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Mitochondrial DNA diversity in the moose, *Alces alces*, from northeastern Poland: evidence for admixture in a bottlenecked relic population in the Biebrza valley

Magdalena Świsłocka[1,*], Mirosław Ratkiewicz[1], Anetta Borkowska[1], Edward Komenda[2] & Jan Raczyński[1]

[1)] *Institute of Biology, University of Białystok, Świerkowa 20 B, PL-15-950 Białystok, Poland (e-mail: magdaswi@uwb.edu.pl)*
[2)] *Forest Division of Knyszyn, Al. Niepodległości 31, PL-19-101 Mońki, Poland*

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In recently deglaciated areas, studies of mtDNA haplotype diversity have often revealed clear phylogeographic structure for many animal species. Here, we assessed mitochondrial DNA variation of the moose, *Alces alces*, in northeastern Poland. Altogether, four haplotypes were found among 45 moose and the haplotype (*h*) and nucleotide (π) diversity estimates were 0.38 and 0.8%, respectively. The most common haplotype, H1 found in the Biebrza valley, NE Poland was exclusively present in this area and was divergent from the remaining mtDNA haplotypes in the European moose lineage. Our results indicate that the moose population in the Biebrza valley experienced severe bottleneck and could be regarded as a relic group of moose that is very distinct from others in Europe. We also found evidence for population admixture due to immigration, both, in the Biebrza valley and in Poland, in general.

Introduction

The patterns of mitochondrial DNA haplotype diversity have been used for inferring historic population processes and subdivided clades of mtDNA lineages that show little or no geographical overlap were detected for many animal species in Europe and North America (Avise 2000). This could be due to cycles of glacials and interglacials that resulted in range fragmentations and population expansions, respectively. For example, Hundertmark *et al*. (2002) described worldwide patterns of mtDNA haplotypes in the moose, *Alces alces*. Molecular data suggest Asia as the region of origin of all extant lineages of moose and three distinct mtDNA lineages, showing very little geographical overlap, have been detected in Europe, Asia and North America (Hundertmark *et al*. 2002). Since the earliest fossil remains of *Alces alces* in Eurasia were dated at approximately 100 000 years ago (Lister 1993), the observed mtDNA divergence must have been of recent origin. Hundertmark *et al*. (2002) explained that low mitochondrial DNA diversity in the moose worldwide is a result of repeated bottlenecks induced by climate oscillations. Hundertmark *et al*. (2003) also revealed a high degree of regional popu-

lation differentiation. For example, haplotypes in Sweden were very distinct from those in Finland (Hundertmark *et al*. 2002). The authors hypothesized that the European moose suffered a recent reduction of effective population size after a late-Pleistocene expansion. In addition, there was a marked decline in the number of moose at the beginning of the 15th century in northern Europe (Markgren 1974). In the 18th and 19th centuries the species was almost extirpated in Norway, Sweden and Finland. The same situation was observed in Poland, and in the middle of the 19th century the moose was only observed in the Biebrza river valley, NE Poland (Brincken 1826). Gębczyńska and Raczyński (2004) hypothesized that this moose population could be a relic group of its previous Holocene range that split very early from the main wave of colonization. The Biebrza river valley is a single northeastern–southwestern ice-marginal valley (53°10´–53°40´N, 22°25´–23°30´E). It is surrounded from the east, the south and the west by morainic plateaux of the last glaciation. It is a large area of wetlands preserved in its natural state (the Biebrza National Park). The wetlands of the Biebrza valley are the largest and most valuable natural complexes of low bogs in central Europe (Okruszko 1990). Ten to twenty moose individuals that survived the Second World War in the Biebrza valley were probably a founding group for the present-day population in Poland. Timely management efforts have allowed this population to increase in numbers and to expand. At present, the moose population in the Biebrza valley is the largest (about 600 individuals in 2004) and the most important population in Poland (Sieńko 2004). In addition, five individuals were successfully translocated from Belarus to the Kampinoski National Park in 1951 (Dzięciołowski & Pielowski 1993). These moose were founders of a contemporary population in central Poland. Moreover, it is highly probable that natural immigration of moose from eastern and/or northern Europe to Poland occurs. As a result, the number of moose in Poland reached its maximum of 6200 individuals in 1981 (Dzięciołowski & Pielowski 1993). It is interesting to study the genetic structure of moose in Poland because they have been affected by both reductions in population size and by admixtures. Unfortunately, so far such studies have not been performed. Thus, the aims of our study were: (i) to estimate mtDNA diversity of the moose population in Poland; (ii) to compare our results with previous studies of the moose in Eurasia; and (iii) to determine whether the moose population in the Biebrza valley is a relic group.

Material and methods

Sampling and individuals analyzed

We analyzed 45 moose samples from Poland. The majority of these individuals ($N = 39$) were from the Biebrza river valley, NE Poland. The remaining six samples were collected in the vicinity of the Narew National Park, 53°10´N, 22°40´E ($N = 3$), the Knyszyńska Forest 53°10´N, 23°50´E, about 1 km from the Belarusian boundary ($N = 1$), the Piska Forest, 53°43´N, 21°30´E ($N = 1$) and the Kampinoski National Park near Warsaw, 52°20´N, 20°40´E ($N = 1$). Total genomic DNA was extracted mostly from animals killed in car accidents, killed by wolves, poached, following a standard protocol supplied with the Genomic Mini Kit (A&A Biotechnology). We used skin and hair bulbs; two stool samples were also taken.

Genetic analyses

The left hypervariable domain of the mitochondrial control region and a potion of the adjoining $tRNA^{thr}$ gene and the entire $tRNA^{pro}$ gene were obtained from all collected samples. Detailed descriptions of the amplification and sequencing processes are provided by Hundertmark *et al*. (2002, 2003). Amplified products were purified with CleanUp system microcolumns (A&A Biotechnology). Cycle sequencing reactions were carried out using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (ver. 3.1, Applied Biosystems). Both primers were used for sequencing both strands. The sequencing products were run on a 3130 Genetic Analyzer (Applied Biosystems). DNA sequences of 518 bp long were aligned in BioEdit ver. 7.0.4 (Hall 1999), and revised manually. For conspicuous

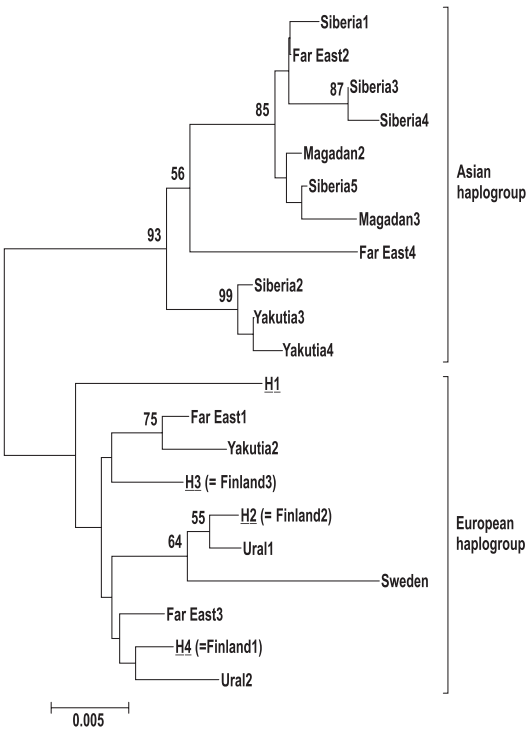

Fig. 1. Phylogenetic relationships among mtDNA haplotypes of the European moose, based on 518 bp sequence revealed by minimum-evolution tree. Haplotypes found in Poland are underlined. Bootstrap values are indicated at nodes if found in more than 50% of the 1000 bootstrap trees. Remaining haplotypes are from Hundertmark *et al.* (2002).

haplotypes, PCR amplification and sequencing were repeated several times. Our 45 mtDNA sequences (4 different haplotypes) have GenBank accession no. EU257814–EU257858. In addition, we compared moose haplotypes from our study with 20 sequences reported by Hundertmark *et al*. (2002, 2003) from Europe and Asia. This allowed us to identify mtDNA haplotypes that were found in previous studies. We also related haplotypes from this study to previously published phylogenetic trees of Eurasian moose haplotypes (Hundertmark *et al*. 2002). Estimates of haplotype diversity (h) and nucleotide diversity (π), and the number of segregating sites were calculated using ARLEQUIN ver. 3.1 (Schneider *et al*. 2000). Phylogenetic relationships among mtDNA haplotypes were determined using a minimum evolution tree constructed in MEGA ver. 3.1 (Kumar *et al*. 2004), using the Kimura-2 parameter model, and confidence in those topologies was assessed by 1000 bootstrap replicates.

Results

We identified four haplotypes among the 45 moose from Poland (Table 1). The most common haplotype (H1), found in 34 individuals (f = 0.75), was present exclusively in the Biebrza valley. The second haplotype (H2) was present in five individuals from the Biebrza valley and was also found in the single sample near the Narew National Park. Thus, as few as two haplotypes (H1 and H2) that differed by 12 substitutions (2.1% sequence divergence) were present in the Biebrza valley. The H2 haplotype was identical with a haplotype from Finland (Finland2; Hundertmark *et al*. 2002). The two remaining haplotypes found in Poland: H3 (the Knyszyńska

Table 1. Measures of intrapopulation variability for the moose samples studied. h = haplotype diversity, π = nucleotide diversity, S = number of segregating sites.

Population	Population size (N)	Number of haplotypes	h (SE)	π (SE)	S
Biebrza	40	2	0.22 (0.08)	0.005 (0.003)	2
Poland (all samples)	45	4	0.38 (0.08)	0.008 (0.004)	15
Finland*	10	3	0.38 (0.18)	0.004 (0.003)	8
Sweden*	6	1	0	0	0
Europe**	58	7	0.61 (0.07)	0.013 (0.007)	23
Asia*	32	19	0.94 (0.03)	0.019 (0.010)	39

*Hundertmark *et al.* (2002), **the pooled sample of the European moose from this study and Hundertmark *et al.* (2002).

Forest, $N = 1$ and the Narew National Park, $N = 2$) and H4 (the Kampinoski National Park, $N = 1$ and the Piska Forest, $N = 1$) were identical with the Finland3 and Finland1 haplotypes, respectively (Fig. 1). The haplotype (h) and nucleotide (π) diversity values for the 45 samples from northeastern Poland were 0.38 and 0.8%, respectively. The corresponding values for moose population from the Biebrza valley were smaller ($h = 0.22$, $\pi = 0.5\%$; Table 1). The number of pairwise differences among haplotypes of the moose in Poland ranged from 2 to 22. Altogether, 15 sites were variable and all substitutions were transitions in our sample. On the other hand, as many as 21 haplotypes were found for the Eurasian moose and they were defined by 45 variable sites. The most common substitutions were transitions (42), two transversions and a single indel were also identified. By combining the data of Hundertmark *et al*. (2002) and ours, the haplotype diversity value decreased and nucleotide diversity value increased for the European moose ($h = 0.61$ and $\pi = 1.3\%$, respectively). The minimum-evolution tree constructed using our sequences and those from the study of Hundertmark *et al*. (2002) identified two main haplogroups: Asian and European (Fig. 1). Eleven haplotypes were assigned to the Asian lineage and ten were specific to the European lineage. All moose haplotypes found in Poland were grouped within European haplogroup. Two European haplotypes exhibited unusually long branches on the tree (Fig. 1). These haplotypes were H1 from Poland and a haplotype possessed by all moose in Sweden. The H1 haplotype from the Biebrza valley was the most distinct from the remaining haplotypes in the European group on the tree (Fig. 1).

Discussion

The moose, *Alces alces*, is a young species in comparison with the evolutionary history of other large mammals (Lister 1993). Despite this, three distinct mtDNA lineages were identified in Europe, Asia and North America (Hundertmark *et al*. 2002). The distribution of these lineages indicates a geographic structure associated with a glacial history. Hundertmark *et al*. (2002) postulated Asia as the region of origin of all extant lineages of moose. Indeed, haplotype and nucleotide diversities were the highest in Asia (Hundertmark *et al*. 2002); *see* also Table 1. Conversely, Europe is characterized by relatively lower h and π values, a signature of a recent reduction in effective population size after a late-Pleistocene expansion (Hundertmark *et al*. 2002). Previous European samples of the moose ($N = 13$, Hundertmark *et al*. 2002) were increased by adding haplotypes found in Poland to $N = 58$. Our results confirmed the low level of mitochondrial DNA diversity of this species in Europe. The new estimate of h (0.61) is even lower than the corresponding value $h = 0.74$ reported by Hundertmark *et al*. (2002). On the other hand, the updated nucleotide diversity (π) of the moose in Europe was slightly greater than the previous one (0.013 *vs* 0.010), most probably due to the occurrence of a distinct lineage in the Biebrza valley, NE Poland. Our study of moose mtDNA diversity in Poland showed much lower h and π values than those in European samples. This is in agreement with Hundertmark *et al*. (2002) who suggested that different Eurasian moose populations have probably passed through a bottleneck. However, the presence of 4 haplotypes in Poland out of 7 recorded in Europe may indicate, that Poland is an important area of moose diversity. Poland seems to be a dispersal corridor for this species. It is remarkable that three rare haplotypes found in Poland were identical with those found in Finland (*see* Fig. 1). Since an individual possessing the H3 haplotype (= Finland3) was sampled very close to the Belarusian boundary, it could be an immigrant from eastern and/or northern Europe. Similarly, the individual sampled in the Kampinoski National Park (KNP) that possessed the H4 haplotype (= Finland1) is likely to be a descendant of females that were introduced to KNP from Belarusia in 1951. This could also be the case for another individual with the H4 haplotype found in the Piska Forest, alternatively it could be an immigrant from eastern countries. The fact that three haplotypes (H2, H3, H4) were shared with Finnish moose could suggest that they are possibly common haplotypes found in the European side of Russia and Belarus, and thus represent immigrants from the east to Poland.

The most common haplotype (H1) found in the Biebrza valley was present only here and it was very distinct from the other haplotypes in Europe. Thus, our molecular data seem to confirm the hypothesis of Gębczyńska and Raczyński (2004) who stated that the moose population in the Biebrza valley, NE Poland, is a relic group of moose that split very early from the main wave of colonization. The presence of highly divergent moose populations within its worldwide range was recorded by Hundertmark *et al*. (2002, 2006). For example, only one haplotype, clearly distinct from the others was found in Sweden. The authors hypothesized that the divergence of that haplotype was a result of drift associated with isolation and it may represent a lineage of moose that colonized the region from the south, perhaps from a different glacial refugium than other lineages in Scandinavia. Similarly, the distribution of haplotypes in Alaska showed a geographic structure related to glacial history and two highly divergent mitochondrial haplotypes with low overlap were recorded (Hundertmark *et al*. 2006). Hofreiter *et al*. (2004) suggested that cycles of retreat of species into refugia during glacial periods followed by incomplete dispersal during the interglacial periods may be responsible for the deep genetic divergences between phylogeographic clusters of mtDNA observed in many species. The genetic structure of the moose population from the Biebrza valley, due to the presence of only two haplotypes that possess highly divergent mtDNA, is very unusual. It is very probable that immigration and admixture of individuals possessing the H2 haplotype (= Finland2) into this relic population occurred. This is quite probable, as the frequency of this haplotype in the Biebrza valley is relatively high (0.15). The rate of admixture must, however, be estimated in the future using nuclear microsatellite loci. We suppose that there could be a contact zone between the two distinct European lineages of moose in the Biebrza valley, and in eastern Poland in general. The contact of genetically differentiated forms from Sweden and Finland have also been proposed (Hundertmark *et al*. 2002) for other species, for example the chequered skipper (*Carterocephalus palaemon*) in eastern Poland (Ratkiewicz & Jaroszewicz 2006) and the field vole in Lithuania (Jaarola & Searle 2002). It is not possible to establish when the immigrant individual(s) possessing the H2 haplotype (= Finland2) entered the Biebrza population. However, it is obvious that the Biebrza valley could not be the place of origin of this haplotype. Before immigration of individuals possessing the H2 haplotype into the Biebrza valley population, there could have been a very low or even no haplotype diversity of moose in this area. Similarly, the presence of a single haplotype in the entire population was found for moose in Sweden (Hundertmark *et al*. 2002). Thus, moose populations in Poland and Sweden may have experienced severe bottlenecks.

There is an opinion that genetically differentiated populations within species should not be merged as they require separate genetic management (Moritz 1995). These populations are referred to as evolutionary significant units (ESU) and moose individuals possessing the H1 haplotype in Poland (the Biebrza valley) could be an example of such a group. There is a need to preserve ESU that have been historically separated as distinct gene pools. On the other hand, such isolated populations could suffer from inbreeding depression. The question arises what are the genetic effects of admixture due to immigration of distinct haplotypes in the moose population in the Biebrza valley. Hofreiter *et al*. (2004) suggested that the existence of phylogeographic patterns of mtDNA in the absence of physical barriers may represent an intermediate state of a spontaneous diffusion process after the removal of a barrier; the past glacial maxima. Thus, no inherent reason exists to assume that the mixing of such "populations" defined by mtDNA clades would have detrimental effects (Hofreiter *et al*. 2004). This may also be true for moose populations, however, before giving any final conclusions, nuclear DNA should be analyzed, which is less likely to show phylogeographic patterns due to their larger effective population size.

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