Spatial structure in European moose (Alces alces): genetic data reveal a complex population history

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ABSTRACT

Aim Moose, Alces alces (Linnaeus, 1758), survived the European Pleistocene glaciations in multiple southern refugia, in a northern refugium near the Carpathians and possibly in other locations. During the second millennium AD, moose were nearly extirpated in Europe and only recolonized their current range after World War II. The number and location of refugia during the Pleistocene and recent population lows may have affected the current genetic diversity. We sought to characterize the genetic diversity in European moose in order to determine its genetic structure and the location of genetic hotspots as a way of inferring its population history and the number of Last Glacial Maximum (LGM) refugia.

Location Europe.

Methods We sequenced 538 nucleotides from the mitochondrial control region of 657 moose from throughout the species’ European range. We estimated diversity within and among 16 sampling localities, and used samova to cluster sampling locations into subpopulations. We constructed phylogenetic trees and median-joining networks to examine systematic relationships, and conducted Bayesian analysis of the coalescent and used mismatch distributions and approximate Bayesian computation to infer demographic history.

Results Estonia had the highest nucleotide diversity, and western Belarus had the highest haplotype diversity. We observed four regional populations from the samova analysis. We found three haplogroups in European moose, probably representing lineages conserved in different refugia during the Pleistocene. European moose underwent spatial expansion after the LGM, but did not undergo demographic expansion. The effective population size has declined markedly within the last 2000 years.

Main conclusions The current levels and distribution of genetic diversity in European moose indicate the effects both of Pleistocene glaciations and of a recent bottleneck, probably associated with anthropogenic influences such as pastoralization and hunting, and a very recent re-expansion. We show that both historical and recent events can influence the diversity and distribution of a large mammal on a large scale.

Keywords Anthropogenic influences, bottleneck, effective population size, expansion, genetic structure, Last Glacial Maximum, mtDNA, north-eastern Europe, phylogeography, refugia.
INTRODUCTION

Quaternary ice ages played a significant role in shaping modern biodiversity by isolating populations in refugia, where genetic lineages diverged and from where post-glacial colonizations started (Stewart & Lister, 2001). Determining whether organisms residing in various refugia successfully recolonized northern habitats informs us about the role of barriers in range shifts and the sources of modern genetic diversity (Klütsch et al., 2012). For temperate species, it is easier to trace range shifts, because species still reside within their refugia. This is in contrast to Arctic and sub-Arctic organisms, which no longer inhabit southern refugial locations, and may have inhabited cryptic northern refugia (Stewart & Lister, 2001). The refugial sources of modern populations can only be inferred or confirmed from fossil evidence and the fate of refugial populations must be determined from modern patterns of genetic diversity on the landscape.

Fossil records indicate that moose, Alces alces (Linnaeus, 1758), existed during the Last Glacial Maximum (LGM; 19–27 ka) in refugia located in northern Italy, the north-western Balkans, and in the more northerly areas of the modern-day Czech Republic and Moldova (Sommer & Nadachowski, 2006), but were probably absent from the Iberian Peninsula (Sommer & Nadachowski, 2006). The range of moose, although probably fragmented, could have been large even during the LGM, based on studies that have confirmed that large areas of eastern and south-eastern Europe had suitable climate and habitats for many boreal species (Markova & Simakova, 1998; Sommer & Nadachowski, 2006; Markova et al., 2009).

After the LGM, moose populations expanded to colonize almost the whole continent. The maximum range of moose in the early Holocene covered Europe from the Pyrenees through eastern and central Europe to Great Britain and Denmark (Schmölcke & Zachos, 2005). Following the Preboreal stage, moose populations declined and died out in most of the species’ range until the Middle Ages (Schmölcke & Zachos, 2005). During this time, however, moose were still numerous in Eastern Europe (Filonov, 1983). The range of moose in Europe was at its smallest in the middle of the 19th century and at the beginning of the 20th century (Filonov, 1983; Dziczołowski & Pielowski, 1993; Schmölcke & Zachos, 2005); at that time, moose survived only in some areas in Scandinavia, Poland, Belarus, Latvia and Lithuania (Karcov, 1903; Mager, 1941; Ryman et al., 1977; Filipov, 1983; Nyrgrén, 1987; Dziczołowski & Pielowski, 1993; Krawczyk, 2010). In the 19th–20th centuries, the only supposedly large moose population in Europe existed in Russia (Filonov, 1983). After World War II, their numbers started to increase (Dziczołowski & Pielowski, 1993; Homolka, 1998) and today the continuous range of European moose covers the northern, central and eastern parts of the continent (Fig. 1). Small, isolated populations of moose exist in the Czech Republic (Homolka, 1998) and migrating individuals have also been recorded in south-eastern Germany (Schönfeld, 2009).

Unsuccessful translocations of moose in Europe occurred in Germany (Schönfeld, 2009). In the mid-20th century, several moose were successfully translocated to the Kampinos Forest, central Poland (Kowalski et al., 2003) and after their re-introduction, the population increased and began to disperse to other areas in Poland and neighbouring countries (Dzięciołowski & Pielowski, 1993).

There have been no comprehensive analyses of the genetic diversity of moose in Europe. Hundertmark et al. (2002) described variation in mtDNA at the Palaearctic scale, but their sample size from Europe (mainly from Scandinavia) was small (19 samples). Nonetheless, they reported limited mitochondrial diversity. Smaller-scale studies have been carried out in Poland (Świslocka et al., 2008), Scandinavia (Ryman et al., 1980; Charlier et al., 2008; Haanes et al., 2011) and Russia (Rozhkov et al., 2009). Aside from the evolutionary history of moose, demographic bottlenecks, human intervention and current ecological factors could have had an impact on the present genetic diversity of moose, as has been reported for other species (Hoelzel et al., 1998; Dalén et al., 2005; Pilot et al., 2006; Jędrezewski et al., 2012). The genetic diversity of the species worldwide is rather low, probably due to several bottlenecks in its history (Hundertmark et al., 2002).

Our study is the first to present data on the genetic variability of European moose populations at a continental scale. The aims of our study were: (1) to describe the genetic diversity of mtDNA of moose over its entire European species range; (2) to compare the present genetic structure of moose with the history of the species in Europe; and (3) to identify the possible refugial areas where moose could have survived the LGM, and assess the roles of LGM refugia and more recent anthropogenic factors in maintaining and shaping the current genetic variability of European moose.
MATERIALS AND METHODS

Sampling, DNA extraction and genotyping
The sampled area consisted of 10 European countries – Belarus, Estonia, Finland, Latvia, Lithuania, Norway, Poland, Russia, Sweden and Ukraine – and covered most of the continuous European range of moose (Fig. 1). We collected and analysed 657 samples from 16 localities (groups) (Table 1). With the exception of samples from Poland, tissue samples were collected from legally hunted animals. In Poland, where moose hunting does not occur, tissue samples were collected from animals that died on the roads or through natural mortality. We stored all samples at −20 °C prior to DNA extraction. We extracted DNA from tissue samples (e.g. muscles, internal organs or skin) using commercial kits (DNeasy Blood and Tissue Kit; Qiagen, Wrocław, Poland) according to the manufacturer’s instructions.

Table 1 Characteristics of the studied moose (Alces alces) populations in Europe calculated using all analysed samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of the local population</th>
<th>n</th>
<th>Na</th>
<th>π</th>
<th>Hd</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>South-central Norway</td>
<td>24</td>
<td>4</td>
<td>0.008</td>
<td>0.70</td>
<td>3.06</td>
</tr>
<tr>
<td>2</td>
<td>Northern Norway</td>
<td>7</td>
<td>2</td>
<td>0.003</td>
<td>0.29</td>
<td>1.32</td>
</tr>
<tr>
<td>3</td>
<td>Northern Finland</td>
<td>22</td>
<td>4</td>
<td>0.009</td>
<td>0.67</td>
<td>2.78</td>
</tr>
<tr>
<td>4</td>
<td>Southern Finland</td>
<td>52</td>
<td>4</td>
<td>0.003</td>
<td>0.28</td>
<td>1.38</td>
</tr>
<tr>
<td>5</td>
<td>Sweden</td>
<td>46</td>
<td>2</td>
<td>0.000</td>
<td>0.04</td>
<td>1.04</td>
</tr>
<tr>
<td>6</td>
<td>Estonia and Latvia – all samples</td>
<td>52</td>
<td>6</td>
<td>0.019</td>
<td>0.60</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>Estonia and Latvia – without H5</td>
<td></td>
<td>50</td>
<td>6</td>
<td>0.019</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Estonia and Latvia – without H5 and H15</td>
<td>48</td>
<td>5</td>
<td>0.009</td>
<td>0.57</td>
<td>2.20</td>
</tr>
<tr>
<td>7</td>
<td>Western Russia (Leningrad, Novgorod and Pskov regions)</td>
<td>78</td>
<td>7</td>
<td>0.007</td>
<td>0.58</td>
<td>2.35</td>
</tr>
<tr>
<td>8</td>
<td>Northern Belarus, Lithuania</td>
<td>110</td>
<td>8</td>
<td>0.008</td>
<td>0.64</td>
<td>2.74</td>
</tr>
<tr>
<td>9</td>
<td>South-eastern Belarus and northern Ukraine</td>
<td>13</td>
<td>4</td>
<td>0.011</td>
<td>0.68</td>
<td>2.68</td>
</tr>
<tr>
<td>10</td>
<td>Western Belarus and eastern Poland</td>
<td>27</td>
<td>7</td>
<td>0.014</td>
<td>0.86</td>
<td>5.84</td>
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<tr>
<td>11</td>
<td>North-eastern Poland and westernmost Russia (Kaliningrad region)</td>
<td>46</td>
<td>3</td>
<td>0.012</td>
<td>0.54</td>
<td>2.14</td>
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<tr>
<td>12</td>
<td>South-eastern Poland and western Ukraine (Polesie)</td>
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<td>0.009</td>
<td>0.67</td>
<td>2.75</td>
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<td>13</td>
<td>Central Poland</td>
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<td>6</td>
<td>0.009</td>
<td>0.50</td>
<td>1.95</td>
</tr>
<tr>
<td>14</td>
<td>East of European Russia</td>
<td>66</td>
<td>4</td>
<td>0.005</td>
<td>0.46</td>
<td>1.82</td>
</tr>
<tr>
<td>15</td>
<td>North of European Russia</td>
<td>40</td>
<td>2</td>
<td>0.004</td>
<td>0.36</td>
<td>1.54</td>
</tr>
<tr>
<td>16</td>
<td>Central Russia (Nizhny Novgorod, Vladimir and Tver regions)</td>
<td>16</td>
<td>2</td>
<td>0.006</td>
<td>0.50</td>
<td>1.88</td>
</tr>
<tr>
<td>Entire study area (all haplotypes)</td>
<td>657</td>
<td>17</td>
<td>0.013</td>
<td>0.73</td>
<td>3.74</td>
<td></td>
</tr>
</tbody>
</table>

n, number of samples; Na, number of haplotypes; π, nucleotide diversity; Hd, haplotype diversity; B, index of haplotype diversity (after Levins, 1968).

We amplified a fragment of the mitochondrial control region (538 base pairs, bp) following Hundertmark et al. (2002, 2003). We performed sequencing reactions using Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA), and then purified the products using ExTerminator (A&A Biotechnology, Gdynia, Poland). We carried out detection of sequencing reaction products on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequencing results were edited and aligned using BioEdit (Hall, 1999).

Statistical analyses
Basic parameters for mtDNA sequences for all moose groups (number of haplotypes, Na; number of polymorphic sites, S; haplotype diversity, Hd; nucleotide diversity, π) were calculated using MEGA 5.05 (Tamura et al., 2011) and ARLEQUIN 3.5 (Excoffier & Lischer, 2010). The diversity of haplotypes was also expressed as B (Levins, 1968), using the formula

\[ B = \frac{1}{\sum p_i^2} \]

where \( p_i \) is the proportion of samples with haplotype \( i \) in a population. The diversity index \( B \) differs from \( H_d \) (which varies from 0 to 1) in that its minimum value is 1 and its upper bound is equal to the number of haplotypes in the sample. \( B \) can thus be interpreted as the effective number of haplotypes in the sample. Population-specific values of \( B \) and \( \pi \) were mapped and values were extrapolated to create spatial representations of diversity. We used the inverse-distance weighting method implemented in the program ArcGIS 9.3.1 (ESRI, Redlands, CA, USA), which assigns values to unknown points by multivariate interpolation of a known set of scattered points. The values of unknown points are computed with a weighted average of the values at the known points.

To present the phylogenetic and phylogeographical relationships among the haplotypes found in the study area, we created a median-joining network using Network (Bandelt et al., 1999). Networks are generally better suited to depicting intraspecific phylogenies than tree algorithms, because they allow for the coexistence of ancestral and descendant alleles in a sample, whereas trees treat all sequences as terminal taxa (Posada & Crandall, 2001). We also created a maximum-likelihood tree with the software MEGA 5 (Tamura et al., 2011) using the molecular clock option. We used mutation rates of 3.14 × 10⁻⁷ substitutions per site per year (Bradley et al., 1996) and 3.93 × 10⁻⁷ substitutions per site per year (Burzyńska et al., 1999) for consistency with the dates estimated by Hundertmark et al. (2002) and Hundertmark & Bowyer (2004). We rooted the tree with a sequence from Siberian moose samples collected in Yakutia (Eastern Siberia, Russia).

Population structure of mtDNA was assessed using spatial analysis of molecular variance, implemented in the software SAMOVA 1.0 (Dupanloup et al., 2002) and based on
maximizing the intergroup portion of genetic variability, $F_{CT}$. SAMOVA calculates genetic structure based on the genetic data and the geographical locations of populations, and requires an a priori definition of the number of groups ($K$). We ran SAMOVA for values of $K$ from 2 to 15. To check that the results were consistent between runs, we ran analyses twice for each $K$-value. Haplotype frequencies, genetic differentiation ($Q_{ST}$), and the numbers of migrants among the subpopulations found by SAMOVA were computed using ARLEQUIN 3.5.

We tested for evidence of an expansion or a bottleneck in the groups of populations by evaluating estimates of Fu’s $F_{S0}$, which is negative after a population expansion and positive after a bottleneck. We used the output from SAMOVA to guide our delineation of populations. Significance was determined by 1000 permutations in ARLEQUIN. The significance level for the entire sample was determined by Fisher’s combined probability test (Fisher, 1932), which combines significance values for individual population groups to test a global hypothesis of expansion or bottleneck.

We tested for spatial (Ray et al., 2003; Excoffier, 2004) and sudden demographic (Rogers & Harpending, 1992) expansions by analysing mismatch distributions in ARLEQUIN. Observations were compared to model predictions based on 10,000 permutations of the data. If observations did not differ significantly from model predictions, we estimated $t$ from the data and calculated the age of expansion ($t$) using the equation $t = 2\mu t$, where $\mu$ is the mutation rate of the sequence, for which we used the rate of $3.14 \times 10^{-7}$ substitutions per site per year multiplied by the number of sites in the sequence. Because $\mu$ is expressed in generations, we used an estimate of 6 years for generation time, based on the median of multiple estimates provided by Gaillard (2007).

We estimated temporal trends in $N_e$ from tree shape using a Bayesian coalescent approach implemented in BEAST 1.7.4 (Drummond et al., 2012) and visualized in TRACER 1.5 (available at: http://beast.bio.ed.ac.uk/Tracer). The populations identified by SAMOVA were used as inputs to comply with the assumption of panmixia. We evaluated models of constant population size, skyline plots (Drummond et al., 2005) and skyride plots (Minin et al., 2008) with uniform smoothing. Models were run for $10^7 \times 10^{11}$ Markov chain Monte Carlo (MCMC) states with the first 10% removed as burn-in. Where effective sample sizes (ESS) for model parameters were below 200, additional independent runs were conducted and the results combined until the ESS exceeded 200. The most appropriate model of molecular evolution for our data (HKY+I) was determined with jModelTest (Posada, 2008). We used a mutation rate of $3.14 \times 10^{-7}$ and allowed rate variation among branches using a lognormal relaxed molecular clock (Drummond et al., 2006). The default values of the MCMC operators were used, with auto-optimization. Model outputs were compared by means of Bayes factors and the most likely model was selected for further analysis. We conducted a model run with no data to sample from the prior only, in order to ensure that the prior did not unduly influence our results.

We evaluated different models of population demographic history to determine if populations were independent prior to the LGM or whether they split after emerging from a common refugium (see Appendix S1 in Supporting Information). We used approximate Bayesian computation (ABC) methods in DIYABC 2.0.3 (Cornuet et al., 2008) in a two-phase approach to estimate posterior probabilities of models of population history. First, we simulated a single population consisting of all of our samples to determine if a bottleneck was most likely. We modelled scenarios of a late bottleneck (within the last few hundred years), an early bottleneck (LGM), two bottlenecks (early and late), and no bottlenecks. The results from that analysis and SAMOVA informed our subsequent analysis of four demographic models, each generating three populations. Our first model simulated a single splitting event generating two populations followed by a second split, simulating the gene tree (see Results). A second model simulated a simple three-way split. The third model simulated a three-way split, followed by two episodes of gene flow between populations to create three mixed populations. The fourth model was similar to the first except that we simulated gene flow between only two of the populations. All models incorporated early and late bottlenecks (see Results and Appendix S1).

**RESULTS**

**Haplotype and nucleotide diversity**

We identified 17 mtDNA haplotypes among 657 moose (Table 1); 35 of 538 (6.5%) sites were polymorphic. Two haplotypes found in two individuals from Estonia were characterized by the 75-bp deletion found in moose in eastern Siberia and North America (Hundertmark et al., 2002). One of those haplotypes (H15) was otherwise identical to haplotype H2, whereas the other (H5) was the only haplotype that was similar to Siberian and North American haplotypes. Given the possibility of undocumented translocations of moose from eastern Asia and the fact that indels tend to disproportionately affect diversity indices, we present diversity indices for Estonia both with and without those haplotypes (Table 1).

The number of haplotypes was correlated with sample size ($r = 0.58$, $P = 0.018$) and was therefore not analysed as an index of diversity. Haplotype diversity and $B$ were clearly greatest in population 10, from western Belarus and eastern Poland. Because these two indices were correlated ($r = 0.78$, $P = 0.0003$), we limited further analysis to $B$. Hotspots of diversity existed in three areas: southern Norway, northern Scandinavia, and the Poland–Belarus area (Fig. 2). Estonia had the greatest nucleotide diversity, but this was largely influenced by the presence of the haplotypes with the 75-bp deletion. Excluding those haplotypes, the nucleotide diversity was greatest in populations 9–11, in the southern part of the range, which included Belarus, Poland, Ukraine and Kaliningrad Region (Table 1, Fig. 2). A second area of high
nucleotide diversity was found in northern Scandinavia (Fig. 2). Nucleotide diversity was the only metric that was correlated with latitude ($r = 0.6$, $P = 0.014$), with diversity decreasing northwards, and only if the two haplotypes with the 75-bp deletion were eliminated from the analysis (Appendix S2).

Phylogeography

Three primary clades (eastern, western and central) were represented in European moose (Fig. 3). A fourth clade was represented by moose with the 75-bp deletion in the median-joining network and by the Asia–America clade in the phylogenetic tree. These representations differ because haplotype H15 is identical to H2 apart from the deletion. The clades are distributed with a strong geographical pattern, representing western, central and eastern moose (Fig. 4). The application of a molecular clock to the tree indicates that the three primary clades diverged 20,000–35,000 years ago.

Population structure

The samova analysis showed that with the highest probability the European moose population divided into four clusters, although a solution for three populations was nearly as probable (Appendix S2). When $K = 4$, one population occurred in southern and central (SC) Norway, the second in central (C) Scandinavia, the third in C Poland, and the fourth – the largest – covered the rest of study area in eastern (E) Europe (Fig. 5). When $K$ was reduced to three, the two Scandinavian clusters merged into one (Fig. 5).

Genetic differentiation among the four subpopulations was high, indicating low levels of gene flow (one effective migrant every 2–4 generations) or no gene flow at all (Table 2). The migration rate was largest between C Poland and E Europe (Table 2). This agrees with the results of the phylogeographical analyses, which showed a large overlap between different clades of moose occurring in this part of Europe (Figs 4 & 5). samova distinguished the subpopulation in C Poland because of the significant proportion of haplotype H11, belonging to the western clade, in this group (see Appendix S2).
Demographic history of European moose

The haplotype network (Fig. 3) does not show a star-like pattern in any of its clades, indicating no recent demographic expansion. Mismatch distributions for C Poland and E Europe did not conform to the demographic expansion model, but fitted expectations under the spatial expansion model (Table 3). SC Norway did not differ from the demographic expansion model and C Scandinavia met the expectations of both models. When SC Norway and C Scandinavia were combined into a single population (i.e. \( K = 3 \) in Samova), all populations met expectations under the spatial expansion model but not the demographic expansion model. Estimates of \( \tau \) for the combined Scandinavian populations and E Europe were very similar, corresponding to expansion estimates of approximately 19,600 and 19,900 years ago. The estimate for Poland was older, corresponding to an expansion at 28,800 years ago, but the confidence intervals were wide enough that we could not detect any significant differences between estimates (Table 3).

Estimates of \( FS \) were positive for all three population groups (\( K = 3 \) under Samova) and the value for C Scandinavia was significantly greater than zero (\( FS = 4.33; P = 0.045 \)). The other groups were also close to this level (E Europe, \( FS = 7.69; P = 0.054 \); Poland–Belarus, \( FS = 4.60; P = 0.053 \)). Fisher’s method generated a global \( P \) of 0.006, which provides strong evidence that at least one bottleneck has occurred in European moose at some point in the past.

The Bayesian skyline model was ranked best by a \( \log_{10} \) Bayes factor of 11.8 in comparison with the constant population size model and 4.5 in comparison with the skyrise model, indicating overwhelming support for the skyline model over these competing models (Kass & Raftery, 1995). The skyline plot shows no early drop in \( N_e \); rather, it shows a constant population size until a decline in very recent times, starting approximately 3000–3500 years ago (Fig. 6). This decline continues into the modern era. Assuming a generation time of 6 years, we estimate the historical effective population size of European moose to be approximately 10,000, declining to 1750 today. This result is without doubt largely influenced by the E Europe population, due to its far greater sample size than the other three populations. Results for the three smaller groups indicate either that their populations remained constant or that there is not enough signal in the data (results not shown) to favour a model of non-constant population size, perhaps due to small sample size. Under the Bayesian framework, the time to the most recent common ancestor (TMRCA) for the two Scandinavian populations under a skyline model was 27,451 years (95% HPD, 6583–62,293), whereas the Polish population had a TMRCA of 50,306 (20,852–85,789) years and E Europe had a TMRCA of 52,597 (25,207–91,846) years.

ABC simulations for the single-population model demonstrated that the model with both early and late bottlenecks was the most probable, although the model with only a late bottleneck also had substantial support (Appendix S1). All models of population splitting therefore incorporated two bottlenecks. The most probable model was model 4, simulating a single three-way split followed by gene flow between
the eastern and central populations, although there was also support for model 1, which represented the gene tree (Appendix S1). The median estimate of population splitting was 27,960 years ago (95% highest posterior density interval, HPDI; 13,560–57,000 years ago), which is consistent with our estimate of lineage formation from the maximum-likelihood tree and corresponds to the youngest estimate for the TMRCA of our populations. The median estimate for the timing of the recent bottleneck is 1842 (95% HPDI, 702–2376) years ago.

**DISCUSSION**

**Genetic diversity of European moose compared to other populations**

There are three major lineages of moose in the world: Asian, European and American (Hundertmark *et al.*, 2002). The Asian lineage is the oldest, with the other two lineages hav-

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**Figure 5** Genetic structure of moose (*Alces alces*) populations in Europe, calculated using *samova*.

**Table 2** Above diagonal, genetic divergences ($\Phi_{ST}$) calculated among the pairs of mtDNA subpopulations of moose (*Alces alces*) in Europe. For all pairs, $P < 0.001$. Below diagonal, number of migrants per generation among all pairs of subpopulations corrected for a small number of demes and assuming that only adjacent populations exchange migrants.

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>C Scandinavia</th>
<th>SC Norway</th>
<th>E Europe</th>
<th>C Poland</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Scandinavia</td>
<td>0.46</td>
<td>0.56</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>SC Norway</td>
<td>0.39</td>
<td>0.48</td>
<td>0.31</td>
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<tr>
<td>E Europe</td>
<td>0.26</td>
<td>—</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>C Poland</td>
<td>—</td>
<td>—</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 3** Statistics for model fit of mismatch distributions (sum of squared deviations, SSD) and estimates of expansion times for the sudden demographic expansion model and the spatial expansion model for moose (*Alces alces*) in Europe. Times of expansion (years ago) and their associated 95% confidence interval (CI) are given only for those instances where the particular model is appropriate for that population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>Central Poland</th>
<th>South-central Norway</th>
<th>Central Scandinavia</th>
<th>Eastern Europe</th>
<th>South-central Norway + central Scandinavia</th>
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<td>Expansion time (95% CI)</td>
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<td></td>
<td></td>
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<td>SSD</td>
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<td>Expansion time (95% CI)</td>
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<td></td>
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*Observed distribution of mismatches differs significantly from model expectations.
The presence of three geographically distinct clades informs our understanding of moose colonization within Europe after the LGM. We hypothesize that moose populations expanded from three refugia, maintaining a large effective population size during the spatial expansion. The evidence of expansion provided by the mismatch distribution implies a spatial rather than demographic expansion (Table 3), and the skyline plot appears to confirm this (Fig. 6). We do not know, however, the effects of the recent bottleneck on these analyses and it may be that this bottleneck has erased any demographic signal from earlier times. We therefore cautiously infer a spatial expansion rather than a demographic expansion after the LGM.

The range of moose was greatest in the early Holocene and covered most of continental Europe, from central France to the Russian Plain and south-east to the Caucasus Mountains (Schmölcke & Zachos, 2005; Sommer & Nadachowski, 2006). During the Holocene, moose disappeared from Western Europe and, by 2000 yr BP, the western border of its range was located in present-day Germany (Schmölcke & Zachos, 2005). Until the 19th–early 20th centuries, when the range of European moose was at its smallest, the only large populations survived in eastern Europe (present-day Belarus, Baltic states and Russia; Filonov, 1983; Dziciolowski & Pielowski, 1993; Schmölcke & Zachos, 2005). The changes in distribution and numbers during the Holocene, caused by both natural and anthropogenic factors, make it difficult to reconstruct the recolonization routes of moose after the LGM.

The large effective population size of moose throughout the millennia of the LGM suggests that the numbers of moose and their geographical range were both large during the LGM. Fossil remains of moose dated to the LGM have been found in northern Italy, Slovenia, Croatia, the Czech Republic and Moldova (Sommer & Nadachowski, 2006). Habitats suitable for moose were, however, more widespread than the scarcity of fossils may suggest. Based on dated macrofossil charcoal from Central and Eastern Europe, Willis & van Andel (2004) reported that, during the period 32–16 ka, boreal forests with pines (Pinus spp.), spruce (Picea spp.), larch (Larix spp.), willow (Salix spp.) and birch (Betula spp.) covered large areas of contemporary Hungary, Romania, Moldova and the Czech Republic, and even occurred in southern Poland. Markova & Simakova (1998) and Markova et al. (2009) report evidence that boreal forests occurred across the Russian Plain during the LGM. Species distribution modelling for 22 European boreal and nemoral trees has also suggested that climatic conditions suitable for boreal forests existed across continental Europe from France and northern Italy to Bulgaria, Romania, Ukraine and into the Russian Plain (Svenning et al., 2008) during the LGM. Thus, newly emerging data and syntheses show that the traditional paradigm of the three main LGM refugia in the southern European peninsulas (Iberian, Italian and Balkan; Taberlet et al., 1998) may not apply to boreal species such as moose.

The results of ABC analyses allowed us to determine the most probable model of demographic changes of the European moose population. The most likely scenario was that the moose population experienced two bottlenecks: one dur-

**Figure 6** Bayesian skyline plots of temporal trend in effective population size for European moose (*Alces alces*).
The effect of the bottleneck is most visible in the part of Russia (Karcov, 1903; Mager, 1941; Filonov, 1983; Belarus, whereas larger populations occurred in the European temporary Poland (the Biebrza River valley), the Baltic states and north-west (to Finland) after deglaciation. The central clade may have originated from the Carpathians and/or the northern Balkans. One of the haplotypes in this clade (H1) was the most common in the Biebrza River valley (NE Belarus) and was identified as a relict haplotype, one of the oldest in Europe (Świslocka et al., 2008). Interestingly, this haplotype was also found in ancient moose DNA from Georgia (M. Meiri and A. Lister, Natural History Museum, London, pers. comm.). Moose of the western clade most probably survived in continental Western Europe (France and as far north as Belgium; Stewart & Lister, 2001), and spread towards the east (to Belarus) and north-east (to the Scandinavian peninsula) after the retreat of glaciers. The colonization of Scandinavia was made possible by a large land bridge that existed between 10 and 8 ka, joining contemporary Denmark with southern Sweden (Rjörck, 1995). Analysis of ancient DNA is needed to verify our proposed scenario of moose history during the LGM and Holocene. Nonetheless, it is now clear that representatives of the eastern clade are the most widespread and numerous, those of the western clade survived only in newly colonized areas and not in refugial areas, and moose belonging to the central clade have barely escaped extinction in historical times (see below) and are now the least numerous clade of European moose.

Recent history in Europe

Our genetic data confirmed the recent bottleneck in European moose populations (Fig. 6, Appendix S1). A significant decrease of moose numbers and shrinkage of their western range began in historical times, probably associated with hunting, pastoralization and anthropogenic habitat fragmentation (Schmöckle & Zachos, 2005). This was also confirmed by the ABC analyses, which showed that the recent bottleneck started about 1800 years ago (Appendix S1). In the late 19th and early 20th centuries, moose survived only in some areas of Fennoscandia (Ryman et al., 1977; Nyrgrén, 1987), contemporary Poland (the Biebrza River valley), the Baltic states and Belarus, whereas larger populations occurred in the European part of Russia (Karcov, 1903; Mager, 1941; Filonov, 1983; Krawczyk, 2010). The effect of the bottleneck is most visible in the central population, which consisted of only four haplotypes, and where the majority of individuals carried a single haplotype (H1). Moose belonging to the central population are likely to have originated from very small populations that survived in north-eastern Poland and Belarus (Karcov, 1903; Dzieciolowski & Pielowski, 1993; Świslocka et al., 2008). The past bottleneck is also well documented in Fennoscandian moose of the western population. During the 19th and early 20th centuries, moose survived only in several small and fragmented populations in Norway, Sweden and Finland (Ryman et al., 1977; Nyrgrén, 1987; Haanes et al., 2011). The bottleneck was least pronounced in the area inhabited by the eastern population, which embraces the largest part of the contemporary range. Even if the number of moose in eastern Europe also decreased in the 19th century, that population would still have been larger (see Filonov, 1983) than those in Central Europe and the Scandinavian peninsula.

The origin of haplotype H5 (Asian–American haplotype), found in two individuals from Estonia, is unclear. It might result from long-distance dispersal, natural introgression of the mtDNA of Asian–American moose into European moose, or of the translocation of moose by humans. The last explanation seems the most plausible, as such cases have been well documented (Karpinski, 1951; Dzieciołowski & Pielowski, 1993; Whitehead, 1993); the genetic effects of translocations have previously been revealed in European red deer (Niedzialkowska et al., 2011, 2012). The origin of haplotype H15 – the other haplotype with the 75-bp deletion – is likely to differ from that of H5, because it is otherwise identical to haplotype H2, which is clearly of European origin. Multiple independent origins of similar indels have been documented in deer (Purdue et al., 2006), demonstrating that the presence of the indel alone is not a reliable phylogenetic marker.

The genetic structure in Europe shown by samova analyses (Fig. 5) reflects both the evolutionary history of moose during and after the LGM and more recent anthropogenic effects. Both samova subpopulations from Scandinavia belonged to the western clade, and the third subpopulation was composed mainly of individuals from the eastern clade. The software did not distinguish individuals from the central clade (the least numerous; Fig. 4) as a separate subpopulation, and combined them with the eastern clade (Fig. 5). Instead, samova differentiated a small population from central Poland, which largely included descendants of moose reintroduced to Kampinos National Park in the 1950s (Dzięciołowski & Pielowski, 1993). The animals used for the reintroduction programme originated from Belarus (Kowalski et al., 2003). It is interesting that haplotype H11, dominating in the subpopulation from central Poland, is one of the two rare haplotypes belonging to the western clade that were found only in a few individuals from Central and Eastern Europe. They could be remnants of the western clade, which naturally occurred in this part of Europe in the past.

In conclusion, the present genetic diversity and population structure of moose in Europe have been shaped by survival during the LGM, range shifts during the Holocene, and the recent impact of humans. Our study provides evidence of the important role of eastern refugial areas in shaping the genetic diversity of European mammals, supporting results obtained for roe deer (Capreolus capreolus; Sommer et al., 2010).
2009). Moreover, the current sites of genetic hotspots are likely to represent refugial areas from recent bottlenecks and areas of convergence of the expanding refugial populations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplemental methods and results for approximate Bayesian computation.
Appendix S2 Supplemental results.

DATA ACCESSIBILITY

The mtDNA sequences described in this study can be found in GenBank http://www.ncbi.nlm.nih.gov with the following accession numbers: KJ831595–KJ831611.

BIOSKETCH

Magdalena Niedziałkowska is a scientist at the Mammal Research Institute, Polish Academy of Sciences in Białowieża, Poland. Her interests concern the genetic diversity of mammals (ungulates, large carnivores and rodents) at regional and continental scales, and factors which affect this diversity, including ecological variables, landscape structure, and the history and origin of the studied populations.

Author contributions: M.N., B.J., K.N. and W.J. conceived the ideas and applied for the financial support; K.N., W.J., M.N., V.E.S., R.V., E.J.S., S.L., H.S., V.S., M.S., J.T., I.M.O., R.J., G.A., V.A.B. and E.A.T. collected the samples; M.N., K.J.H. and B.J. analysed the data and wrote the paper; M.G. performed the GIS analyses and prepared the maps; all authors contributed to data interpretation and revising the manuscript.

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