpopulations. A similar geographic pattern was observed for the mean number of pairwise differences (MPD) per sampling location, which ranged from 0.909 to 4.200 (mean = 3.061) (Table 2).

Only two Y chromosome haplotypes, 2.02 and 3.09, were distributed across the entire study area (Figure 2, Table 1), with 2.02 previously found to be present also in the Ural region (Bidon *et al.* 2014), consistent with an old origin of these Y chromosome haplotypes (e.g. Morral *et al.* 1994). In contrast, more than half of the haplotypes (19 of 36) were found in only one sampling location (Table 1). However, eight of these were found in Northern Russia, which, despite its low number of samples, displayed the highest haplotype diversity (Table 2). Hence, more extensive sampling efforts are needed in Russia to investigate the full haplotype diversity there. A detailed presentation of location-specific Y chromosome haplotype distribution can be found in the Figures S2-S4 in appendix S1. The constructed median-joining network is shown in Figure 3. Qualitative assessment showed that genetically close haplotypes, were not restricted to locations that were geographically close to each other.

Contemporary population genetic structure of male lineages

The cluster analysis used to group sampling locations according to haplotype frequencies recovered six Y chromosome haplotype clusters separated into two main groups (Figure 4). The first main branch contained the southern Swedish and southern Norwegian sampling locations, which clustered in an east-west direction across the Swedish-Norwegian border, with a southern and a northern group (groups 1 and 2, light red and dark red colors, respectively; Figure 4a and b). The second main branch, which contained all of the other sampling locations, split again into two subbranches. The first subbranch contained the sampling locations of TR, NB, and FLL (group 3, yellow color; Figure 4a and b). The second subbranch formed three clusters along a north-south direction; the first being AN, PA, and KA in the North (group 4, green color; Figure 4a and b), the second being the southern sampling locations FSK, FNK and RNK (group 5, light blue color; Figure 4a and b), and the third being the southern and eastern RSK and AOKM (group 6, dark blue color; Figure 4a

and b). The few samples constituting the latter cluster were distributed across a large area. Haplotype frequencies indicated that groups 1, 2, and 3 were different from the other groups (Figure 5).

Overall, the AMOVA showed that among-group genetic variation was high (20.4 %) compared to within-group genetic variation (1.1 %). This was expected, as groupings were chosen to maximize the former and minimize the latter. However, the magnitude of among-group variation differed geographically according to recovery history. The AMOVA run for the western groups 1-3 only (i.e. Sweden and Norway without AN and PA) showed a similar high among-group variation (18 %) as for the analysis for the total dataset, whereas AMOVA on the eastern groups 4-6 only (i.e. AN, PA with the Finnish and Russian groups) displayed an among-group variation of only 5.7 % (Table 3). Also the within-group variation indicated a stronger spatial genetic structuring in the western subpopulation than in the rest of Northern Europe: when all groups in the western subpopulations were combined, within-group variation increased by 6.4 %, whereas the same treatment of all eastern groups resulted in an increase of only 2.5% (alternative grouping resulted generally in an increase of within-group variation as shown in appendix S2). Additionally, pairwise F_{ST} values among sampling locations, which ranged from -0.057 (NT-ST) to 0.517 (RNK - NO), largely supported the grouping based on allele frequencies, with both lower absolute values and a lower degree of clustering in PCoA among hclust/AMOVA-group members than among nonmembers (Figure S5, Table S5). Exceptions were AN, which was displayed at an equal distance between group 3 and group 4, and FSK, which clustered closely with KA and PA, as opposed to the clustering analysis (Figure S5). Our analysis strategy of using clustering analysis based on haplotype frequencies offered a simple alternative to hierarchical pooling of predetermined groups based on population differentiation estimates, as our results showed a reasonable concordance between the two approaches.

The subsequent second estimation of genetic differentiation, using the results of the clustering analysis, the AMOVA, and the pairwise F_{ST} values to pool sampling locations, resulted in pairwise F_{ST} values among these groups ranging from 0.039 to 0.354, with all of the values being significant (Table 4). Again, the PCoA analysis of pairwise F_{ST}-values displayed a divide in terms of genetic differentiation between the western groups 1-3 and eastern groups 4-6 (Figure 6 and S5), indicating less gene flow and/or a higher impact of genetic drift within Scandinavia than in the east and thus increasing the robustness of our findings.

Pre-bottleneck Y chromosome haplotype diversity of brown bears samples from Norway (1750-1950)

We also investigated the genetic impact of the demographic bottleneck by assessing temporal changes to the genetic composition of the post-bottleneck population. We collected 215 historic (1750-1950) samples from 130 individuals from the extinct brown bear population in southern Norway. We were able to amplify 62 bears successfully, 20 females and 42 males, with the gender test (see Methods). Of these 42 male historical samples, we obtained a nearly complete Y chromosome haplogroup and Y chromosome haplotype profile (i.e. one to two Y-SNPs and seven to nine Y-STRs) for 14 samples. An overview of the locations of the individuals in Norway and their Y chromosome haplotypes are shown in Figure 7 and the year of origin and Y chromosome haplotype and -group profile are given in Table S3. We detected four haplotypes, each in only one individual, that were not present among the 443 bears sampled during 2006-2012, nor were they present in the data on brown bears from across the global distribution zone (Bidon *et al.* 2014). Furthermore, seven individuals were assigned to Y chromosome haplotype 2.05, which is currently most frequent in Northern

Scandinavia. The remaining three individuals were assigned to Y chromosome haplotype 2.08, which occurs at a relatively high frequency across most of Scandinavia today.

Discussion

Most large terrestrial carnivores experienced extensive bottlenecks during the last centuries (Woodruff 2001). Gene flow may be important for the successful recovery of these previously extirpated or isolated populations (Vilà *et al.* 2003; Fredrickson *et al.* 2007; Hedrick *et al.* 2014; Whiteley *et al.* 2015). Using Y-chromosomal markers identified using genomic sequences of the brown and the polar bear (Bidon *et al.* 2014), we have documented a clear pattern of Y chromosome haplotypes across the reexpanding post-bottleneck populations of brown bears in northern Europe. Specifically, we show that a lack of range-wide male gene flow during the recovery process has left a dramatically reduced Y chromosome haplotype diversity in the post-bottleneck brown bear subpopulation in central and southern Scandinavia. In this area, we also find a low degree of Y-haplotyp admixture, providing no evidence of male-driven genetic rescue, which in turn suggests that male gene flow probably had little or no impact on the demographic recovery process in Scandinavia.

Regional differences in Y chromosome haplotype diversity

Our previous data on autosomal STRs showed extensive gene flow between Finnish and Russian brown bears (Kopatz *et al.* 2012, 2014), but low gene flow farther westwards (Schregel *et al.* 2012; Kopatz *et al.* 2014). These different gene flow histories, i.e. low versus high immigration, between the western and eastern populations (Kopatz *et al.* 2014) may have led to a permanent difference in their Y chromosome haplotype diversity following the demographic bottleneck. In the populations in the northern and eastern parts of Northern Europe, connected by a substantial amount of gene flow (Kopatz *et al.* 2012, 2014), we found

35 different Y chromosome haplotypes among 207 males. Eastern European Russia has been identified as the main source population for the postglacial brown bear colonization of Northern Europe using mtDNA analyses (Taberlet & Bouvet 1994; Korsten *et al.* 2009; Keis *et al.* 2013) and as a source of more recent immigration with autosomal STRs (Kopatz *et al.* 2014; Hagen *et al.* 2015). Thus, a decrease in haplotype diversity from east to west can be expected (Excoffier *et al.* 2009; DeGiorgio *et al.* 2011). This is consistent with the gradual decrease in Y chromosome haplotype diversity from Russia towards Finland and northern Norway observed in our study. In contrast to this, we found exceptionally low diversity of male lineages in central and southern Scandinavia, with only four Y chromosome haplotypes among 192 extant males. We sampled all areas that have a substantial number of brown bears today, except Jämtland in Sweden, and assume that we have discovered most or all common haplotypes in the area. The demographic bottleneck in Finland was similar in extent to that in Sweden and Norway (Swenson *et al.* 1995; Ermala 2003; Danilov 2005), yet Y chromosome haplotype diversity observed in Finland was comparable to that of Northwest Russia. Thus, although the demographic bottleneck seems to have had a severe and, in the absence of sufficient immigration, long-lasting effect on Y chromosome haplotype diversity in Sweden and Norway, it seems that extensive immigration from Russia has had an alleviating effect on Y chromosome haplotype diversity in Finland.

Male gene flow as a driver in the population recovery process?

Given the pronounced male-biased dispersal behavior in brown bears (Støen *et al.* 2006; Zedrosser et al. 2007a), we hypothesized that male gene flow would play an important role in the ongoing range-wide demographic recovery process. Instead, we found that the different immigration histories in the eastern and western brown bear populations in Northern Europe were associated with different degrees of spatial genetic structuring of the post-bottleneck

populations. Whereas the eastern brown bears displayed a high degree of Y chromosome haplotype admixture, the western brown bears displayed regionally distinct Y chromosome haplotype frequencies, suggesting the existence of barriers to male gene flow. The Scandinavian brown bear population in the west has increased from ~130 to over 3000 individuals between the 1970s and 2010 (Kindberg et al. 2011). Further east, in Finland, a demographic increase of a similar magnitude has been documented (Ermala 2003; Kojola & Heikkinen 2006). In spite of this substantial population growth in both areas, the PCoA of pairwise F_{ST}-values displayed a clear difference in Y chromosome haplotype structure between the east and west. The AMOVA showed that the overall among-group genetic variation of ~20% was mostly due to the local substructure in the west. Thus, our results suggested a large variability in the degree of male gene flow across the study area, rather than homogeneous gene flow and admixture. Hence, we believe that other mechanisms than male gene flow underlie the large-scale demographic recovery of the Northern European brown bear population. In humans, sex-biased genetic structure decreases with geographical distance, suggesting that long-distance gene flow is relatively independent of sex (Wilder *et al.* 2004a,b, Heyer *et al.* 2012). Even though the male bias in dispersal behavior is distinct, also female brown bears have been shown to disperse more frequently and across farther distances in Sweden than previously assumed (Støen *et al.* 2006). Thus, both males and females may contribute to large-scale genetic connectivity, which should be investigated further.

History as a determinant of genetic structuring in Scandinavia

It has been proposed that the spatial genetic structure of the post-bottleneck brown bear population in Scandinavia reflects the recovery from refuge areas (Waits *et al.* 2000; Manel *et al.* 2004), combined with the impact of postglacial colonization history (Bray *et al.* 2013),

resulting in three genetic clusters in the contemporary subpopulation (Manel *et al.* 2004). These three clusters correspond roughly to the AMOVA groups 1 to 3 in our study. In the absence of Y-linked data, it was assumed that the observed clusters were connected via male gene flow (Manel *et al.* 2004). In contrast, our data suggested that contemporary restrictions to male gene flow prevented or slowed a dissolving of population structure and therefor that the genetic structure observed today is not merely an echo of the fragmentation caused by the human persecution. A similar structuring of the pre- and post-bottleneck populations, suggesting historic, ecological causes for the observed spatial genetic structure rather than anthropogenic ones was also recently proposed by Xenikoudakis et al. (2015). Y chromosome haplotypes are nonrecombinant and directly inherited from father to son, so that the reproductive success of an immigrant male and its descents should be reflected by the spread of a nonlocal haplotype into a new area. The generation time of brown bears in Scandinavia has been estimated to be ca. 10 years (Tallmon *et al.* 2004), so at least three to four generations have passed since brown bears received protection (Swenson *et al.* 1995). Male brown bears produce between 0.42 and 1.02 offspring per year, with the age of successfully reproducing males ranging from 3 to 27 years (Zedrosser *et al.* 2007b). It seems reasonable to expect a relatively high degree of Y haplotype admixture in the population, if dispersing males able to produce viable, male offspring. Based on this, the clear difference in Y chromosome haplotype distribution (and hence limited male gene flow) we observed between Västerbotten and Norrbotten was not expected. These two adjacent Swedish counties have no obvious geographical barriers between them, which suggests that other mechanisms are behind the presumed lack of Y chromosome haplotype exchange. Støen *et al.* (2006) showed inversely density-dependent natal (offspring) dispersal and that indicated both potential social restraints on dispersal and a stronger-than-previously-assumed territoriality in brown bears. Our noninvasive genetic capture-recapture monitoring records of brown bears

regularly identify migrants among the different areas in Sweden (22 females vs. 81 males; unpublished data). Although at least two females and 11 males have dispersed from Västerbotten into Norrbotten (unpublished data), such dispersal has apparently not reduced genetic dissimilarities, at least in regard to Y-STRs. This may support the conclusions of Støen *et al*. (2006) regarding an impact of social mechanisms and higher-than-previously-assumed territoriality on population genetic structuring in brown bears, making it difficult for male immigrants to establish their own territory and/or mate successfully with a resident female. However, if a similar behavioral pattern can be assumed for brown bears in the eastern subpopulation, it is unclear why it does not result in a similar effect on the population structure there. One possible difference may be the occurrence of illegal hunting, which seems to be generally more accepted and occurring at a higher rate in northern Sweden compared to southern Scandinavia (Gangaas et al. 2013; Rauset 2013). It has been shown that this has a strong influence on the mortality rates of large carnivore populations (Andrén et al. 2006; Persson et al. 2009; Liberg et al. 2011). If disproportionately more dispersing males are killed, this would limit male gene flow among regions considerably and might contribute to the occurrence of the observed spatial patterns. However, we are not aware of any data available for the rate of occurrence and/or acceptance of illegal hunting in the area of the eastern subpopulation, so that a targeted study should be conducted to test this hypothesis.

The effect of the bottleneck on Y chromosome haplotype diversity and population genetic structure

The observed low diversity in the western subpopulation could be expected, because of the very low estimated number of bottleneck survivors (Swenson et al. 1995). Our results suggest that the impact of the bottleneck on male diversity and number may have been more devastating than assumed, with apparently only four male lineages surviving the bottleneck.

This was corroborated by the data obtained from the historical samples. Among 14 genotyped historical samples from southern Norway (1780-1920), we found two haplotypes also present in today's population, but also four haplotypes not present among the modern samples. The high coverage of sampling of extant males in Scandinavia strongly suggests that these four Y chromosome haplotypes were not present in the post-bottleneck population, and thus that the diversity of male lines and haplotypes in Scandinavia (i.e. Norway and Sweden) was higher historically than it is today in the recovered population. This is supported by a recent study comparing historic and modern Scandinavian brown bears, which showed both a strong reduction of mtDNA diversity and a significant loss of autosomal allelic richness in the same area (Xenikoudakis *et al.* 2015). In contrast, Waits *et al.* (2000) who tested statistically for a genetic bottleneck using only modern samples, found weak evidence for this in Sweden and only in the southern parts of the country using autosomal STRs. Similarly, using autosomal STRs and modern samples from Norway, Sweden, Finland and Western Russia, also Kopatz et al. (2014) found only weak signs of a genetic bottleneck in Sweden and Norway. Moreover, several studies have found that autosomal STR diversity is relatively high and similar across the Northern European distribution zone (Waits *et al.* 2000, Tammenleht *et al.* 2010; Schregel *et al.* 2012, Kopatz *et al.* 2014), despite of the dramatic demographic decline (Swenson *et al.* 1995). Our results, using Y-chromosomal diversity, differ from these studies, probably because the Y chromosome has an effective population size (N_e) equivalent to only 1/4 of that of autosomes. Consequently, Y-linked genetic diversity is more sensitive to demographic bottlenecks than autosomal genetic diversity (Greminger *et al.* 2010).

The Y chromosome haplotypes 2.08 and 2.05 were found in three and seven historic bears, respectively, indicating that they may have been as frequent before the bottleneck as they are in the present population. However, although 2.08 currently occurs at relatively high

frequency across most of Scandinavia, 2.05 is more common in the northern parts of Scandinavia, being the dominant haplotype in Norrbotten. The other abundant haplotype in the extant Scandinavian population, 3.09, was not found among the historical samples. This may suggest a shift in Y chromosome haplotype distribution and diversity following the demographic bottleneck and subsequent demographic changes, although the low number and limited geographical distribution of our historical samples may not give an accurate representation of the pre-bottleneck population. Thus, if possible, both the area of sampling and the number of samples should be extended to investigate this issue further. MtDNA studies indicate that the Scandinavian Peninsula was colonized from two directions in the postglacial period, from the northeast into northern Scandinavia and from the south (Taberlet & Bouvet 1994; Bray *et al.* 2013). A contact zone between the southern and northern mtDNA clades may have been located in the area of Sør- and Nord-Trøndelag in Norway and Jämtland and Gävleborg in Sweden (Bray *et al.* 2013). All of our historical samples were obtained from the area of the southern mtDNA clade. The four haplotypes we found only in the historical samples may thus be "southern" haplotypes, which may have reached Scandinavia in the postglacial recolonization from the south. This may also be the case for the haplotypes 2.08 and 2.05. Due to the bottleneck in the 19th and 20th centuries, haplotype distribution apparently shifted and several male lineages were eradicated, resulting in a population with a possibly very different genetic composition than the original population, thus indicating a founder effect (Hundertmark & Van Daele 2010; Andersen *et al.* 2014).

Perspectives on conservation

Demographic population recovery from only a very small number of surviving individuals has been shown to occur in many populations with very low autosomal genetic variability, providing exceptions to the widely-held assumption that high genetic diversity is vital for

population viability and recovery (e.g. Visscher *et al.* 2001; Hoelzel *et al.* 2002; Reed 2010; Chan *et al.* 2011; Baldursdottir *et al.* 2012; Taft & Roff 2012). However, rather than shifting the paradigm of the importance of genetic variability for long-term survival, these examples highlight that the viability of a population does not depend on genetic diversity alone, but is influenced by a complicated relationship between genetic, environmental and life history factors (Reed 2010). Despite examples to the contrary, a large number of studies show that a significant reduction in genetic variability is generally correlated with reduced viability, mostly due to increased levels of inbreeding depression (e.g. Liberg *et al.* 2005; Hogg *et al.* 2006; O'Grady *et al.* 2006; Heber *et al.* 2013; Hostetler *et al.* 2013). We are unaware of a study investigating whether sex-chromosomal marker variability has a similar significance for population viability, so it is not possible to evaluate the relevance of the drastic reduction of Y chromosome haplotype diversity during the demographic bottleneck in Sweden and Norway for the long-term survival of the brown bears. The functionality of the Y chromosome is still poorly understood (Sayres *et al.* 2014), but recent research has suggested that the Y chromosome is more than just a determinate of the sex of its bearer, and is essential for male survival (Bellott *et al.* 2014). Regulatory functions on the chromosome may influence gene expression across the entire genome and hence biological functions throughout the lifetime (Bellott *et al.* 2014; Clark 2014; Cortez *et al.* 2014). Based on previous studies, estimates of genetic bottlenecks and diversity in autosomal markers suggested widespread genetic recovery, i.e., the recovery of genetic variability to a viable level, across the entire Northern European brown bear population (Waits *et al.* 2000, Schregel *et al.* 2012, Kopatz *et al.* 2014). However, our results showed that this process is incomplete among Swedish and most Norwegian bears and management actions that are aimed at increasing gene flow among regions may be needed to ensure long-term viability of this population.

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Author contributions
JS, HGE and SBH designed and conceived the study; JS, HGE, FAG, FH, JA, AJ and SBH developed the methods; HGE, FAG, IK KT, PD, AR, EP and SBH collected, compiled and quality controlled the samples. Main analyses were performed by JS, HGE and SBH. The manuscript was written by JS, HGE and SBH with input from all other authors.

Data Accessibility
The raw data (i.e. the genetic profiles) have been archived at the Dryad depository under accession number: http://dx.doi.org/10.5061/dryad.t25mt

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Table 1: Y-chromosomal haplotype frequencies of extant male brown bears by sampling location in Northern Europe, Scandinavian locations listed from South to North: **HO**=Hedmark/Oppland, **DV**=Dalarna/Värmland, **GA**=Gävleborg, **ST**=Sør-Trøndelag, **NT**=Nord-Trøndelag, **NO**=Nordland, **VB**=Västerbotten, **TR**=Troms, **NB**=Norrbotten; Finnmark/Lappland and Finland/Russia listed from West to East: **AN**=Anarjohka, **FLL**=Lappland, **PA**=Pasvik , **KA**=Kainuu, **RNK**=Russian Northern Karelia, **FNK**=Finnish Northern Karelia, **FSK**=Finnish Southern Karelia, **RSK**=Russian Southern Karelia, **AOKM**=Arkhangelsk/Komi. **hpt** = Haplotype names: the first digit of the respective haplotype names indicates the haplogroup (Y-SNP alleles); the last two digits were assigned according to the order in which the haplotypes were registered during STR genotyping.

	Sampling location																		Total
	Western subpopulations										Eastern subpopulation								
hpt	HO (n=50)	DV (n=16)	GA (n=14)	ST (n=12)	NT (n=12)	NO (n=12)	VB (n=12)	TR (n=18)	NB (n=26)	FLL (n=14)	AN (n=15)	PA (n=54)	KA (n=28)	RNK (n=2)	FNK (n=26)	FSK (n=2)	RSK (n=2)	AOKM (n=2)	(n=443)
1.01	-	-	-	-	-	-	-	-	-	0.071	0.200	0.352	0.267	-	0.039	0.154	-	-	0.086
1.13	-	-	-	-	-	-	-	-	-	-	-	-	0.033	-	-	-	-	-	0.002
1.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
1.23	-	-	-	-	-	-	-	-	-	-	0.067	-	-	-	-	0.039	-	-	0.009
1.25	-	-	-	-	-	-	-	-	-	-	0.067	0.019	-	-	-	-	-	-	0.005
2.02	0.103	0.063	0.071	0.083	0.075	0.083	0.025	0.389	-	-	-	0.037	0.067	-	-	-	0.143	0.134	0.065
2.05	0.034	-	0.071	0.167	0.175	0.167	0.250	0.444	0.731	0.500	0.333	0.056	-	-	-	-	-	-	0.149
2.06	-	-	-	-	-	-	-	-	-	-	-	0.019	0.033	-	0.039	-	0.143	0.067	0.014
2.07	-	-	-	-	-	-	-	0.111	-	-	0.133	0.093	0.200	0.308	0.192	0.269	-	-	0.070
2.08	0.414	0.563	0.429	0.583	0.575	0.750	0.575	-	0.115	0.143	-	-	-	-	-	-	-	-	0.239
2.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.143	-	0.005
2.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.039	0.039	0.071	-	0.007
2.12	-	-	-	-	-	-	-	-	-	-	-	-	-	0.077	0.077	-	0.071	-	0.009
2.14	-	-	-	-	-	-	-	-	-	0.071	-	0.019	0.033	0.077	0.039	-	-	-	0.011
2.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
2.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.134	0.005
2.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
2.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.077	-	-	0.005
2.24	-	-	-	-	-	-	-	-	-	-	-	0.019	-	-	-	-	-	-	0.002

2.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.039	-	-	0.002
2.28	-	-	-	-	-	-	-	0.056	-	-	-	-	-	-	-	-	-	-	0.002
2.29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
2.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
2.31	-	-	-	-	-	-	-	-	-	-	-	-	0.100	-	-	-	-	-	0.007
2.32	-	-	-	-	-	-	-	-	-	0.071	-	-	-	-	-	-	-	-	0.002
3.03	-	-	-	-	-	-	-	-	-	0.071	-	0.056	0.033	-	-	0.231	-	-	0.025
3.04	-	-	-	-	-	-	-	-	-	-	-	0.185	0.067	0.231	-	-	-	0.067	0.036
3.09	0.448	0.375	0.429	0.167	0.175	-	0.150	-	0.154	-	-	0.037	0.133	0.154	0.346	0.154	0.214	0.067	0.185
3.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.134	0.005
3.20	-	-	-	-	-	-	-	-	-	-	0.133	0.037	0.033	-	-	-	-	-	0.011
3.21	-	-	-	-	-	-	-	-	-	0.071	0.067	0.074	-	-	-	-	-	-	0.014
3.26	-	-	-	-	-	-	-	-	-	-	-	-	-	0.077	-	-	-	-	0.002
3.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.002
3.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.039	-	-	-	0.002
3.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.077	-	-	-	0.005
3.36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.115	-	-	-	0.007

Table 2: Y-STR diversity of extant male brown bears per sampling location in Northern Europe calculated with *Arlequin 3.5.1.2*; n=sample size. **no. hpt.**= number of haplotypes found, **MPD** = Mean number of pairwise differences, **hd** = haplotype diversity, both listed with their respective standard deviation (± s.d.).

	Region	Sampling location	Country	n	no. hpt.	MPD	± s.d.	hd	± s.d.
Western subpopulation	Southern Scandinavia	**HO** Hedmark. Oppland	Norway	58	4	3.180	1.668	0.627	0.034
		DV Dalarna. Värmland	Sweden	16	3	3.100	1.698	0.575	0.080
		GA Gävleborg	Sweden	14	4	3.396	1.848	0.670	0.082
	Central Scandinavia	**ST** South Trøndelag	Norway	12	4	2.515	1.455	0.652	0.133
		NT North Trøndelag	Norway	40	4	2.421	1.341	0.618	0.067
		NO Nordland	Norway	12	3	0.909	0.676	0.439	0.158
		VB Västerbotten	Sweden	40	4	2.249	1.264	0.600	0.062
	Troms	**TR** Troms	Norway	18	4	2.092	1.225	0.673	0.069
	Norbotten	**NB** Norrbotten	Sweden	26	3	1.975	1.154	0.446	0.105
	Lappland	**FLL** Lappland	Finland	14	7	2.945	1.640	0.758	0.116
Eastern subpopulation	Anarjohka	**AN** Anarjohka/Lemmenjoki	Norway/Finland	15	7	3.600	1.934	0.857	0.065
	Pasvik	**PA** Pasvik/Inari/Pechenga	Norway/Finland /Russia	54	13	3.708	1.903	0.832	0.038
	Karelia	**KA** Kainuu	Finland	30	11	4.200	2.146	0.876	0.036
		RNK Russian Northern Karelia	Russia	13	7	3.603	1.953	0.872	0.067
		FNK Finnish Northern Karelia	Finland	26	10	3.317	1.760	0.843	0.053
		FSK Finnish Southern Karelia	Finland	26	8	3.649	1.909	0.849	0.037
		RSK Russian Southern Karelia	Russia	14	8	4.044	2.147	0.923	0.044
	Northern Russia	**AOKM** Arkhangelsk/Komi Oblast	Russia	15	12	4.190	2.204	0.971	0.033

Table 3: Results of the AMOVA based on Y-STR data, performed on male brown bears in Northern Europe; the percentages of variation displayed where calculated for the entire dataset (AMOVA groups 1-6), and separately for the western (AMOVA groups 1-3) and eastern part of the population (AMOVA groups 4-6). See Figure 4 for the sampling locations per group.

	Percentage of variation		
	AMOVA groups 1 - 6	AMOVA groups 1 - 3	AMOVA groups 4 - 6
among groups	20.40**	17.95**	5.68*
within groups among sampling locations	1.14^{ns}	-0.21^{ns}	2.82*
within populations	78.45**	82.26**	91.50**

* $p<0.05$; ** $p<0.001$; ns=non significant

Table 4: Pairwise F_{ST} values among groups of sampling locations of male brown bears in Northern Europe, based on Y-STR data, using number of different alleles as measure of genetic distance. The groups are as follows: **group 1**=HO, DV, GA; **group 2**=ST, NT, NO, VB; **group 3**=TR, NB, FLL; **group 4**=AN, PA, KA; **group 5**=RNK, FNK, FSK; **group 6**=RSK, AOKM.

	Group 1	Group 2	Group 3	Group 4	Group 5
Group 2	0.148**				
Group 3	0.242**	0.158**			
Group 4	0.175**	0.315**	0.250**		
Group 5	0.139**	0.354**	0.290**	0.071**	
Group 6	0.087*	0.293**	0.270**	0.080**	0.039*

* $p < 0.05$; ** $p < 0.001$

Figures

Figure 1: Y chromosome haplogroup distribution of extant male brown bears in Northern Europe. Historical samples are shown separately in Figure 7. Symbols correspond to the major (SNP-based) Y chromosome haplogroups: red circles = haplogroup 1, black cross = haplogroup 2, blue squares = haplogroup 3. The location of sampling areas are indicated as follows: **HO**=Hedmark/Oppland, **DV**=Dalarna/Värmland, **GA**=Gävleborg, **ST**=Sør-Trøndelag, **NT**=Nord-Trøndelag, **NO**=Nordland, **VB**=Västerbotten, **TR**=Troms, **NB**=Norrbotten; **AN**=Anarjohka, **FLL**=Finnish Lappland, **PA**=Pasvik Valley, **KA**=Kainuu, **RNK**=Russian Northern Karelia, **RSK**=Russian Southern Karelia, **FNK**=Finnish northern Karelia, **FSK**=Finnish southern Karelia, **AOKM**=Arkhangelsk/Komi. The geographical location of the proposed division between the western and eastern subpopulations is shaded red.

Figure 2: Geographical distribution of the detected Y chromosome haplotypes among extant male brown bears in Northern Europe. Each sample is depicted with the symbol used for the haplotype to which it was assigned. Brackets in the legend: total frequency of each haplotype. Haplotypes occurring in Sweden and the southern and centrals parts of Norway (HO, ST, NT and NO) are depicted with a circle and indicated with bold characters in the legend. The location of sampling areas are indicated as follows: HO=Hedmark/Oppland, DV=Dalarna/Värmland, GA=Gävleborg, ST=Sør-Trøndelag, NT=Nord-Trøndelag, NO=Nordland, VB=Västerbotten, TR=Troms, NB=Norrbotten; AN=Anarjohka, FLL=Finnish Lappland, PA=Pasvik Valley, KA=Kainuu, RNK=Russian Northern Karelia, RSK=Russian Southern Karelia, FNK=Finnish Northern Karelia, FSK=Finnish Southern Karelia, AOKM=Arkhangelsk/Komi. The geographical location of the proposed division between the western and eastern subpopulations is shaded red.

Figure 3: Phylogenetic relationship among Y chromosome haplotypes of extant male brown bears in Northern Europe, constructed with the help of the program Network v4.6.1.2.

(Fluxus Technology) by using the RM-MJ method, based on the unweighted Y-STR and Y-SNP data. Only Y chromosome haplotypes with an occurrence of >1 were used for the construction. The area of each pie chart reflects the frequency of the respective haplotype in the extant study population, colors correspond to the sampling location of the haplotypes. The names of the haplotypes are written in black; the number of mutations between each node of the tree, i.e. difference in repeat number and/or difference in Y-SNP sequence, is given in red for each branch (if >1). Haplotypes of haplogroups 1 and 3 are indicated by boxes, all remaining haplotypes belong to haplogroup 2.

Figure 4: Geographic distribution of the AMOVA groups. a) Overview of the geographic location of the different haplotype groups of male brown bears in Northern Europe formed from the *hclust* analysis and the subsequent AMOVA. The color of each circle represents the group to which the individuals were assigned. The colors correspond to those given in inset b). The location of sampling areas are indicated as follows: **HO**=Hedmark/Oppland, **DV**=Dalarna/Värmland, **GA**=Gävleborg, **ST**=Sør-Trøndelag, **NT**=Nord-Trøndelag, **NO**=Nordland, **VB**=Västerbotten, **TR**=Troms, **NB**=Norrbotten; **AN**=Anarjohka, **FLL**=Finnish Lappland, **PA**=Pasvik Valley, **KA**=Kainuu, **RNK**=Russian Northern Karelia, **RSK**=Russian Southern Karelia, **FNK**=Finnish Northern Karelia, **FSK**=Finnish Southern Karelia, **AOKM**=Arkhangelsk/Komi. b) Result of the clustering analysis performed with the function *hclust* in R.; the colors correspond to those used in the map. Groupings for the subsequent AMOVA are indicated by the colored rectangles, the number in each rectangle indicates the name of the respective AMOVA group.

Figure 5: Y chromosome haplotype frequencies of extant male brown bears in Northern Europe calculated for each AMOVA group, the colors correspond to the ones used in Figure 4. All x-axes are identical, each bar representing the respective haplotype frequencies in each AMOVA group. Haplotypes are listed in consecutive order, beginning with haplotypes of haplogroup 1. The black vertical lines in each plot separate the haplogroups from each other.

Figure 6: PCA ordination graph of the genetic differentiation based on matrices of pairwise F_{ST} between AMOVA groups. The circles are colored corresponding to Figure 4. The percentage values on the axes indicate how much variation is explained by the respective axis.

Figure 7: Location and assigned haplotype of 14 historical male brown bear samples in Norway (1780-1920). Circles symbolize the haplotypes that also were found in the extant brown bear population and squares indicate the four haplotypes exclusive for the historical samples.