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The history of Crimean red deer population and *Cervus* phylogeography in Eurasia

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The present distribution of many species is a result of climatic changes during the Pleistocene and human activity. The impact of climate has been accompanied by restrictions of populations into refugia during glacial periods, and subsequent expansions during more favourable conditions, whereas human influence has been associated with hunting practices and translocations. One mammalian species that has been subject to such transformations is the red deer, *Cervus elaphus*, but the exact nature of these changes has been difficult to determine using only modern DNA. In this study, we obtained new cytochrome *b* sequences from subfossil remains of deer found in the Crimean Peninsula. A comparison of these sequences with the available recent and ancient sequences allowed to us to reconstruct phylogeographic relationships between *Cervus* lineages and to determine their potential migration routes at both local and Eurasian scales. Our analyses showed that the Crimean Peninsula was not a glacial refugium for red deer, but rather that red deer colonized Crimea in three independent waves from both Western and Eastern red deer populations. The immigrations were related to local extinctions and replacements of native populations.

ADDITIONAL KEYWORDS: ancient DNA – *Cervus elaphus* – Crimea – phylogeny – phylogeography – Pleistocene – red deer.

INTRODUCTION

During the Late Pleistocene, both environmental change and human activity influenced the distribution and genetic diversity of many species (Hewitt, 1996; Burney & Flannery, 2005; Stewart *et al.*, 2010; Lorenzen *et al.*, 2011; Sandom *et al.*, 2014; Cooper *et al.*, 2015). These factors led to the extinction of many taxa and shaped the modern-day phylogeographic patterns of those that survived. Genetic analyses suggested repeated retreats of ancient populations into refugia during unfavourable conditions and subsequent expansions after a suitable environment stabilized (Hewitt, 1996; Taberlet *et al.*, 1998; Bennett & Provan, 2008).

One of the species subjected to such climatic and environmental change was the red deer (*Cervus elaphus*). Its current distribution is assumed to be strongly influenced by its colonization history during the Late

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Pleistocene and early Holocene and by human activities throughout the Holocene. Phylogeographic analyses showed two main clades of this species (Kuwayama & Ozawa, 2000; Ludt *et al.*, 2004; Skog *et al.*, 2009). Individuals in Asia and North America have been assigned to the Eastern clade, and European, Middle Eastern and African populations belong to the Western clade. These clades represent populations that are adapted to different climatic conditions (Geist, 1998). The European red deer has a mixed feeding strategy, whereas the Eastern deer is a cold-adapted open-country grazer (Geist, 1998). Estimates of divergence times among the Western and Eastern lineages differ among studies and range from less than a million (Kuwayama & Ozawa, 2000; Mahmut *et al.*, 2002; Polziehn & Strobeck, 2002) to 6–7 Mya (Randi *et al.*, 2001; Ludt *et al.*, 2004).

Phylogeographic analyses of European red deer populations have shown that their isolation in Pleistocene refugia had a major impact on their genetic diversity and distribution (Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011). These studies found three mtDNA lineages, marked as haplogroups A, B and C. It has been inferred that these haplogroups correspond to isolation in Iberian, African/Sardinian and Balkan refugia, respectively. Nowadays, haplogroup A is present in Western, Northern and Central Europe. Haplogroup B is restricted to North Africa and Sardinia, whereas haplogroup C is found in Southern and Eastern Europe. Human management over the last centuries is believed to have disrupted the genetic diversity of red deer populations in Europe that arose during the long-term isolation and subsequent post-glacial decolonization. Niedziałkowska *et al.* (2011) found that haplogroups from lineages A and C occur together in Poland, Belarus and Lithuania. This could be a result of either natural overlap and contact zones or human-mediated translocations that occurred at the end of the 19th and beginning of the 20th centuries. A similar mixture of mtDNA lineages has been found in populations from Bulgaria, Hungary and the Czech Republic (Markov *et al.*, 2012, 2015; Krojerová-Prokešová, Barančeková & Koubek, 2015). Red deer translocations have also been suggested for populations in Germany (Ludt *et al.*, 2004), Scotland (Carden *et al.*, 2012) and Spain (Fernández-García *et al.*, 2013). Moreover, Kuznetsova *et al.* (2007) found the mtDNA lineages of both Western and Eastern red deer in the Ukrainian population, which probably resulted from the introduction of *Cervus elaphus maral* to Europe.

Therefore, to better understand the impact of climate and environmental conditions on the natural distribution of red deer populations during in the last glaciation, it is crucial to analyse samples from the period before the intensive human management. This can be achieved by ancient DNA (aDNA) analyses, as demonstrated in earlier studies of red deer remains, which indicated that the Late Quaternary history of red deer was more complex than previously assumed (Stankovic *et al.*, 2011; Meiri *et al.*, 2013). Its past genetic diversity was greater than at present, and the distribution of mtDNA lineages was also different. Meiri *et al.* (2013) noticed that mtDNA lineages only found today in Southern and Eastern Europe were also present in England and Spain before the Last Glacial Maximum (LGM). Analyses of Crimean red deer (Stankovic *et al.*, 2011) indicated that both Western and Eastern lineages were present on this peninsula in the Late Pleistocene, suggesting that conclusions about isolation by distance, which were based on contemporary samples, could be misleading.

The Crimean Peninsula has been proposed as a Pleistocene refugium for several species. Biome reconstructions have revealed that forest and steppe-forest vegetation with pine and broadleaved species persisted in Crimea through the last glaciation (Markova, Simakova & Puzachenko, 2009), although Cameron, Pokryszko & Horsák (2013) have argued that full forest cover probably did not survive. Environmental conditions appear to have been stable enough to create a refugium for small mammals, snails and trees (Gerasimenko, 2007; Markova *et al.*, 2009; Markova, 2011; Cameron *et al.*, 2013). Black Sea-level fluctuations in the Pleistocene may periodically have cut off the peninsula from the mainland. The sea level during the Eemian interglacial was few metres higher than today, which could result in an island (Richter, 2005). Crimea became the part of the mainland during colder periods due to sea-level decrease. This level was lower by 30–120 m than present during the Vistulian Interpleniglacial (MIS3), when Crimea was connected with Dobruja, a historical area located in the eastern part of Romania and Bulgaria (Stanko, 2007; Demidenko, 2008). The sea-level fluctuations may therefore have influenced the history of the red deer in the region. Deer populations may have been isolated in the peninsula during warmer events and migrations from adjacent regions may have occurred during colder phases. Similar landscape changes did not happen in other European refugia, which makes Crimea an unusual and interesting area for studying species' response to Pleistocene climate and geographic changes.

In order to test the hypothesis by Stankovic *et al.* (2011) that the Crimean Peninsula served as Europe's refugium, from which red deer colonized the Eurasian continent, we obtained additional aDNA from red deer specimens dated to the Late Pleistocene and Holocene. If this hypothesis is correct, we would expect that the Late Pleistocene red deer population would survive

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the LGM. Therefore, the genetic continuity between samples from before and after the LGM should be observed. Since ancient samples from Crimea represented both Western and Eastern lineages of red deer (Stankovic *et al.*, 2011), we also included all Eurasian available red deer sequences published so far, to reconstruct phylogeographic relationships and determine their potential migration routes at a Eurasian scale.

MATERIAL AND METHODS

Samples and DNA analyses

We analysed 33 samples from red deer remains excavated from ten caves located on three plateaus in the Crimean Mountains (Fig. 1; Supporting Information, Table S1). The majority of the samples were obtained from the Emine-Bair-Khosar Cave (EBK), which is an important palaeontological site rich in vertebrate remains (Vremir & Ridush, 2005; Ridush *et al.*, 2013) (Supporting Information, Fig. S1 and Table S2). Seventeen samples yielding mtDNA were radiocarbon dated in the Poznań Radiocarbon Laboratory and Gliwice Absolute Dating Methods Centre in Poland. Five other samples had been dated previously. The age of the samples ranged from *c*. 47 000 to 900 cal yr BP (Fig. 2; Supporting Information, Table S1), and thus covered a broad time range from the Late Pleistocene to the Holocene.

The DNA extraction was performed by the Institute of Genetics and Biotechnology of the University of Warsaw, in a laboratory dedicated to aDNA analyses. The amplification of the cytochrome *b* (cytb) sequences (1140 bp) was performed in multiplex PCR using 12

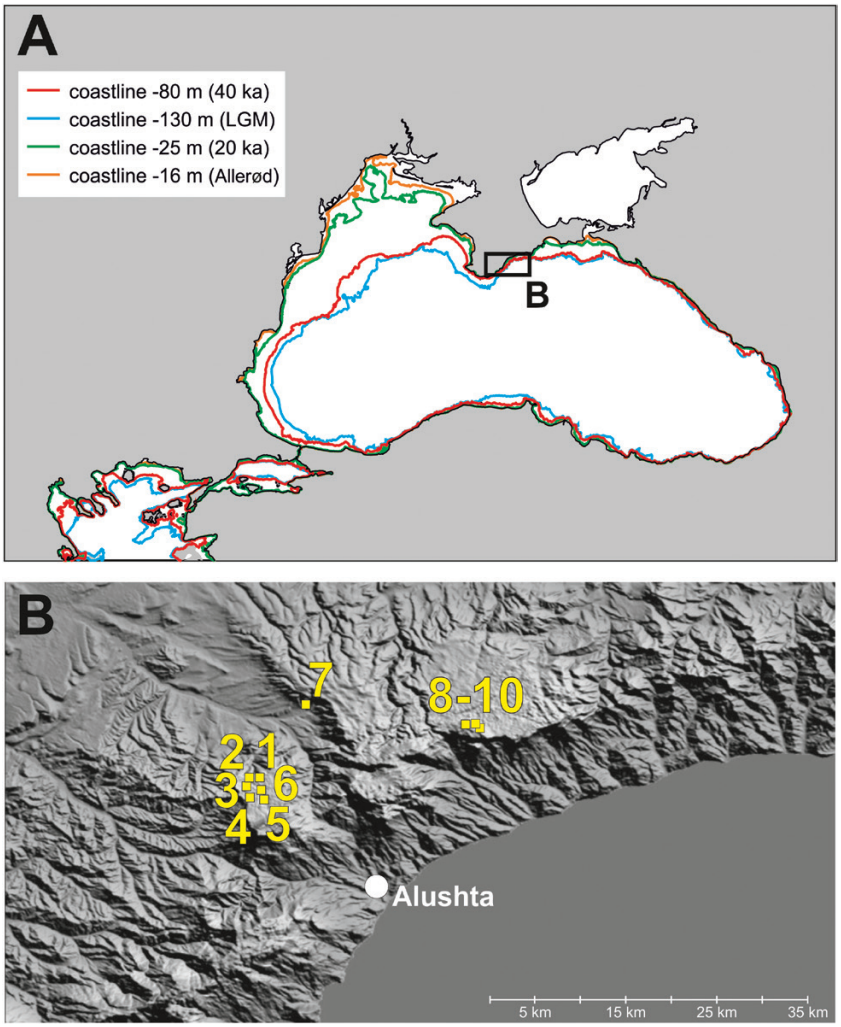

Figure 1. A, Crimean Peninsula with the research area (in rectangle) and palaeocoastlines in four time periods. B, research area with the location of caves: 1 – Emine-Bair-Khosar, 2 – K18, 3 – Pavucha, 4 – Primula, 5 – Binbash Koba, 6 – Krapivnyi Grotto, 7 – Dizel, 8 – Bilosnizhka. 9 – Lavrushka, 10 – Sokil.

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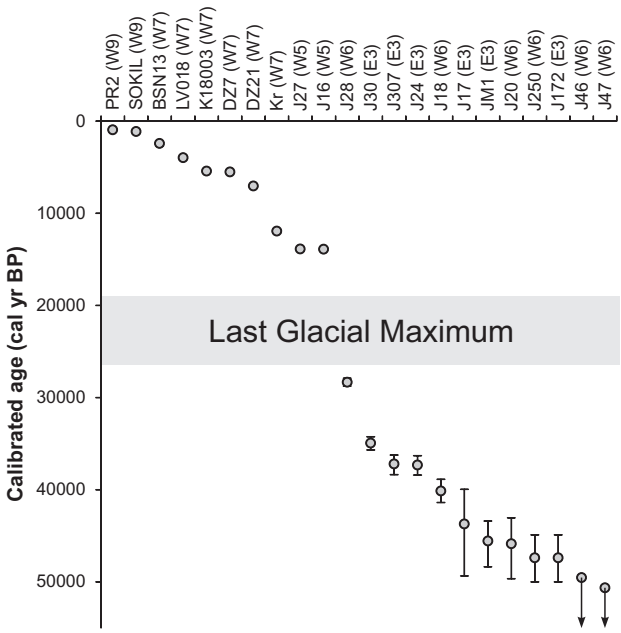

Figure 2. Distribution of calibrated radiocarbon dates in time for red deer samples from Crimea. Median (circle) and 95.4% confidence interval (whiskers) are shown. The circles with arrows mean open dates. Phylogenetic clades, to which the samples belong, are shown in parentheses.

overlapping primer pairs described in Stankovic *et al.* (2011). Full details regarding DNA extraction and amplification are given in Supporting Information.

Multiplex PCR products were used to prepare libraries for pyrosequencing following the protocol from Stiller *et al.* (2009) and were sequenced on a Roche 454 sequencing platform at the Institute of Biochemistry and Biophysics, PAS. The resulting sequences were trimmed in Bioedit v. 7.2.5 (Hall, 1999) and assembled into contigs using SeqMan Pro software (DNASTAR, Inc.). To ensure authenticity of the sequence data, each sample was amplified in two independent multiplex PCRs and contigs from both replicates were used to create consensus sequences according to guidelines proposed by Stiller *et al.* (2009).

Phylogenetic analyses

Data sets were obtained by comprehensive searches of the GenBank database for cytochrome *b* homologs in members of the Cervini tribe. Many of the red deer sequences studied here, including fossil samples, were obtained by various research groups, such as Ludt *et al.* (2004), Skog *et al.* (2009), Kuznetsova, Danilkin & Kholodova (2012) and Meiri *et al.* (2013, 2014). Phylogenetic analyses were based on two alignments comprising non-redundant sequences: a longer set of 190 sequences, 1140 bp in length and a shorter set of 175 sequences with 423 bp.

We applied five approaches for phylogenetic reconstruction: Bayesian inference in MrBayes (Ronquist *et al.*, 2012) and PhyloBayes (Lartillot, Lepage & Blanquart, 2009), maximum likelihood analyses in TreeFinder (Jobb, von Haeseler & Strimmer, 2004) and morePhyML (Criscuolo, 2011) based on PhyML (Guindon *et al.*, 2010), and the neighbor-joining method in PAUP* (Swofford, 1998).

In the MrBayes analyses, we assumed separate mixed + I + Γ(5) models for each of three codon positions and two independent runs, each using 32 and 16 Markov chains for the longer and shorter sets, respectively. Trees were sampled every 100 generations for 10 000 000 generations. In the final analysis, we selected trees from the last 3 062 000 (the longer set) and 4 664 000 (the shorter set) generations that reached convergence. In the PhyloBayes approach, two independent Markov chains were run through 100 000 (the longer set) and 200 000 (the shorter set) cycles assuming the CAT GTR + Γ(5) model. After obtaining convergence, the last 50 000 and 10 000 trees from each chain were collected to compute the posterior consensus, respectively.

In TreeFinder, we applied a search depth set to 2, separate substitution models for each of three codon positions according to the TreeFinder Propose Model (Supporting Information, Table S3). Trees inferred with morePhyML and PAUP were based on the best-fit substitution models found in jModeltest (Darriba *et al.*, 2012) among 1624 candidate models (Supporting Information, Table S3). The best search algorithm NNI + SPR was applied in morePhyML. To assess the significance of branches, non-parametric bootstrap analyses were performed on 1000 replicates in TreeFinder, PhyML and PAUP.

Molecular dating

Divergence times were estimated with the software BEAST (Drummond *et al.*, 2012) on the full-length alignment comprising 216 sequences, using as many samples as possible with assigned dates and geographic localizations. We used a fixed MrBayes tree that was inferred according to the procedure described above, but with 32 Markov chains and the last 3 861 000 generations to summarize the final tree. Based on that, we created an ultrametric tree using Sanderson's approach (Sanderson, 2002) in the chronopl function in R (R Core Team, 2014). We applied separate substitution models for each of three codon positions selected in jModeltest among 1624 candidate models (Supporting Information, Table S2). The age of the root of the tree was set as a uniform prior with the

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lower and upper limits 5.1 and 7.3 Mya. This age corresponds to the separation time of *Axis* and *Rucervus* from other Cervini based on three methods (Hassanin *et al.*, 2012). Moreover, we used 25 tip dates associated with sequences obtained from fossil and historical samples. To unify the dates, we applied the same calibration procedure in OxCal 4.2.3 (Ramsey, Scott & van der Plicht, 2013) using curve IntCal 13 (Reimer *et al.*, 2013) for original uncalibrated dates. We tested strict and uncorrelated lognormal relaxed clock models with a coalescent constant size tree and separate clock models for particular data partitions. Finally, we selected the relaxed clock model because the SD of the relaxed clock revealed significant variation among branches. Posterior distributions of parameters were estimated for 50 000 000 generations with a sampling frequency of 1000 steps. The convergence and sufficient sampling were checked using Tracer (Rambaut *et al.*, 2014). The phylogenetic trees were summarized in TreeAnnotator (Drummond *et al.*, 2012) with a 10% burn-in.

Approximate Bayesian computation

Based on archaeological and historical data, we defined a set of the 12 most likely models that described the temporal pattern in genetic diversity of Crimean red deer (Fig. 3). The best-fitted model was selected by approximate Bayesian computation (ABC) (Beaumont, Zhang & Balding, 2002). For defining the models, we considered the following scenarios:

- Two possible scenarios regarding the pre-LGM origin of Crimean population: one including a long-standing panmictic population (Fig. 3A, C, E, G, I, K) and the second assuming an admixture of Western and Eastern populations (Fig. 3B, D, F, H, J, L);
- Two possible scenarios regarding the post-LGM (until *c.* 2000 cal yr BP) origin of the Crimean population: in one scenario, Crimea was populated by deer that survived locally (Fig. 3A–F). In the other, Crimea was colonized by Western European lineages (Fig. 3H–L);
- Three scenarios regarding the present origin of the Crimean population: in one scenario, the present population was derived from the population inhabiting Crimea before 2000 cal yr BP (Fig. 3A, B, K, L); in the second scenario, the present population split from a common ancestor with the population that colonized Crimea after the LGM and until 2000 cal yr BP. Here we considered two split times: before the LGM (Fig. 3I, J) and after the LGM (Fig. 3C, D). In the third scenario, the present population was colonized from Western European lineages that were unrelated to the post-LGM (till *c.* 2000 cal yr BP) population (Fig. 3E–H).

The 12 tested models resulted from combining these scenarios. Simulations were performed under a coalescent framework, where unknown parameters and their uncertainness were assessed by Bayesian prior

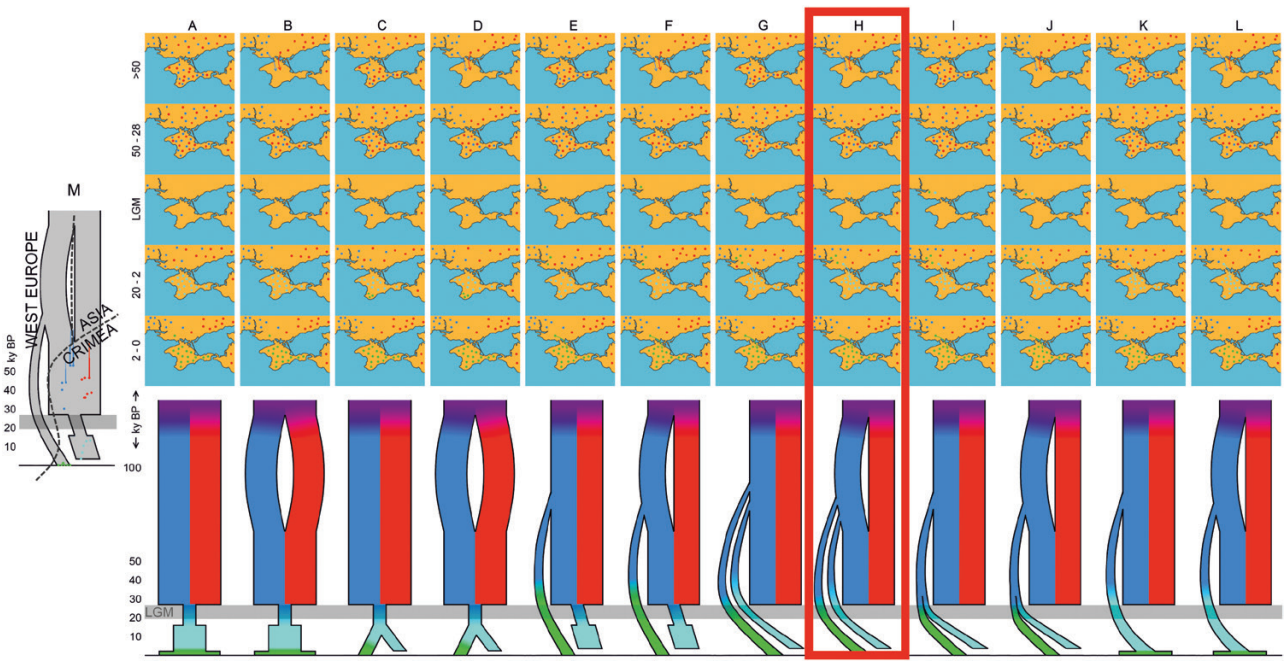

Figure 3. Population models considered in approximate Bayesian computation analyses. The most likely model H assuming 5.14 and 10% mutation rate per Myr was outlined by the red rectangle.

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probability distributions. We carried out two rounds of pilot simulations to optimize the parameters. The priors for effective population size (N_e) had an exponential distribution in pilot simulations to better screen an ample range. Then, we replaced them with uniform distributions in the final simulations. The times to population splits or demographic changes were set by uniform priors as well. Uncertainty in radiocarbon dating of sample ages was included by assigning normal priors (except in four cases where exponential priors were used because of infinite radiocarbon dates; see Supporting Information). We employed a 4-year generation time, based on the literature (Pérez-Espona *et al.*, 2009; Rosvold *et al.*, 2012) and a transition/transversion (Tr/Ts) bias of 0.945, a gamma shape parameter of 0.05 and a mutation rate of 5.14% per Myr (Skog *et al.*, 2009). We calculated the basic parameters: the number of haplotypes, private haplotypes and segregating sites and nucleotide diversity for three sets of samples grouped by time periods: pre-LGM, post-LGM to 2000 cal yr BP and the present. In addition, we employed the number of pairwise differences and F_{ST} among pairs of consecutive samples (Supporting Information, Table S4). We ran 2 000 000 simulations for each model in the final analyses.

The best-fitting model was selected with Bayes factors (BFs) that were calculated as ratios of model likelihoods between pairs of models for all 66 pairwise comparisons of the 12 models (Supporting Information, Table S5). The model likelihoods were estimated by (1) a direct approach where they were directly equalled to their relative acceptance ratios, (2) a weighted approach, which also employs the acceptance ratios after being weighted with an Epanechnikov kernel evaluated in the Euclidean distances to the observed data and (3) a logistic regression in which summary statistics represent explanatory variables of model likelihoods (Fagundes *et al.*, 2007). The consistency of the model likelihoods and the BFs was assessed by applying the procedure with 10 different acceptance proportions (from 0.001 to 0.01%).

Since the mutation rate, and possible dependency on temporal scale (Ho, 2014), could impact our estimated parameters and model choice, we tested the robustness of our results with a range of different mutation rates: 10, 20 and 30% per Myr.

Finally, to assess the probability of correct model selection, we estimated the statistical power of the analyses with pseudo observed data sets (PODs), where simulated data sets play the role of real ones. We generated 1000 PODs, which were analysed with the same reference tables and 2 000 000 simulations each. Simulations, model choice and POD analyses were performed in the software BaySICS (Sandoval-Castellanos, Palkopoulou & Dalén, 2014), whereas the Tr/Ts bias and gamma parameter were calculated in MEGA 6 (Tamura *et al.*, 2013).

RESULTS

Phylogenetic relationships within red deer lineages including Crimean deer sequences

We successfully amplified and obtained cytochrome *b* sequence from 24 samples, which gave us a 73% success rate of DNA extraction for the subfossil samples. We identified 16 haplotypes. None of the negative controls yielded DNA. The sequences were aligned to all available cytochrome *b* sequences from Cervini members including ancient and modern samples of *C. elaphus*. The five applied approaches produced similar trees. Deer sequences assigned to the *Cervus* genus do not form a monophyletic clade but are split into Western and Eastern subclades, with strong and moderate support, respectively (Supporting Information, Figs S2, S3). The Eastern subclade is very significantly grouped with the Thorold's deer *Przewalskium albirostris* sequences.

All newly obtained sequences from the Crimean samples group significantly either with the Western or Eastern lineages (detailed phylogenies in Figs 4, 5; Supporting Information, Figs S4, S5). To study the detailed relationships between *Cervus* sequences, we considered the shorter set (Supporting Information, Figs S4, S5) in addition to the longer one (Figs 4, 5) because it contained important subfossil samples.

In the Eastern lineage, two main well-supported groups representing wapiti, *Cervus canadensis*, and sika deer, *Cervus nippon*, can be recognized (Fig. 4; Supporting Information, Fig. S4). The wapiti group consists of clades marked as E1 (only in the tree for the smaller set, Supporting Information, Fig. S4), E2 and E3. The basal clade E1 comprises exclusively ancient sequences from Alaska and north-eastern Siberia (Meiri *et al.*, 2014). The pre-LGM samples from north-eastern Siberia (Meiri *et al.*, 2014) are located in the E2 and E3 clades too. The E2 clade also includes other samples from Central and central-eastern Asia. The deer samples in the E3 clade show a much wider distribution. Besides Central Asia, north-eastern Siberia and South Korea, they were also found in North America and Crimea, where they are represented by only pre-LGM samples. In the tree for the longer set, all Crimean sequences branch off at the base of E3 clade, but for the shorter set, they are placed inside the clade without any support (Fig. 4; Supporting Information, Fig. S4).

In the sika deer group, four clades can be recognized (Fig. 4; Supporting Information, Fig. S4) including Asian (clades E4 and E5) and Japanese deer separated

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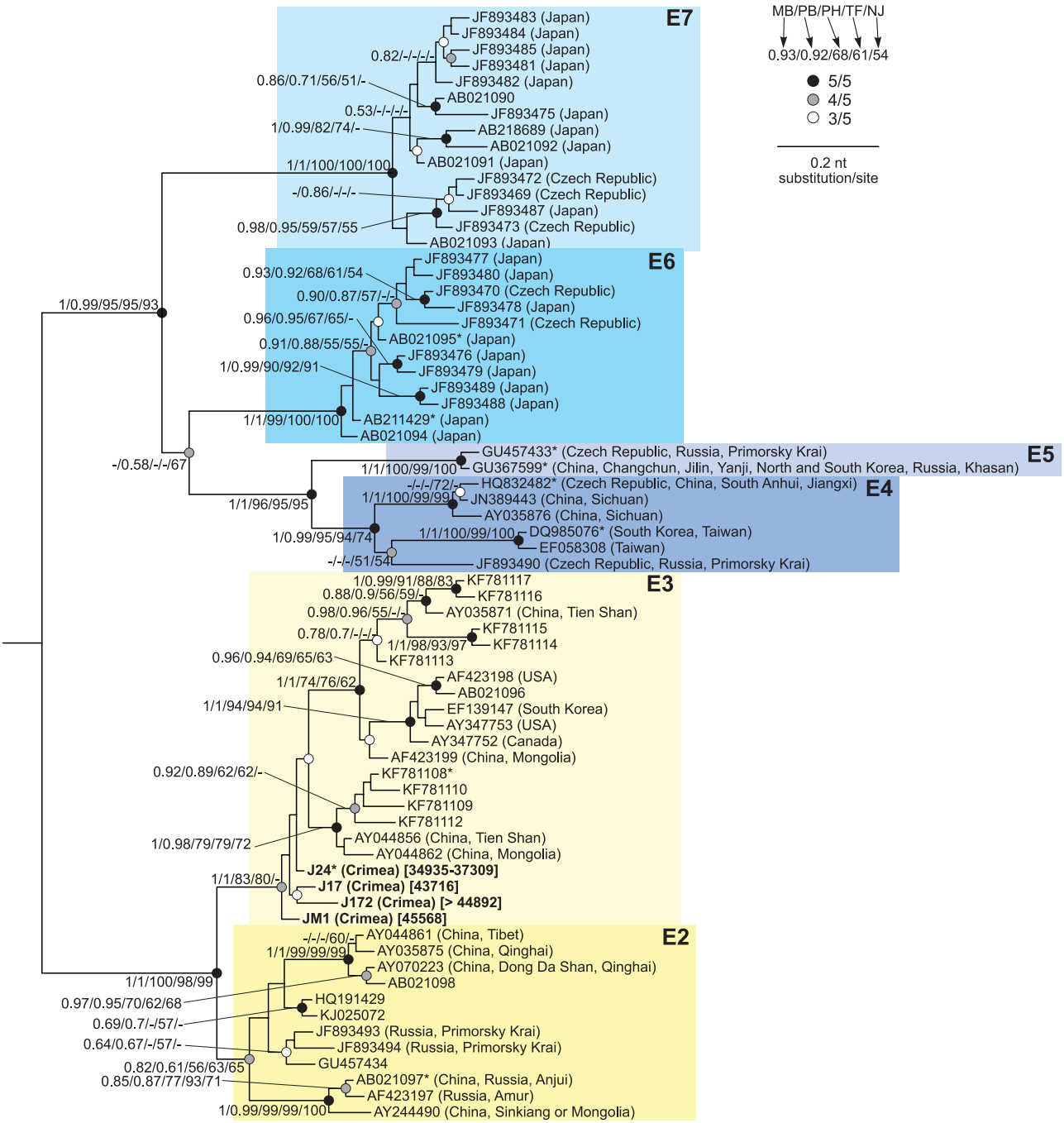

Figure 4. MrBayes tree based on the longer alignment for the Eastern lineage of red deer (the clade E in Supporting information, Fig. S2). Clades E2 and E3 represent *C. elaphus canadensis* (wapiti), whereas E4–E7 *C. elaphus nippon* (sika). Sequences newly obtained in this study from Crimea specimens are in bold. The asterisk indicates that a given sequence is represented by more than one accession number. The shaded circles at nodes correspond to the number of methods (out of five) that recovered the given node. Probable age, time period or calibrated dates of samples are shown in square brackets. Numbers at nodes, in the order shown, correspond to posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB), bootstrap values obtained in PhyML (PH), TreeFinder (TF) and PAUP by the neighbor-joining (NJ) method. Values of the posterior probabilities and bootstrap percentages ≤ 0.50 and 50%, respectively, were omitted or indicated by a dash '–'.

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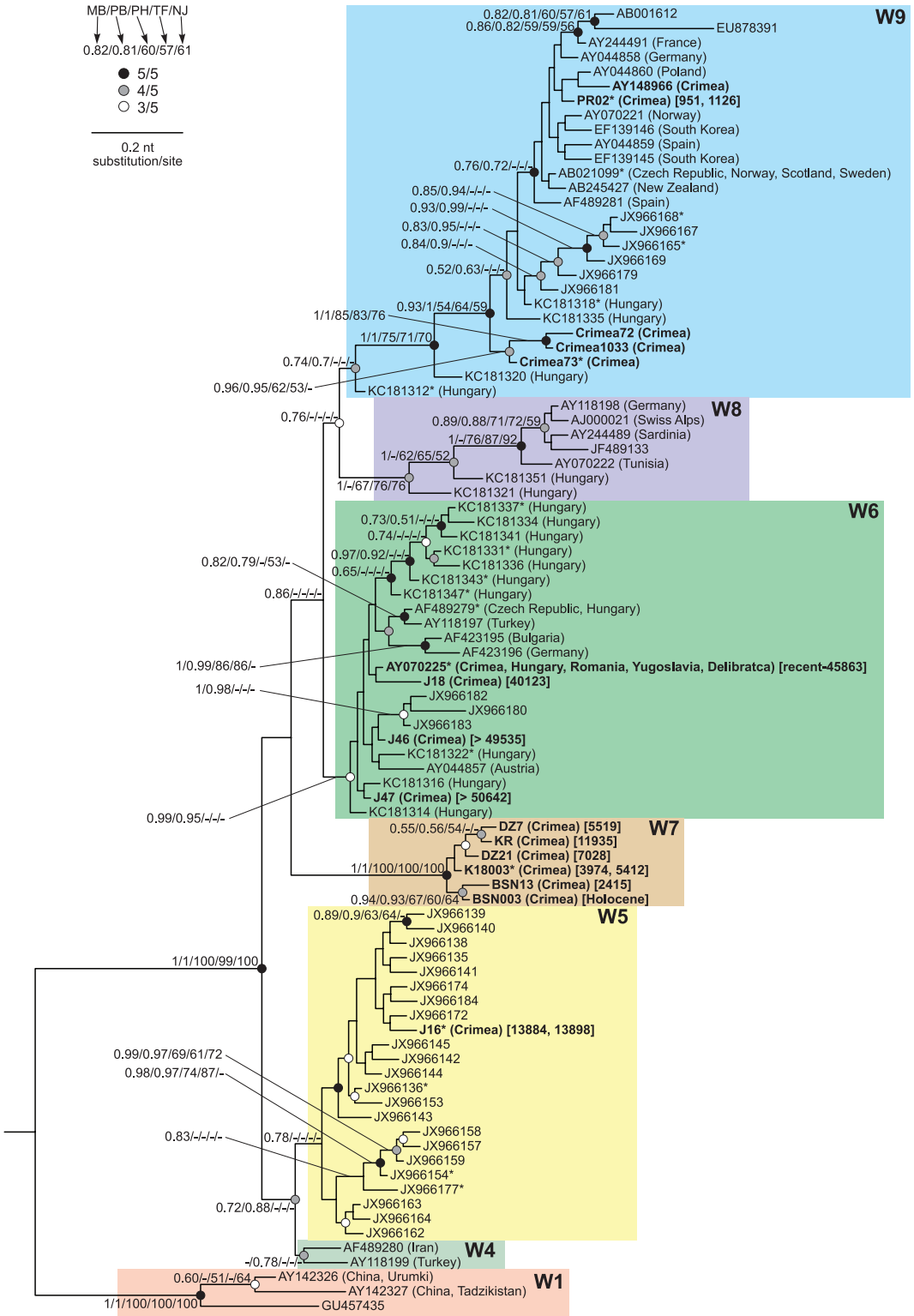

Figure 5. MrBayes tree based on the longer alignment for the Western lineage of red deer (the clade W in Supporting Information, Fig. S2). Sequences newly obtained in this study from Crimean specimens are shown in bold. For further explanation, see Figure 4.

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into its 'northern' (clade E6) and 'southern' (clade E7) (Nagata *et al.*, 1999; Groves, 2005). Deer introduced to the Czech Republic are also present in these clades. However, no Crimean samples were reported among the sika deer clade.

The Western red deer lineage is diversified into nine distinct clades, which are inferred by at least three out of the five methods applied (Fig. 5; Supporting Information, Fig. S5). Sequences from Crimean deer are distributed among four clades (W5, W6, W7 and W9). The Late Pleistocene samples from Crimea (post-LGM) are present in clade W5, which also contains other sequences from the southern part of Eastern Europe: modern samples from Krasnodarsky Krai, Voronezh and the Caucasus (from the pre-LGM period) (Meiri *et al.*, 2013). Much older Crimea sequences from the pre-LGM are grouped with sequences from the Balkans, Italy, Central Europe and Turkey in the W6 clade (corresponding to haplogroup C). In the smaller set tree (Supporting Information, Fig. S5), this clade also includes pre-LGM specimens from the Caucasus and the Urals (Meiri *et al.*, 2013) as well as a modern Crimean sequence. In the longer alignment tree (Fig. 5), the W7 clade contains exclusively post-LGM Crimean sequences, whereas in the shorter set tree (Supporting Information, Fig. S5), it includes additionally a modern sequence from Italy, two post-LGM sequences from Serbia and two ancient sequences from England, with pre- and post-LGM ages (Meiri *et al.*, 2013), respectively. The W9 clade (corresponding to haplogroup A) has the highest sequence diversity. Besides Mediaeval (eighth to 11th centuries) and modern sequences from Crimea, it comprises deer mainly from Western, Central and Northern Europe including Great Britain, and sequences from regions far to the east, in Russian Karelia and Belarus, and deer introduced to South Korea and New Zealand. In the tree based on the short alignment (Supporting Information, Fig. S5), the basal position of this clade is occupied by a pre-LGM sample from Belgium (Meiri *et al.*, 2013). Post-LGM specimens from Spain are branched off next.

The deer from Crimea was not found in the basal clades of the Western lineage: W1 including *Cervus elaphus yarkandensis* and *bactrianus* from China and Uzbekistan; W2 (in the shorter alignment tree only, Supporting Information, Fig. S5) containing exclusively post-LGM sequences from Spain (Meiri *et al.*, 2013); W3 (in the shorter alignment tree only, Supporting Information, Fig. S5) with a historical Jordan sample (Meiri *et al.*, 2013); and W4 comprising the sequences of *C. elaphus maral* from Turkey and Iran. The W8 clade (corresponding to haplogroup B) is also absent from Crimean samples and includes deer from Tunisia, Sardinia, Hungary, the Swiss Alps, a deer introduced to Germany and pre-LGM samples from the Caucasus – Supporting Information, Figure S5.

Population history of Crimean deer

In the ABC model choice analysis, the same model H was selected for mutation rates of 5.14 and 10% per Myr (Fig. 3H; Table 1; Supporting Information, Table S5). This model assumed that the pre-LGM population was an admixture of Eastern (Asian) and Western (Europe) lineages, whereas the two post-LGM populations (post-LGM to 2000 yr BP, and present) resulted from two unrelated waves of colonization that originated in the West before 50 ka. The BF of the chosen

Table 1. Model likelihoods and Bayes factors among the 12 models compared by approximate Bayesian computation

Model	Model likelihoods	A	B	C	D	E	F	G	H	I	J	K	L
A	0.0006	–	0.348	0.071	0.128	0.021	0.021	0.004	0.001	0.006	0.003	0.095	0.034
B	0.0018	2.871	–	0.205	0.368	0.059	0.059	0.012	0.004	0.017	0.009	0.272	0.097
C	0.0087	14.000	4.876	–	1.793	0.289	0.288	0.056	0.020	0.085	0.044	1.327	0.474
D	0.0049	7.806	2.719	0.558	–	0.161	0.161	0.031	0.011	0.048	0.024	0.740	0.264
E	0.0300	48.419	16.865	3.459	6.202	–	0.996	0.194	0.068	0.295	0.151	4.590	1.640
F	0.0302	48.613	16.933	3.472	6.227	1.004	–	0.195	0.068	0.296	0.152	4.609	1.647
G	0.1547	249.355	86.854	17.811	31.942	5.150	5.129	–	0.348	1.519	0.779	23.639	8.448
H	**0.4439**	**715.903**	**249.360**	**51.136**	**91.707**	**14.785**	**14.727**	**2.871**	–	**4.362**	**2.237**	**67.869**	**24.255**
I	0.1018	164.129	57.169	11.724	21.025	3.390	3.376	0.658	0.229	–	0.513	15.560	5.561
J	0.1985	320.032	111.472	22.859	40.996	6.610	6.583	1.283	0.447	1.950	–	30.339	10.843
K	0.0066	10.548	3.674	0.753	1.351	0.218	0.217	0.042	0.015	0.064	0.033	–	0.357
L	0.0183	29.516	10.281	2.108	3.781	0.610	0.607	0.118	0.041	0.180	0.092	2.798	–

The Bayes factors have a row to column interpretation (the value at row i and column j is $BF_{ij} = ML_i/ML_j$). These values correspond to the analysis with a mutation rate of 5.14% per Myr, after averaging the five smallest acceptance proportions (0.001–0.005%) of the closest simulated data sets to the observed one. The best-fit model is shown in bold.

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model was relatively large (Table 1; Supporting Information, Table S5). For mutation rates of 20 and 30% per Myr, model G instead obtained the highest support (Supporting Information, Tables S6 and S7). The model G assumed that the pre-LGM Crimean population was panmictic and long standing.

Four models obtained higher likelihood support than the rest (Figs 2I, 3G–J). In all four models, the most recent populations originated before the LGM in two waves of colonization. These models had BF values in the range of 7.5–360, 4.3–3800, 8.6–1560 and 18.0–10^5 for mutation rates of 5.14, 10, 20 and 30% per Myr, respectively. These results provide strong support for the presence of at least three waves of sequential colonization (and extinction/replacement) of Crimean red deer by unrelated Western populations, and reject that the present-day population originated from a local pre-LGM population or even a post-LGM population that existed until 2000 yr BP. For mutation rates of 5.14 and 10% per Myr, the BF was > 2.3 for the models with the admixed pre-LGM population (Fig. 3H, J), and it was > 1.5 for the models G and I where the pre-LGM population was panmictic (Fig. 3G, I). However, for mutation rates of 20 and 30% per Myr, the converse occurred (BF = 0.12 and 0.06, respectively). Comparison of models also indicates that the support for a Western origin of post-LGM populations in at least two colonization waves is strong and consistent.

Finally, the POD analysis showed low statistical power for several models (Supporting Information, Table S8) ranging from 0.22 to 0.48, which rose to 0.28–0.83 when only BFs equal to or larger than the observed ones were taken into account. The lack of statistical power resulted from 'competition' between similar models. An exploratory analysis showed that if similar models (e.g. models of Fig. 3G, H) were mixed into one, the statistical power rose to over 0.9 even for the least supported scenarios. Therefore, the POD analysis showed that the model choice procedure is able to separate correctly between scenarios, although it is less sensitive to distinguishing between very similar models.

DISCUSSION

Phylogeography of *Cervus* lineages including Crimean samples

We analysed the most sequence-rich set of cytochrome *b* from different Cervini using the greatest variety of methods so far. The obtained global tree topologies are generally in agreement with results of other authors (Pitra *et al.*, 2004; Hassanin *et al.*, 2012) – Supporting Information, Figure S2. Divergence times for particular lineages of Cervini estimated by us are close to those by Gilbert, Ropiquet & Hassanin (2006), although slightly younger (Supporting Information, Fig. S3). Our phylogenetic study clearly recovered two monophyletic clades of *Cervus*, corresponding to the eastern and western part of its distribution (Randi *et al.*, 2001; Ludt *et al.*, 2004; Pitra *et al.*, 2004; Skog *et al.*, 2009). The analyses of a large number of deer samples, including fossils from Crimea, also enabled us to infer branching order and migration routes of *Cervus* in more detail.

The significant grouping of the Eastern lineage with *Przewalskium* from China suggests that this lineage emerged somewhere in Asia. The phylogenies are based on an mtDNA marker, which reflects the history of the maternal lineage. Therefore, nuclear markers should be studied to verify the inferred relationships. About 1 Mya, the Eastern lineage separated into sika (*C. nippon*) and wapiti (*C. canadensis*) clades, which further differentiated in Central and Eastern Asia (Fig. 4; Supporting Information, Figs S3, S4). The former colonized Japanese islands (Nagata *et al.*, 1999; Groves, 2005) and the latter reached north-eastern Siberia in the pre-LGM period (Meiri *et al.*, 2014). Wapiti deer carrying mtDNA haplotypes belonging to the E3 clade showed the most widespread distribution because they not only colonized North America but also migrated as far west as Crimea, where they survived to ~35 ka. This implies long-distance migrations of the eastern deer, originally confined into Central and Eastern Asia. The colonization of North America was a single event (Meiri *et al.*, 2014) (Fig. 4) and probably started from north-eastern Siberia (Supporting Information, Fig. S3). Our molecular dating indicates that the migration occurred between 12.4 and 75.9 ka. The deer that crossed the land bridge across the present Bering Strait to North America carried at least two mitochondrial haplotypes, represented by clades E1 and E3.

The ancestor of the Western lineage most likely lived in Central Asia, as indicated by the earliest diverged W1 clade (Fig. 5; Supporting Information, Fig. S5). This clade includes Yarkand deer (*C. elaphus yarkandensis*) and Bactrian deer (*C. elaphus bactrianus*) from China and Uzbekistan, closely related to *C. elaphus hanglu* from Indian Kashmir (Lorenzini & Garofalo, 2015). Lorenzini & Garofalo (2015) proposed to raise these three deer to species level as *Cervus hanglu*. The migration of red deer to Europe happened likely about 1 Mya, as implied by the oldest fossils of *C. elaphus* (Brugal & Croitor, 2007) – Figure 6A. This is supported by our phylogenies, where an early separated clade with Spanish samples (W2) is basal to the rest of the sequences (Supporting Information, Fig. S5). This is also consistent with our estimated split time between clade W1 and the rest: 1.08 (0.57–1.72) Mya

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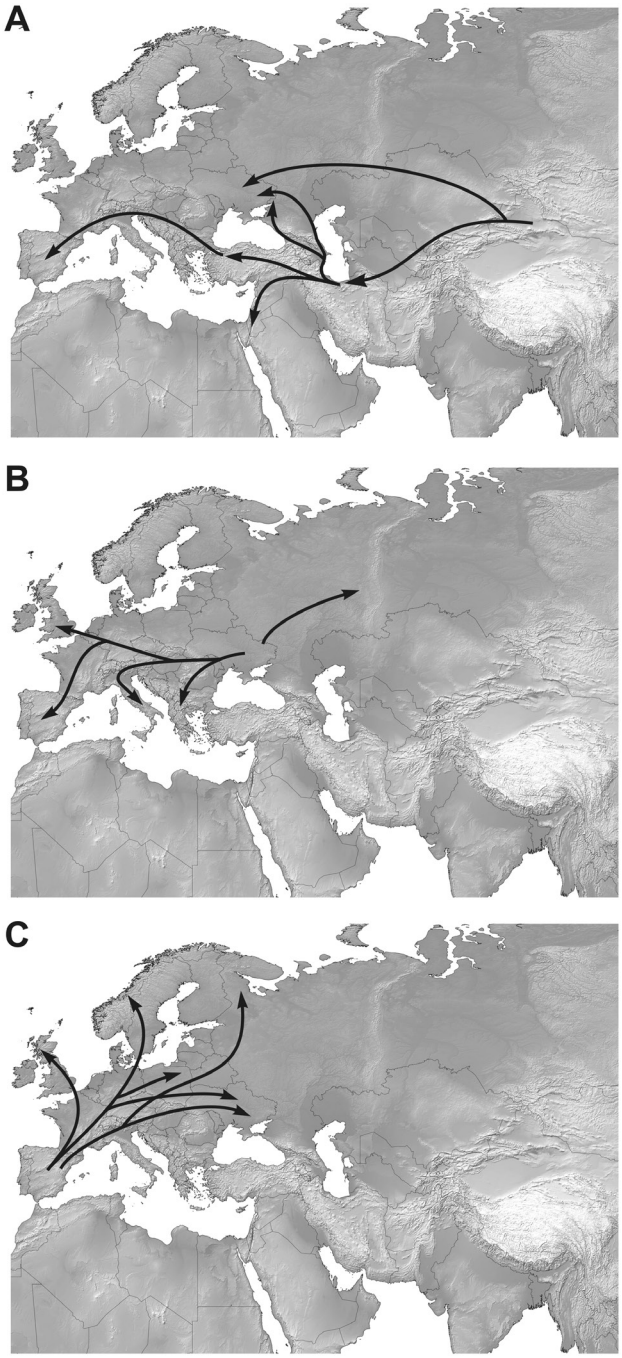

Figure 6. Inferred migration routes of red deer in Europe. A, the first settlement of Europe from Central Asia across Near East and Asia Minor as well as migrations to the southern region of Eastern Europe including the Crimean Peninsula. B, further migrations from the latter region to Europe and towards the Ural Mountains. C, post-LGM recolonization of the rest territory of Europe including the Crimean Peninsula from Iberian refugium. The role of the Balkan and Apennine refugia is not shown.

(Supporting Information, Fig. S3). The ancient Spanish samples are dated to post-LGM and do not group with other Spanish sequences, which separated much later (the W9 clade), suggesting that they represent an old lineage that became extinct without descendants. A similar placement of these sequences was obtained in the phylogenetic tree by Meiri *et al.* (2013). The migration route to Europe probably extended across the Near East and Asia Minor (Fig. 6A) because sequences from Iran, Jordan and Turkey clades are closely related with those from Spain (Supporting Information, Fig. S5).

The next diverged W5 clade includes sequences from the Caucasus, Krasnodarsky Krai, Voronezh and Crimea, which suggests that populations from the Near East also migrated north along the western or/and eastern seashore of the Caucasus and inhabited the southern part of Eastern Europe, including Crimea (Fig. 6A). The first migration may have occurred around 0.6 Mya because the oldest red deer from the Caucasus is dated to 583 ka (Blackwell *et al.*, 2005). This agrees with our calculation of separation time between the lineage including Turkey and Iran sequences and other clades, that is 512 (265–832) ka (Supporting Information, Fig. S3). Samples from Crimea and the Caucasus are separated into different clades with short internal branches, which may indicate a quite diversified ancestral population or several rapid migration events to the north. Some deer from the region migrated to the northeast (Fig. 6A) as indicated by ancient sequences from the Urals, identical to those from the Caucasus (Supporting Information, Fig. S5).

At least four westward migrations could have occurred from the southern part of Eastern Europe (Fig. 6B) or different haplotypes were carried by a larger population during a smaller number of migration events. This is suggested by clades W6–8 including pre-LGM sequences from Crimea and the Caucasus and from different regions of Europe (Fig. 5; Supporting Information, Fig. S5). In the W6 and W8 clades, these sequences occupy a basal position to others (Supporting Information, Fig. S5). The separation of these clades appears to have begun roughly 468 (244–758) ka (Supporting Information, Fig. S3).

Drastic cooling during the LGM (23–19 ka) caused the distribution of red deer in Europe to be limited to the Balkan, Italian and Iberian Peninsulas, southwestern France and the region east of the Carpathians (Banks *et al.*, 2008; Sommer *et al.*, 2008; Skog *et al.*, 2009; Sommer & Zachos, 2009; Meiri *et al.*, 2013). After the LGM with the start of Bølling-Allerød warming (15–13 ka), deer expanded from refugial areas into other regions of Europe. Our results are in agreement

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with those studies. In clade W9 (corresponding to haplogroup A), the basal position is occupied by Spanish sequences dated from *c.* 19 ka to the Neolithic (Meiri *et al.*, 2013) – Supporting Information, Figure S5. Other Spanish sequences are younger and are intermingled in the tree with sequences from Western and Central Europe, in line with expectations under a scenario where the red deer that colonized other regions of Europe originated in the Iberian Peninsula (Ludt *et al.*, 2004; Sommer *et al.*, 2008; Skog *et al.*, 2009; Sommer & Zachos, 2009; Niedziałkowska *et al.*, 2011; Meiri *et al.*, 2013) – Figure 6C. Sequences from Crimean deer are separated in the W9 clade, which suggests two independent waves of migration into the Crimean Peninsula or one colonization event by a deer population that carried different haplotypes. A subcluster in clade W6 (corresponding to C haplogroup) with sequences from Southern and Central Europe may correspond to the Balkan refugium. Sequences from Turkey are also present in this clade, which could imply a migration between Europe and Asia Minor. Alternatively, deer from the Balkan refugium could have spread further to the south-east. The W8 clade (corresponding to B haplogroup), besides harbouring sequences from Corsican and Tunisian red deer (*C. elaphus corsicanus* and *C. elaphus barbarus*), also includes sequences from Hungary and the Swiss Alps. These most likely represent human translocations from Italy.

Detailed population history of Crimean red deer

The goal of our study was to investigate the role of the Crimean Peninsula as a possible Eastern European refugium. In agreement with Stankovic *et al.* (2011), we found that red deer from both the Eastern and Western lineages were present in the Crimean Peninsula before the LGM, even though these populations have otherwise been isolated since their divergence (Kuwayama & Ozawa, 2000; Mahmut *et al.*, 2002). Our ABC analyses, assuming the mutation rates of 5.14 and 10% per Myr, showed that the pre-LGM Crimean population was probably the result of a recent mixture of these lineages. However, for higher mutation rates, the best-supported model (G) assumes that the pre-LGM population was panmictic. This discrepancy results from the model selection procedure, where the POD analysis had difficulties distinguishing between very similar models. Based solely on the ABC results, we cannot therefore be certain which of the scenario regarding the pre-LGM population is more probable.

However, the scenario presented in model H is in agreement with other results: the early divergence estimate for the two red deer lineages (*c.* 1.54 Mya), the distribution of pre-LGM specimens in separate clades, E3 and W6 from the Eastern and Western lineages, and radiocarbon dates and the distribution of red deer samples in cave layers. The specimens from clade W6 were usually found in the lower part of sediments in Emine-Bair-Khosar Cave (Supporting Information, Fig. S1). This layer (I) was dated with pollen to the Eemian stage (MIS5e) when the climate was warmer than now (Avdeienko, 2015) – Supporting Information, Table S2. The oldest deer remains (J46 and J47) with ^{14}C radiocarbon dates close or above the upper limit of this method also belong to clade W6. On the other hand, samples from the eastern population, located in clade E3, were frequent in the upper layer (H), dated to the Middle Pleniglacial (MIS3) by ^{14}C and pollen (Avdeienko, 2015) – Supporting Information, Figure S1 and Table S2. The deer lived in a boreal climate, which was cooler than now, and became extinct before the LGM when the accumulation of allochthonous material in the cave stopped. The youngest pre-LGM sample J28 (28 332 cal yr BP) again belongs to clade W6. The data suggest that the deer from clade W6 dominated in Crimea earlier, next the deer from clade E3 appeared and again the deer from clade W6 returned (Fig. 2). However, some radiocarbon dates of samples from these two lineages overlap. Therefore, we cannot exclude that the different deer could interact in some periods (e.g. between 47 400 and 43 700 cal yr BP), although there were also periods when only one deer clade existed, for example before *c.* 50 000 cal yr BP or between 37 300 and 34 900 cal yr BP.

We did not observe a genetic continuity between pre- and post-LGM samples from Crimea (Fig. 2), which suggests a local extinction hypothesis. This is also supported by the lack of red deer remains from the LGM period in Emine-Bair-Khosar Cave, where such remains are generally abundant (Vremir & Ridush, 2005). However, it cannot be excluded that bone accumulation was impossible in the MIS 2 due to a snow-ice plug that covered the entrance to the cave (Ridush *et al.*, 2013). In this time, the Scandinavian Ice Sheet reached its maximum extent, the climate became colder and drier, the sea level dropped and many temperate species retreated to refugial areas in the southern peninsulas (Hewitt, 2004; Hubberten, 2004; Clark *et al.*, 2009). Environmental conditions in Crimea also deteriorated at the end of MIS 3. Pollen, palaeontological and stable isotope analyses indicated that the climate in the LGM was cold and dry, which reduced broadleaved trees and extended steppes (Gerasimenko, 2007; Markova, 2011; Gąsiorowski *et al.*, 2014). Those unfavourable conditions could have contributed to the extinction of the pre-LGM red deer population. However, the results of Markova (2011) revealed that despite climate deterioration, Crimea was a refugium for small mammals. Therefore, we

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cannot exclude that climate was the only factor that influenced the red deer extinction.

The post-LGM samples are distributed into three clades that belong to the Western lineage and were not descendants of the pre-LGM population, as is indicated by our phylogenetic and ABC analyses. Thus, the pre-LGM population became extinct and was replaced by immigrants. Our data suggest that the population replacement started around 27–14 ka when the pre-LGM deer disappeared from Crimea (Fig. 2). The oldest immigrant population is represented by samples J16 and J27 (*c*. 13 900 cal yr BP), which were probably derived from Russian and Caucasian populations (assigned in Fig. 5 as J16* and in Supporting Information, Fig. S5 as JX966135* in clade W5). They could get into Crimea during the Bølling-Allerød interstadial along the Black Sea seashore from the Transcaucasia refugim, but the lineage vanished in the next Younger Dryas cooling. Younger samples (from Kr to BSN13 dated to *c*. 12 000–2400 cal yr BP, in Fig. 2) belong to clade W7 and are grouped together with sequences from Serbia and Italy and pre- and post-LGM specimens from England. This suggests that Crimea was colonized from the Balkans in the beginning of Holocene. Noticeably, in the late Pleistocene and early Holocene, the Black Sea level was lower than today (Fig. 1). As a consequence, the Balkans and the Caucasus were connected to Crimea by land, which made the migration between these areas possible (Stanko, 2007; Demidenko, 2008). This is also supported by similarities in material culture from the Late Palaeolithic and Mesolithic sites in Bulgaria, Romania and Crimea, suggesting direct contacts among humans from these localities (Stanko, 2007).

Despite more stable and favourable climatic conditions since the beginning of the Holocene, the post-LGM red deer population from clade W7 did not survive to modern times in Crimea. This population disappeared *c*. 2.5 ka and was not an ancestor of contemporary deer. Causes for this Holocene extinction are less clear. Based on soil development and pollen analysis, Cordova & Lehman (2005) reconstructed the environmental changes in Crimea during the Holocene. Their study revealed climate fluctuations and two events of dry and cool phases with a decrease in arboreal vegetation and spreading steppe taxa, dated to 11–7.5 and 4.2–3.5 ka, respectively. This climatic deterioration, however, is unlikely to have caused the extinction of the Holocene population, given that similar conditions are present today in some parts of Crimea, where red deer exist. Cordova & Lehman (2005) also found an increase in phryganoid plants, weeds and other cultivated species around 3.5 ka, suggesting a human impact on the Crimean environment. Between seventh and fifth centuries BC (2.65–2.45 ka), Greeks started to colonize Crimea. One of the earliest settlements on the northern Black Sea coast was established *c.* 700 yr BC (2.65 ka) on Berezan Island, which was then a part of the mainland (Dolukhanov & Shilik, 2007). The Greek colonization contributed not only to increasing amounts of pastoral plants but also to intensive deforestation, which took place *c.* 2.5 ka (Cordova & Lehman, 2005; Cordova, 2007). This human activity corresponds well with the observed Holocene red deer extinction, suggesting that intensive landscape management led to red deer habitat fragmentation and possibly to its extinction. Humans have been postulated as a cause of faunal extinction in many other studies (Burney *et al.*, 2004; Burney & Flannery, 2005; Lorenzen *et al.*, 2011; Rawlence *et al.*, 2015). Some of these concentrated on the overkill hypothesis, while others suggested less direct reasons such as habitat alteration, biological invasion or diseases (Burney & Flannery, 2005).

The second recolonization of the peninsula appears to have started ~1000 years after the post-LGM extinction of red deer, between the Late Antic and Mediaeval periods, as is supported by two Crimean specimens (SOKIL and PR2) dated to 1.2–0.9 cal ka (eighth to 11th centuries AD) – Figure 2. These, together with present samples, belong to Western haplogroup A (clade W9). This haplogroup originated on the Iberian Peninsula and spread across the whole of Europe (Skog *et al.*, 2009; Niedziałkowska *et al.*, 2011; Meiri *et al.*, 2013). This suggests that Crimea was probably colonized naturally by immigrant red deer from Western Europe. However, translocations by humans cannot be excluded, as it is well known that this happened in Sardinia, Corsica, Crete and Ireland during the Neolithic period (Hajji *et al.*, 2008; Carden *et al.*, 2012; Harris, 2014). The reasons for such management are not well known, but studies by Sykes *et al.* (2006, 2011) and Davis & MacKinnon (2009) have showed that fallow deer (*Dama dama*) was introduced to Britain and Portugal by the Romans, probably for keeping them in special parks (*vivaria*), which enhanced their owner's status. In the tenth century AD, Crimea was a part of the Eastern Roman Empire. Historical and archaeological studies suggest that deer was a totemic animal for Romans and a symbol of wealth and power. Moreover, hunting for red deer in Roman Britain was an expression of status (Allen *et al.*, 2014). Therefore, a potential extinction of natural red deer population due to Greek activities in Crimea may have induced the need for its reintroduction. More studies on ancient red deer populations, especially from potential migration routes from Iberia to Crimea, are crucial to resolve the question about the origin of the contemporary red deer population in Crimea.

Comparison of the 12 models with ABC analyses revealed that after the LGM, red deer likely colonized

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the Crimean Peninsula in two independent waves: at the end of the Pleistocene and in the Holocene. Those events were related to extinctions and replacements of populations. Similar events have been reported for the collared lemming, where population turnovers were probably caused by climate fluctuations and changes in vegetation (Brace *et al.*, 2012; Palkopoulou *et al.*, 2016). Red deer, however, has a wider environmental tolerance and is generally well adapted to various environments (Straus, 1981; Geist, 1998). Thus, climate as a single factor driving extensive changes in its population may be less likely than for small mammals. Correspondingly, red deer as an important game species has been hunted, translocated and introduced since ancient times (Hartl, Zachos & Nadlinger, 2003; Zachos & Hartl, 2011).

We find no support for the hypothesis that Crimea was a Pleistocene refugium for red deer. At least two extinction events in the late Quaternary are in conflict with the classical refugium definition. Considering that our analyses were based on an mtDNA marker and thus reflect only the maternal lineage history, it would be interesting to also analyse nuclear markers to confirm our conclusions on red deer population history in Crimea. Nonetheless, the Crimean samples analysed here provide significant insights into the phylogeography and migration routes of red deer at a global scale.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Detailed information about samples used in the study. Radiocarbon dates obtained in previous studies are marked by asterisks. GenBank accession numbers in italics indicate partial cytb sequences published by Stankovic *et al.* (2011).

Table S2. Chronostratigraphy of layers in Emine-Bair-Khosar Cave.

Table S3. Nucleotide substitution models for alignment sets used in three programs. In all models, we assumed five discrete categories in the case of gamma distributed rates.

Table S4. Summary statistics used in ABC analysis. N – number of individuals; h – number of haplotypes; S – segregating sites; π – nucleotide diversity; PairDiff – pairwise differences between groups.

Table S5. Model likelihoods and Bayes factors among the 12 models compared by approximate Bayesian computation. The Bayes factors have a row to column interpretation (the value at row i and column j is $BF_{ij} = ML_i/ML_j$). These values correspond to the analysis with a mutation rate of 10.0% per million years, after averaging the five smallest acceptance proportions (0.001 to 0.005% of the closest simulated datasets to the observed one). The best-fitted model was bolded.

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Table S6. Bayes factors among the 12 models compared by approximate Bayesian computation. The Bayes factors have a row to column interpretation (the value at row *i* and column *j* is $BF_{ij} = ML_i/ML_j$). These values correspond to the analysis with a mutation rate of 20.0% per million years, after averaging the ten smallest acceptance proportions (0.001–0.01% of the closest simulated datasets to the observed one). The number of acceptance proportions averaged for this result was higher than in tables with lower mutation rates due to many models presented a likelihood of 0.0 in the first five proportions. The best-fitted model was bolded.

Table S7. Bayes factors among the 12 models compared by approximate Bayesian computation. The Bayes factors have a row to column interpretation (the value at row *i* and column *j* is $BF_{ij} = ML_i/ML_j$). These values correspond to the analysis with a mutation rate of 30.0% per million years, after averaging the ten smallest acceptance proportions (0.001 to 0.005% of the closest simulated datasets to the observed one). The number of acceptance proportions averaged for this result was higher than in tables with lower mutation rates due to several models presented a likelihood of 0.0 in the first five proportions. The best-fitted model was bolded.

Table S8. Rate of error and statistical power in the model choice analysis by approximate Bayesian computation, as assessed by pseudo observed data sets (PODs).

Figure S1. Cross section through layers in Emine-Bair-Khosar Cave. The position of red deer remains was marked. Uncalibrated radiocarbon dates of these samples and membership of DNA from the samples to phylogenetic clades were given.

Figure S2. MrBayes tree based on the longer alignment of Cervini members with simplification of Eastern (clade E) and Western (clade W) lineages of red deer. The asterisk indicates that a given sequence is represented by more than one accession number. The shading of circles at nodes corresponds to the number of methods (out of five) which recovered the given node. Numbers at nodes, in the order shown, correspond to: posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB), bootstrap values obtained in PhyML(PH), TreeFinder (TF) and PAUP by neighbor-joining methods (NJ). Values of the posterior probabilities and bootstrap percentages ≤ 0.50 and 50%, respectively, were omitted or indicated by a dash '–'.

Figure S3. Simplified maximum clade credibility tree obtained in BEAST for the longer alignment of Cervini members. Median ages expressed in ka BP are shown for all nodes. Sequences obtained from Crimea specimens are bolded. The Eastern (clade E) and Western (clade W) lineages of red deer were indicated.

Figure S4. MrBayes tree based on the shorter alignment for the Eastern lineage of red deer. Clades E1-E3 represent *C. canadensis* (wapiti), whereas E4-E7 *C. nippon* (sika). Sequences newly obtained in this study from Crimea specimens are bolded. The asterisk indicates that a given sequence is represented by more than one accession number. The shading of circles at nodes corresponds to the number of methods (out of five) which recovered the given node. A probable age or calibrated dates are shown in square brackets. Numbers at nodes, in the order shown, correspond to: posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB), bootstrap values obtained in PhyML (PH), TreeFinder (TF) and PAUP by neighbor-joining methods (NJ). Values of the posterior probabilities and bootstrap percentages ≤ 0.50 and 50%, respectively, were omitted or indicated by a dash '–'.

Figure S5. MrBayes tree based on the shorter alignment for the Western lineage of red deer. Sequences newly obtained in this study from Crimea specimens are bolded. The asterisk indicates that a given sequence is represented by more than one accession number. The shading of circles at nodes corresponds to the number of methods (out of five) which recovered the given node. A probable age or calibrated dates are shown in square brackets. Numbers at nodes, in the order shown, correspond to: posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB), bootstrap values obtained in PhyML (PH), TreeFinder (TF) and PAUP by neighbor joining methods (NJ). Values of the posterior probabilities and bootstrap percentages 0.50 and 50%, respectively, were omitted or indicated by a dash '–'.

Box S1. Input file for scenario A with mutation rate 5.14% per million years for final simulations.

Box S2. Input file for scenario B with mutation rate 5.14% per million years for final simulations.

Box S3. Input file for scenario C with mutation rate 5.14% per million years for final simulations.

Box S4. Input file for scenario D with mutation rate 5.14% per million years for final simulations.

Box S5. Input file for scenario E with mutation rate 5.14% per million years for final simulations.

Box S6. Input file for scenario F with mutation rate 5.14% per million years r for final simulations.

Box S7. Input file for scenario G with mutation rate 5.14% per million years for final simulations.

Box S8. Input file for scenario H with mutation rate 5.14% per million years for final simulations.

Box S9. Input file for scenario I with mutation rate 5.14% per million years for final simulations.

Box S10. Input file for scenario J with mutation rate 5.14% per million years for final simulations.

Box S11. Input file for scenario K with mutation rate 5.14% per million years for final simulations.

Box S12. Input file for scenario L with mutation rate 5.14% per million years for final simulations.

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