

Subspecies dynamics in space and time: A study of the red deer complex using ancient and modern DNA and morphology

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Abstract

Aim: The status of geographical units within species and species complexes is debated for many taxa, with many molecular studies failing to detect phenotypically defined subspecies. The history and longevity of geographical patterns are also generally very poorly understood. We examine Holarctic red deer (*Cervus elaphus* and related forms), incorporating ancient DNA to ask whether the present phylogeography has persisted through climatic perturbations or is a relatively recent phenomenon.

Location: Holarctic (Europe, northern Asia and North America).

Methods: We obtained 21 modern and 30 Holocene and Late Pleistocene samples, which together with published data resulted in 180 individuals spanning 21 nominal extant and one extinct subspecies. Phylogenetic analyses were carried out on 748-bp of mitochondrial DNA (cytochrome *b* and control region). Where possible, the morphology of DNA-yielding ancient samples was examined to assess subspecies identity.

Results: Major clades within the red deer complex are upheld, but subspecies within them receive varying support. The ancient phylogeographical structure conforms in significant part to the modern situation, but some haplogroups no longer survive. Moreover, there have been substantial shifts in geographical ranges through time. Wapitoids spread as far west as Romania in the last glaciation, and elaphoids reached eastward to the Ural Mountains. A possible contact zone between the two lineages stretched from the Urals through the Crimea to Eastern Europe.

Main conclusions: Ancient DNA and morphology are strongly complementary in elucidating population history. Through the past 50 kyr, the major lineages of red deer, and some of the subspecies groups within them, have maintained their genetic and morphological integrity and their core geographical distributions, despite range expansions and contractions and likely contact between the haplogroups (with potential for hybridization).

KEYWORDS

ancient DNA, *Cervus canadensis*, *Cervus elaphus*, *Cervus hanglu*, mitochondrial DNA, morphology, phylogeography, red deer

1 | INTRODUCTION

Geographical variation is widespread within animal species. This variation often reflects local adaptation, has been used to delimit conservation units, and can be significant for speciation. It is also commonly used to define subspecies, although the concept of subspecies has been fiercely debated (e.g. Mayr, 1982; Phillimore & Owens, 2006) and many molecular studies have failed to detect phenotypically defined subspecies (e.g. Patten, 2015, and references therein).

The taxonomy, distribution and inferred history of mammal species are generally based on modern populations alone. Current phylogeographical patterns have been explained as resulting from separation between populations in refugia during the last glaciation, and rapid recolonization afterwards (e.g. Hewitt, McKinnon, Lascoux, & McKinnon, 2004; Taberlet, Fumagalli, Wust-Saucy, & Cosson, 1998). Ancient DNA studies have shown, however, that modern phylogeographical patterns are often not seen before the Last Glacial

Maximum (LGM), c. 21 kyr (e.g. brown bears: Hofreiter et al., 2004; Valdiosera et al., 2007), raising the question whether present geographical patterning within species has persisted through climatic perturbations or is a relatively recent phenomenon.

The red deer complex, with its broad Holarctic distribution (Figure 1), provides an excellent model for examining these issues. It is divided into a western lineage ("elaphoids") across Europe, North Africa and Western Asia as far as the Tarim region, and an eastern lineage ("wapitoids," elk or wapiti) in Eastern Asia and North America (Figure 1). These lineages have historically been further subdivided into at least 25 extant, and two extinct, subspecies (Dolan, 1988; Flerov, 1960; Geist, 1999; Groves & Grubb, 1987; Grubb, 2004; O'Gara, 2002; Trense, 1989). However, the status of many of these has been questioned, as some type specimens may be individual variants, or reflect the direct effects of environmental conditions (Flerov, 1960; Geist, 1999; Groves & Grubb, 1987; Grubb, 2004; Ludt, Schroeder, Rottmann, & Kuehn, 2004; O'Gara, 2002).

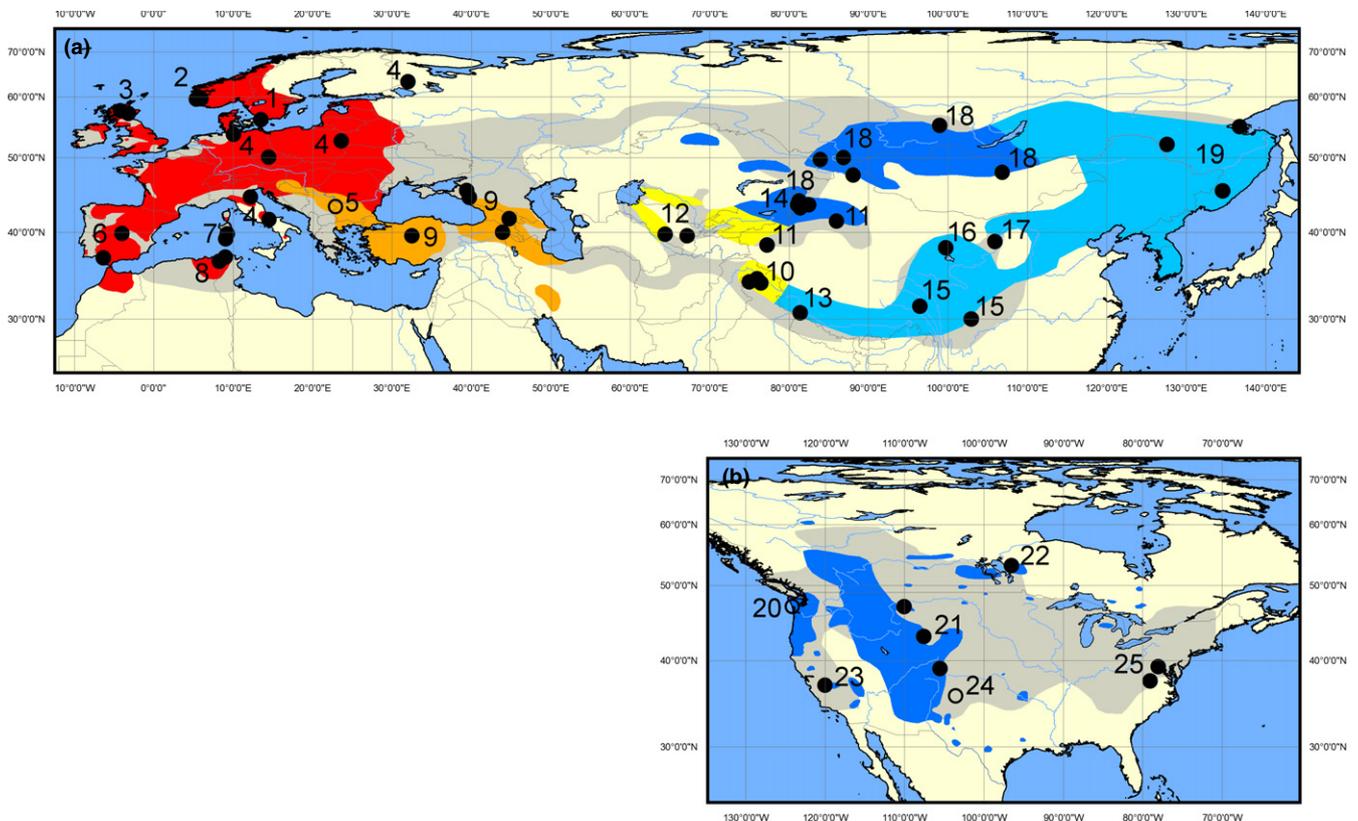


FIGURE 1 Holarctic red deer distribution in (a) the Palearctic and (b) Nearctic. Filled circles represent modern sample locations, open circles unsampled subspecies. Numbers correspond to subspecies names: **Elaphoids**: 1. *C. e. elaphus* 2. *C. e. atlanticus* 3. *C. e. scoticus* 4. *C. e. hippelaphus* (including *italicus*) 5. *C. e. montanus (pannoniensis)* 6. *C. e. hispanicus* 7. *C. e. corsicanus* 8. *C. e. barbarus* 9. *C. e. maral* 10. *C. h. hanglu* 11. *C. h. yarkandensis* 12. *C. h. bactrianus*. **Wapitoids**: 13. *C. c. wallichii* 14. *C. c. songaricus* 15. *C. c. macneilli* 16. *C. c. kansuensis* 17. *C. c. alashanicus* 18. *C. c. sibiricus* 19. *C. c. xanthopygus* 20. *C. c. roosevelti* 21. *C. c. nelsoni* 22. *C. c. manitobensis* 23. *C. c. nannodes* 24. *C. c. merriami* (extinct) 25. *C. c. canadensis* (extinct). Colours correspond to the major taxonomic groupings (cf. Table 1): **blue**, Asian wapitoids: *sibiricus*, *songaricus* and North American wapitoids: *roosevelti*, *nelsoni*, *manitobensis*, *nannodes*; **turquoise**, *xanthopygus* and Central Asian wapitoids: *kansuensis*, *macneilli*, *wallichii* & *alashanicus*; **yellow**, Central Asian elaphoids: *bactrianus*, *yarkandensis*, *hanglu*; **orange**, "maraloid" elaphoids: *maral*, *pannoniensis*; **red**, western and central European elaphoids: *elaphus*, *scoticus*, *hippelaphus*, *atlanticus*, *corsicanus*, *barbarus*. **Grey**, areas indicate historic distribution, integrating documentary and archaeological records spanning c. 2,000–100 BP. Modern species map from IUCN (2016); historical and subspecies distributions from Flerov, 1960; Heptner, Nasimovich, & Bannikov, 1966; Whitehead, 1972; Hall, 1981; Banwell, 2009. The *C. e. scoticus* specimen from New Zealand is not shown. For more details see Appendices S2 & S3

Nonetheless, on the basis of heritable characters such as antler branching and coat pattern, significant agreement on the validity and relationships of subspecies was reached by Groves and Grubb (1987) and Geist (1999) (Table 1). Genetic studies (e.g. Ludt et al., 2004; Mahmut et al., 2002; Niedzialkowska et al., 2011; Pitra, Fickel,

Meijaard, & Groves, 2004; Skog et al., 2009) support many of these groupings and have identified strong phylogeographical structuring, but could not recover all named subspecies.

The divergence time between wapitoids and elaphoids is also contested. Published molecular estimates range from 7–3 Myr

TABLE 1 Consensus morphological taxonomy of Holarctic red deer based on Groves and Grubb (1987) and Geist (1999), with mtDNA clades identified by Mahmut et al. (2002); Ludt et al. (2004); Pitra et al. (2004); Skog et al. (2009); Niedzialkowska et al. (2011); Lorenzini & Garofalo (2015). Numbers in brackets in the subspecies column correspond to subspecies number on the map in Figure 1

Main divisions	Location	Subspecies	Comments	clades	
Elaphoid forms of Europe and western Asia	Western and Central	(1) <i>elaphus</i> (2) <i>atlanticus</i>	Should be combined as <i>C. e. elaphus</i> (see also Lowe & Gardiner 1974). Other authors (e.g. Banwell, 2009), however, recognise differences worthy of subspecies distinction.	●●	
	European	(3) <i>scoticus</i> (4) <i>hippelaphus</i> (6) <i>hispanicus</i>			
	Southeast Europe	(5) <i>pannoniensis</i>	<i>C. e. montanus</i> is a synonym (Banwell 1998). Probably related to <i>maral</i> .	●	
	Corsica/Sardinia	(7) <i>corsicanus</i>	Distinct, however some (e.g. Flerov, 1960, Groves & Grubb 2011), have synonymised with <i>barbarus</i>	●	
	North Africa	(8) <i>barbarus</i>	Distinct, however some (e.g. Flerov, 1960, Groves & Grubb 2011), have synonymised with <i>corsicanus</i>	●	
	Turkey and the Caucasus	(9) <i>maral</i>	Distinct	●	
	Central Asian forms of uncertain affinity	Kashmir	(10) <i>hanglu</i>	Affinities debated, but antler configuration generally 'elaphoid'	●
		Tian Shan	(11) <i>yarkandensis</i>	Supposedly 'primitive' features and neither typically elaphoid nor wapitoid, but most likely primitive elaphoids.	●
		Central Asia (Amu Darya/Syr Darya)	(12) <i>bactrianus</i>		●
Western China		(13) <i>wallichii</i>	Supposedly 'primitive' morphology and of unclear affinity, but most likely linked to wapitoids.	●	
Western China		(15) <i>macneilli</i>	Likely synonyms. Supposedly 'primitive' morphology and of unclear affinity, but most likely linked to wapitoids.	●	
Wapitoid forms of eastern Asia and North America	Western China	(16) <i>kansuensis</i> (17) <i>alashanicus</i>	Known only from two specimens. Geist (1999) described it as a 'primitive wapiti' but Groves & Grubb (1987) say the antlers are not typical for wapiti.	●	
	Eastern China and SE Siberia	(19) <i>xanthopygus</i>	A small, distinct, wapiti with 'primitive' features, but Geist (1999) describes it as pedomorphic, so its characters could be secondarily derived.	●	
	Tian Shan Altai	(14) <i>songaricus</i> (18) <i>sibiricus</i>	Synonyms.	●	
	North America	(20) <i>roosevelti</i> (21) <i>nelsoni</i> (22) <i>manitobensis</i> (23) <i>nannodes</i> (24) <i>merriami</i> (extinct) (25) <i>canadensis</i> (extinct)	Geist (1999) and Groves and Grubb (2011) unite all the 'advanced wapitis' of Siberia and N America into one taxon, <i>C. e. canadensis</i> or <i>C. canadensis</i> .	●	

(Lorenzini & Garofalo, 2015; Ludt et al., 2004), to c. 0.8–0.2 Myr (Kuwayama & Ozawa, 2000; Polziehn & Strobeck, 2002). The older estimates rely on deep calibration points (Ho et al., 2008) and are highly unlikely since the earliest known fossil red deer is c. 0.9 Myr (Lister, Parfitt, Owen, Collinge, & Breda, 2010).

A related issue is whether the elaphoid and wapitoid groups should be treated as one, two or more species. Geist (1999) indicated that the two lineages breed in captivity but retain their distinctiveness in nature despite geographical proximity. However, he still classified both as *C. elaphus*. Mattioli (2011) separated *C. elaphus* and *C. canadensis*, and added *C. wallichii* (including *C. w. hanglu*). Groves and Grubb (2011) subdivided red deer into 12 species, mostly by elevating former subspecies to specific rank, a move strongly contested by Zachos et al. (2013); see further, Groves (2013) and Zachos and Lovari (2013).

In this study, we examine red deer from across its Holarctic range, incorporating modern and ancient DNA to ask whether the present phylogeography has persisted through climatic perturbations or is it a relatively recent phenomenon. We follow Lorenzini and Garofalo (2015) and the IUCN (2016) in recognizing *C. elaphus* and *C. canadensis*, and provisionally *C. hanglu*, as separate species. All are informally named “red deer,” but we retain the term “elaphoids” for *C. elaphus* and *C. hanglu*, and “wapitoids” for *C. canadensis*. We also consider sika deer *C. nippon*, distributed in Eastern Asia and Japan, as a fourth species within this complex according to the DNA evidence (e.g. Kuwayama & Ozawa 2000; Polziehn & Strobeck 1998; and see below).

2 | MATERIALS AND METHODS

2.1 | Sample collection

Adding to data from our previous studies (Meiri et al., 2013, 2014), we sampled 21 modern red deer specimens (Figure 1), including eight hangul or Kashmir stag, *C. h. hanglu*, and 30 ancient red deer (Appendix S1 & S2). We received four modern DNA extracts from Dr F. Zachos. Hog deer (*Axis porcinus*), and Eld's deer (*Cervus eldii*) served as outgroups, following Pitra et al. (2004) and Gilbert, Ropiquet, and Hassanin (2006).

2.2 | Radiocarbon dating

Ten ancient samples yielding DNA were dated at the Oxford Radiocarbon Accelerator Unit. Other dates were taken from the literature (Appendix S2 & S3). Finite radiocarbon dates were calibrated (to 2σ) in OxCal 4.1 (<https://c14.arch.ox.ac.uk/oxcal/OxCal.html>) using the IntCal09 calibration curve (Reimer et al., 2009). All dates in the text are median calibrated ages.

2.3 | Morphological assessment

Where possible, the morphology of DNA-yielding ancient samples was examined to assess subspecies identity. Body size in elaphoid

deer broadly increases from west to east, whereas many wapitoids are significantly larger still (Geist, 1999), especially in the postcranial skeleton (Parfitt & Lister, 2013). European and Western Asian elaphoid antlers are characterized by a transverse upper fork, in the European forms generally developed into a multi-point “crown,” whereas the wapitoid antler top is characterized by a series of forks in the sagittal plane (Groves & Grubb, 1987).

2.4 | DNA extraction

DNA of modern specimens was extracted in one laboratory, and that of ancient specimens in another, dedicated ancient DNA laboratory where no modern red deer had been analysed. DNA from soft tissue was extracted using QIAamp DNA Micro Kit (QIAGEN, Inc, Crawley, UK), following the protocol for forensic casework samples. DNA from bone, tooth and antler was extracted according to a method modified from Yang, Eng, Waye, Dudar, and Saunders (1998) as described in Meiri et al. (2013, 2014). Every 8th or 10th sample served as a negative extraction control with no bone powder (Appendix S1).

2.5 | DNA amplification and sequencing

We amplified two mitochondrial markers: the 5' of the cytochrome *b* (*cyt b*) gene (423 bp), and the 3' of tRNA-Pro and 5' of the control region (CR) (358–436 bp). Both regions have been extensively used in phylogeographical studies, including those of red deer (e.g. Ludt et al., 2004; Randi, Mucci, Claro-Hergueta, Bonnet, & Douzery, 2001). The two markers were concatenated (Meiri et al., 2013, 2014), using the slower mutation rate of *cyt b* to recover deeper splits in the tree.

For the ancient samples, five and four primer pairs were designed to amplify sections of *cyt b* and CR, respectively, consisting of overlapping fragments of around 150 bp (Meiri et al., 2013, 2014; Appendix S1).

2.6 | Data analyses

To the new sequence data we added 79 modern and 67 ancient published DNA sequences (Lorenzini & Garofalo, 2015; Meiri et al., 2013, 2014; Stankovic et al., 2011). Sika deer (*Cervus nippon*) was added to the ingroup following Ludt et al. (2004) and Pitra et al. (2004).

The best-fit partitioning scheme was identified by PARTITIONFINDER 1.1.0 (Lanfear, Calcott, Ho, & Guindon, 2012) and the substitution model parameters estimated independently for each partition. Phylogenetic relationships were estimated using Maximum likelihood (ML) (Felsenstein, 1981), Bayesian inference (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), and minimum spanning network (Excoffier, Laval, & Schneider, 2005). For more details see Appendix S1.

We used bootstrap and posterior probability to assess clade support. We consider bootstrap values of 70%–89% to indicate

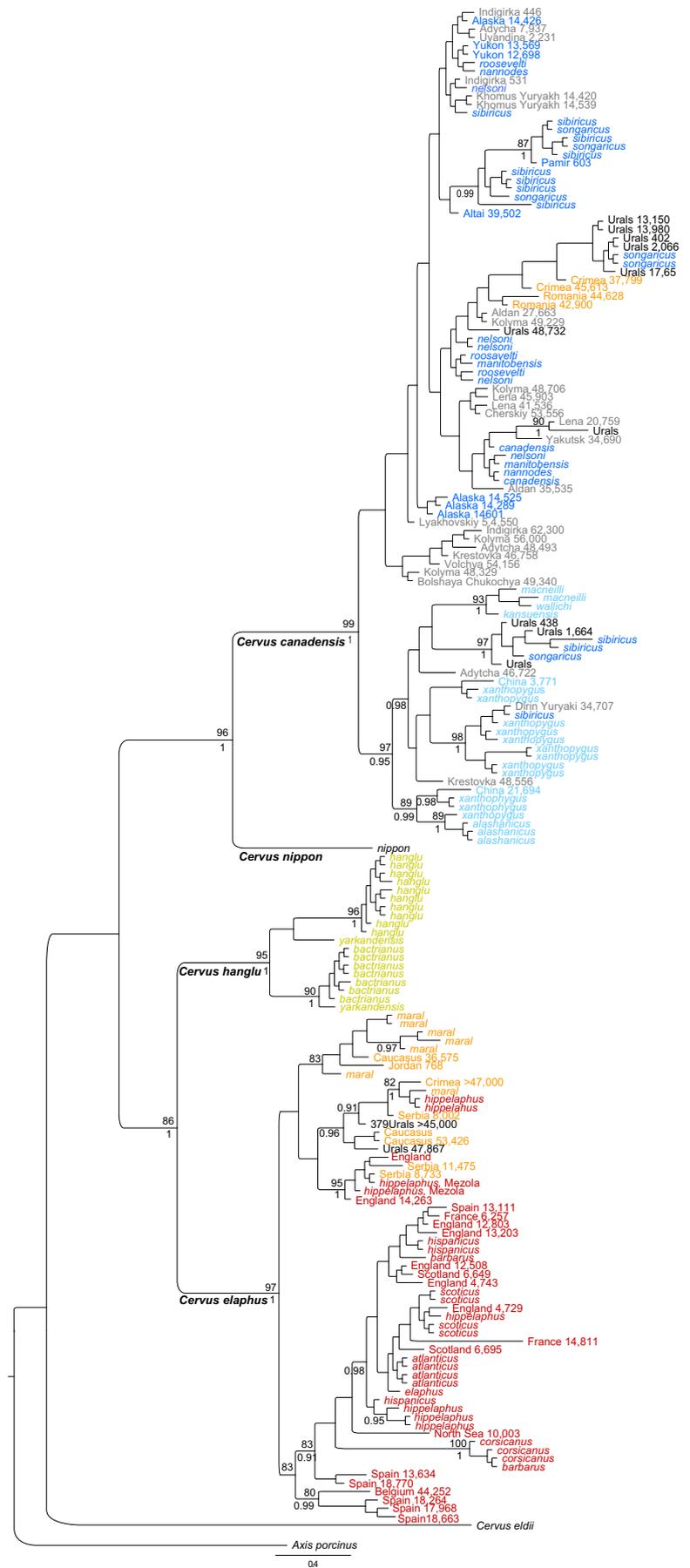


FIGURE 2 Bayesian phylogenetic tree obtained using 748 bp of combined mitochondrial *cyt b* and CR from 183 ancient and modern specimens. Hog deer (*Axis porcinus*) and Eld's deer (*Cervus eldii*) serve as outgroups. Bootstrap support (above 80%) and Bayesian posterior probabilities (above 0.9) are shown above and below the branches, respectively. Modern samples are represented by subspecies name in italic, whereas ancient samples are represented by location and median radiocarbon date (when available). The colours correspond to geographical location of the samples divided according to the modern distribution of haplogroups (cf. Figure 1): red, W & C Europe and North Africa; orange, SE Europe and SW Asia; yellow, C Asia; light blue, C & E China; dark blue, E Asia and N America; black, ancient samples from the Urals; grey, ancient samples from NE Siberia. For more details, see Appendices S2 & S3

moderate support, whereas highly supported branches are defined as values above 90% and 0.95 for bootstrap values and posterior probabilities respectively (Hillis & Bull, 1993; Huelsenbeck & Rannala, 2004).

To estimate the divergence date between wapitoids and elaphoids, we used the earliest fossils of red deer in Europe, at 0.9 Myr (Lister et al., 2010), and 62 radiocarbon dates of ancient samples with finite dates as calibration points (tip calibration). Uncorrelated relaxed-lognormal clock (Drummond, Ho, Phillips, & Rambaut, 2006) analyses were performed using BEAST 1.8.4 (Drummond & Rambaut, 2007; Drummond, Rambaut, Shapiro, & Pybus, 2005) (Appendix S1).

3 | RESULTS

3.1 | Radiocarbon dates

The uncalibrated and calibrated radiocarbon results are shown in Appendices S2 & S3. The dates range from beyond the limit of radiocarbon dating, to finite dates at c. 48,000 yr BP, to very recent, c. 400 yr BP dates.

3.2 | New DNA sequence data

Of the 21 new samples of modern red deer, 19 yielded DNA, of which one gave only a partial sequence. Of 30 new ancient specimens, 12 (40%) yielded DNA, one of which yielded a partial sequence (Appendix S2).

Sequence data were consistent between fragments generated with different primer pairs. Repeated extracts and amplification of the same samples yielded the same sequences. No stop-codons or indels were found in *cyt b*. Seven indels were found in the control region and removed from the analyses together with tandem repeats not present in elaphoids. The total fragment length used for phylogenetic analyses was 748 bp.

3.3 | Analysis of modern DNA sequence data

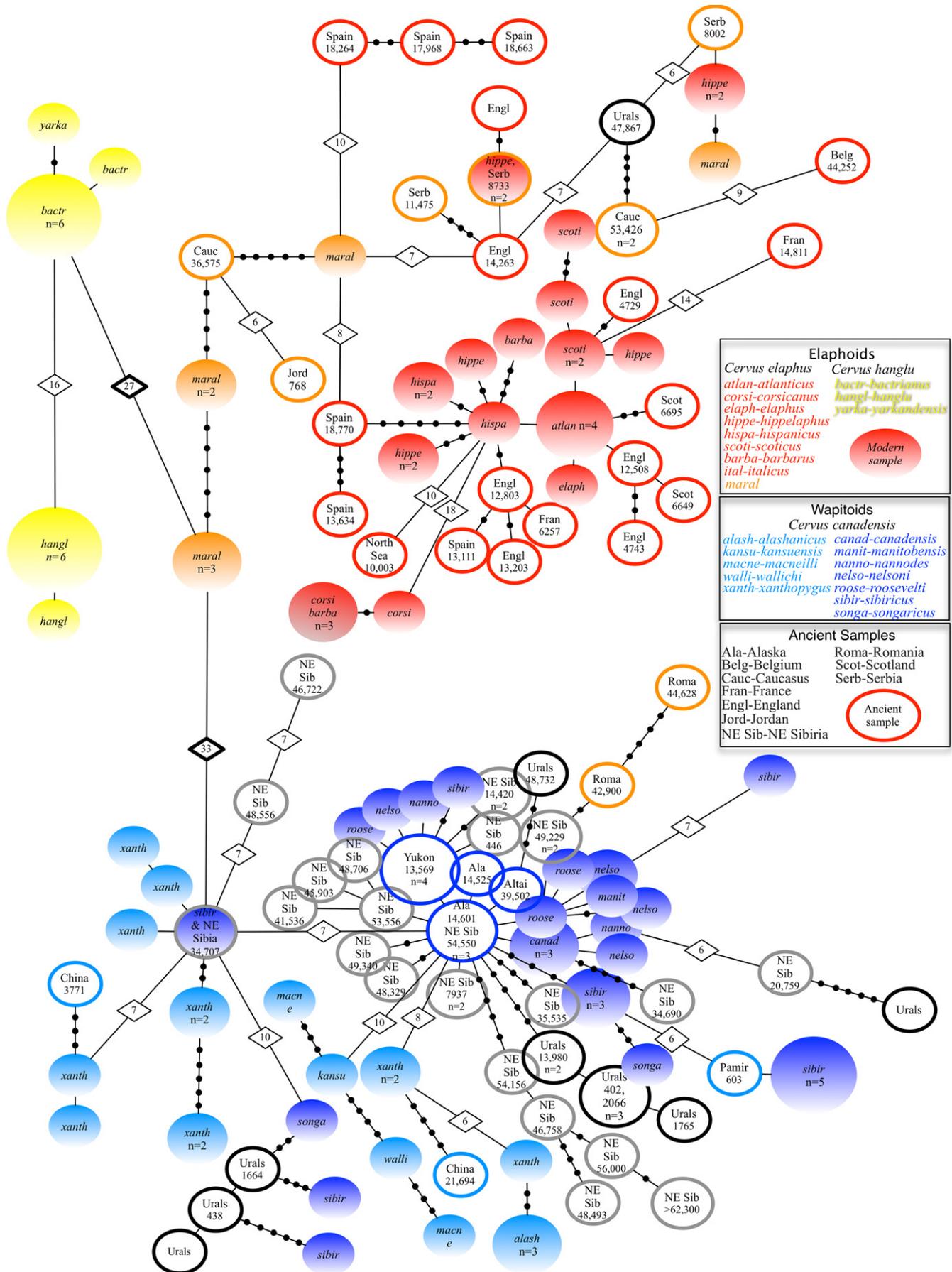
We analysed 101 modern red deer specimens and one sika deer (*Cervus nippon*) (Appendices S2 & S3). Maximum Likelihood and Bayesian analyses yielded similar tree topologies (Figure 2).

The minimum spanning network (Figure 3) highlights how ancient specimens provide transitional haplotypes “missing” from the modern network, and in some cases filling the centre of a radiation. This network, based on pairwise differences among 141 haplotypes of red deer only, represents 172 sequences (partial sequences were excluded).

The analyses indicate the following:

- 1 A major split between *C. canadensis* (wapitoids) and *C. nippon* (sika) on the one hand, and *C. elaphus* and *C. hanglu* (both elaphoids) on the other (Table 1). The taxonomic separation of *C. hanglu* restricts the *C. elaphus* clade to deer from Europe, the Caucasus, Near East and North Africa.
- 2 A clade of N & C European deer includes *C. e. elaphus*, *scoticus*, *atlanticus*, *hispanicus* and most *hippelaphus*. The network reveals little structure within the group and they are all close, with one to three base-pair differences (Figure 3).
- 3 As sister-group to this clade, *C. e. corsicanus* is distinct, differing by 19–20 bp from the core Spanish haplotype. One of the two Corsican haplotypes is shared with Tunisian (*barbarus*) deer (Figure 3). From our analyses, *C. e. corsicanus* is more closely related to the Spanish population than to that of northern Italy (Figures 2 & 3). However, studies using microsatellites show the opposite (Hajji et al., 2008; Hmwe et al., 2006; Zachos, Hartl, Apollonio, & Reutershan, 2003); this may be due to different markers capturing distinct aspects of population history.
- 4 In the tree (Figure 2), all modern *C. e. maral*, from Turkey and the Caucasus, form a clade in sister-group relationship to the western and Central European clades, although its support is weak to moderate. Historical specimens identified as *C. e. maral* from Voronezh (SW Russia), close to the mapped *maral* range, also fall here. Interestingly, two groups of modern Italian deer, conventionally *hippelaphus*, also fall within this group. These findings are corroborated in the network (Figure 3): the *maral* haplotypes from Turkey and the Caucasus are highly genetically diverse, falling far from the main Western/Central European cluster, but group with the Italian samples from the southern Apennines and Mesola. The latter were recently suggested as a separate subspecies, *C. e. italicus*, by Zachos, Mattioli, Ferretti, and Lorenzini (2014), and their mitochondrial relationship to marals has been supported based on fossil and molecular evidence (Borowski et al., 2016; Croitor & Cojocar, 2016). *C. e. maral* distantly connects Western Asian and European deer to the Central Asian subspecies (*C. h. yarkandensis*, *bactrianus* and *hanglu*) and to East Asian wapitoids (Figure 3).
- 5 Central Asian elaphoids, *C. h. bactrianus*, *yarkandensis* and *hanglu*, together form a monophyletic clade, with *bactrianus* Rom. *hanglu* each monophyletic. *C. h. hanglu* is 17–18 bp away from *bactrianus*, whereas one sample of *yarkandensis* differs by only 2 bp from *bactrianus* (Figure 3). Our data support Lorenzini and Garofalo (2015) in placing the endangered *hanglu* as part of this group, and not with *wallichi* and *macneilli* as suggested by Mattioli (2011), although it is the most genetically divergent of the three. This group is provisionally treated as a species, *C. hanglu* (IUCN, 2016).

FIGURE 3 Minimum spanning network of 141 haplotypes that represent 172 sequences. Small black circles indicate missing haplotypes, lines between them represent a single mutation, and numbers within diamonds indicate the number of missing haplotypes where this is more than five. Modern samples are represented as filled circles and the name of the subspecies, whereas ancient samples are represented as open circles and location and radiocarbon date (if applicable). The colours correspond to modern distributions of haplogroups as in Figure 2 (for more details see Appendices S2 & S3)



- 6 Chinese subspecies (*C. c. xanthopygus*, *alashanicus*, *macneilli*, *wallichii*, *kansuensis*) form a well-supported wapitoid clade (97% and 0.95 bootstrap value and posterior probabilities, respectively). Within it, *macneilli*, *kansuensis* and *wallichii* are monophyletic (with posterior probability support of 0.98), but with no internal structure that corresponds to these taxa.
- 7 North American subspecies (*C. c. nelsoni*, *manitobensis*, *roosevelti*, *nannodes*) form a second wapitoid clade but with less support and there is no structure that corresponds to these individual taxa. They are very close on the network (Figure 3), connecting to the above “Chinese” wapitoid clade at a somewhat greater distance (8–11 bp).
- 8 *C. c. songaricus* (Tian Shan) and *sibiricus* (Transbaikal, Mongolia and Altai) cannot be distinguished based on mtDNA, as suspected on morphological grounds (Geist, 1999; Groves & Grubb, 1987). They occur across both wapitoid clades.
- 9 The sika deer (*Cervus nippon*) is sister-group to *C. canadensis* based on mtDNA (see also Ludt et al., 2004; Pitra et al., 2004; Randi et al., 2001). It was formerly assumed to be a “primitive” form, close to the ancestry of the red deer complex as a whole (Geist, 1999; Groves & Grubb, 1987; Lister, 1984).

3.4 | Estimating divergence times among the major clades

The data set comprised 95 modern sequences of red deer and one of sika, together with 62 ancient sequences with finite radiocarbon dates.

The divergence time between elaphoids (including *C. hanglu*) on the one hand, and wapitoids+sika on the other, is relatively similar whether using constant population size (CPS) or Bayesian skyline plot (BSP) demographic models. With CPS the divergence time is 1.40 Myr with a 95% higher posterior density (HPD) of 0.73–2.20 Myr. Using the BSP model the divergence time is 1.38 Myr BP (95% HPD: 0.75–2.10 Myr).

The most recent common ancestor (MRCA), using CPS and BSP, respectively, is estimated as 0.74 Myr (95% HPD: 0.25–1.33), or 0.73 Myr (0.29–1.25) for wapitoids and sika deer; 0.30 Myr (0.14–

0.48 Myr) or 0.30 Myr (0.15–0.47 Myr) for *C. canadensis*; 0.73 Myr (0.52–0.95 Myr) or 0.69 Myr (0.47–0.91 Myr) for *C. elaphus*; and 0.23 Myr (0.053–0.45 Myr) or 0.21 Myr (0.056–0.41 Myr) for *C. hanglu*.

3.5 | Ancient DNA and morphology

We analysed DNA sequences of 79 ancient red deer specimens. A proportion of these also provided usable morphological information. Measurement data are given in Appendix S4 and compared with modern male Asiatic and North American wapiti, and fossil elaphoids from the Last Glaciation of the UK.

3.5.1 | Western and Central European elaphoid deer

All ancient mtDNA haplotypes from this region, LGM to Holocene, are “elaphoid,” and most are in the modern Western-Central European haplogroup (Meiri et al., 2013). This conforms to morphological evidence and suggests integrity of this group through time: antlers preserving the diagnostic upper part are rare, but where available they conform to the elaphoid “coronate” type (e.g. von Koenigswald, 2002; fig. 98). The same is true of images of red deer in cave art from the last glaciation (Figure 4; Aujoulat, 2005). Many of these deer are larger than those living in the same areas today (Lister, 1987), but are nonetheless smaller than West Siberian and Eastern European wapitoids (see below).

Pre-LGM, one individual from Belgium falls in this group on mtDNA, although there is admixture from the modern *maral* group, shown by pre- and post-LGM specimens from Belgium and England (Figure 3; Meiri et al., 2013).

3.5.2 | Eastern Europe and SW Asian (maral group) elaphoid deer

Pre-LGM samples from the Crimea and Caucasus group mitochondrially with modern marals that occupy the same or nearby ranges

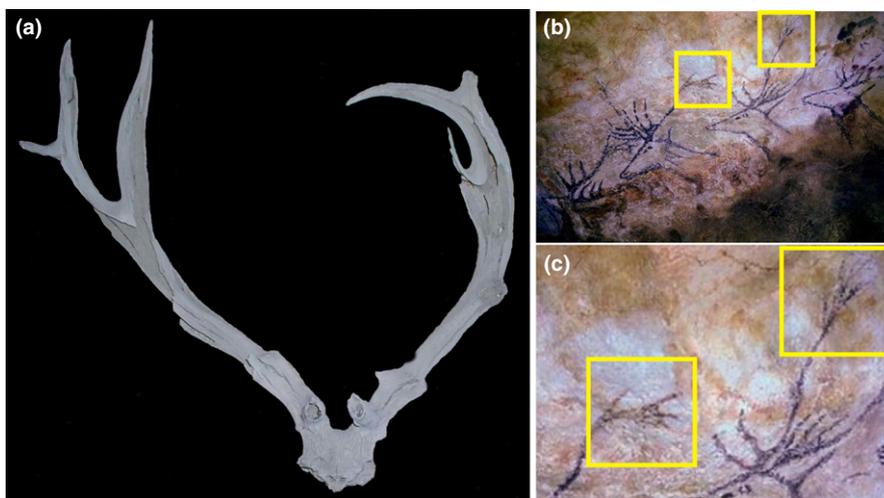


FIGURE 4 Ancient red deer antlers. (a) Skull roof and antlers from Pamir (Altai region, Russia), c. 600 BP, showing “wapitoid” sagittal fork at top of right antler (to left of picture; left antler distorted). (b, c) “Swimming deer” from Lascaux Cave, France (c. 20 kyr) —“elaphoid” antler crowns are marked in (b) and enlarged in (c)

today. DNA from a late Holocene sample from Jordan conforms to evidence that the species persisted in the Levant until the 12th Century (Tsahar et al., 2009), and indicates relationship to the geographically closest modern subspecies, *C. e. maral*, to the north (Figure 1). Early Holocene samples from Serbia (Meiri et al., 2013) also group here—they are in the historical range of *C. e. pannoniensis*, which is regarded as a “maraloid” deer (Banwell, 1998; Croitor & Cojocaru, 2016). These data suggest integrity of the maral group though time.

Radiocarbon dates and mtDNA indicate that pre-LGM (>45 kyr), maraloid deer reached the Ural Mountains, a substantial extension compared to their modern range.

3.5.3 | Siberian wapitoids

The pre-LGM Altai specimen conforms in mtDNA to modern *C. c. sibiricus/songaricus* inhabiting that area (Meiri et al., 2014). The specimen (a metatarsal, c. 39.5 kyr BP) is measurable and strongly conforms to identity as a wapitoid; it is even larger than living North American wapiti (Appendix S4).

Antlers from the Pamir Mountains (to the west of Tien Shan, c. 0.6 kyr BP), with wapitoid mtDNA of the Siberian/North American clade, show a strong upper tine followed by a fork which in the right antler is in the sagittal plane. The left antler is twisted, but following the original line of the beam the orientation was sagittal, i.e. of wapitoid form (Figure 4). This find is geographically at the centre of a triangle formed by the isolated modern populations of the *C. hanglu* subspecies *bactrianus*, *yarkandensis* and *hanglu*. A relatively small southern extension of the Altai *C. c. sibiricus/songaricus* range seems the most likely explanation.

Wapitoid deer, from both major mtDNA clades, extended through Western Siberia to the Urals, with dates spanning from c. 22–0.4 kyr (Figures 2, 3 & 5). The size of the specimens is not diagnostic but is consistent with their wapitoid genotype (Appendix S4): the Ural astragalus (c. 1.7 kyr BP) is in the overlap zone of size between North American and Asian wapiti and European last-glacial elaphoids; the West Siberian tarsal (undated) and Ural molar (c. 1.7 kyr BP) are almost as large.

The former presence of both elaphoid and wapitoid deer in the Ural region, not occupied by red deer today, is noteworthy. The dates range from at least 50 kyr up to only 300 years ago (Figure 5a–d). This is corroborated by historical evidence that red deer distribution was continuous in an east-west band across the whole of Eurasia (Heptner et al., 1966; Figures 1 & 5), with the Urals acting as a boundary between the elaphoids and wapitoids.

Furthermore, several pre-LGM specimens from Crimea (Niedzalkowska et al. 2011) and Romania fall in the clade of Siberian and NE Chinese wapitis, their haplotypes forming a small subclade with some ancient Ural sequences. The five limb bones from Romania (Peștera cu Oase, c. 44 kyr BP), one of which yielded DNA, are of exceptionally large size (Parfitt & Lister, 2013). The mandible (c. 46 kyr BP) is also genetically wapitoid but belongs to a different individual. It is relatively smaller, possibly due to sexual dimorphism

or to a smaller dental to postcranial ratio in wapitoids (Parfitt & Lister, 2013). Proxy data from the Oase site indicate a productive environment, potentially contributing to the large size of the deer; the cave bears from this site are also among the largest known (Trinkaus, Constantin, & Zilhao, 2013). However, with the exception of a metacarpal from Tornewton Cave, England (Lister, 1984; Appendix S4), which has not been tested for DNA, the Oase red deer are larger than any other known from the European Late Quaternary, implying a genetic basis, at least in part.

3.5.4 | NE Chinese wapitoids

From Jilin (Yushu) a metacarpal (c. 4 kyr BP) and upper molars (undated) fall within the mtDNA clade currently occupying the region, *C. e. xanthopygus*. The fossils are relatively small (Appendix S4), corresponding to living *xanthopygus* which are the smallest living Eurasian wapitis (Geist, 1999).

3.5.5 | NE Siberia and N America

Based on mtDNA data, wapitoid deer occupied NE Siberia over considerable intervals of time (Meiri et al., 2014). Heptner et al. (1966) indicate that in the Lena River region, the historical range extended to 61°N. Our samples include specimens from Yakutia up to 73°N, the individuals spanning both major wapitoid clades. Four of the DNA-yielding specimens are morphologically wapitoid: antlers from Duvanny yar, Kolyma (>35 kyr BP) (Boeskorov, 2005; Figure 2e), and the Indigirka basin (>62 kyr BP) have a classic wapitoid upper part. A later antler from the Kolyma (c. 14.5 kyr BP; Meiri et al., 2014) lacks the diagnostic upper part but the large basal circumference (21 cm) is consistent with wapitoids, whereas a tibia from the Kolyma (undated) exceeds in size a small sample of modern N. American wapiti (Appendix S4).

All North American deer, fossil and living, post-date the LGM and are closely related to each other and to one clade of *C. c. sibiricus/songaricus* (Meiri et al., 2014), with 1–3 base pair differences (Figure 3).

4 | DISCUSSION

Our mtDNA data identify several monophyletic clades within the red deer complex, corresponding in some cases to groupings of named subspecies, but questioning the number of subspecies, especially in Europe and in North America. Moreover, while our core topology agrees with previous studies (Ludt et al., 2004; Mahmut et al., 2002; Skog et al., 2009), our broader sampling of the region between the Altai and Caucasus shows that some subspecies (*maral*, *yarkandensis* and especially *songaricus* and *sibiricus*) are not monophyletic, at least as far as mtDNA is concerned. Conversely, the distinctive nature of other subspecies, particularly *C. e. corsicanus* and *C. h. hanglu*, is affirmed; both are classified as endangered by USFWS and *C. h. hanglu* as endangered by IUCN (2016).

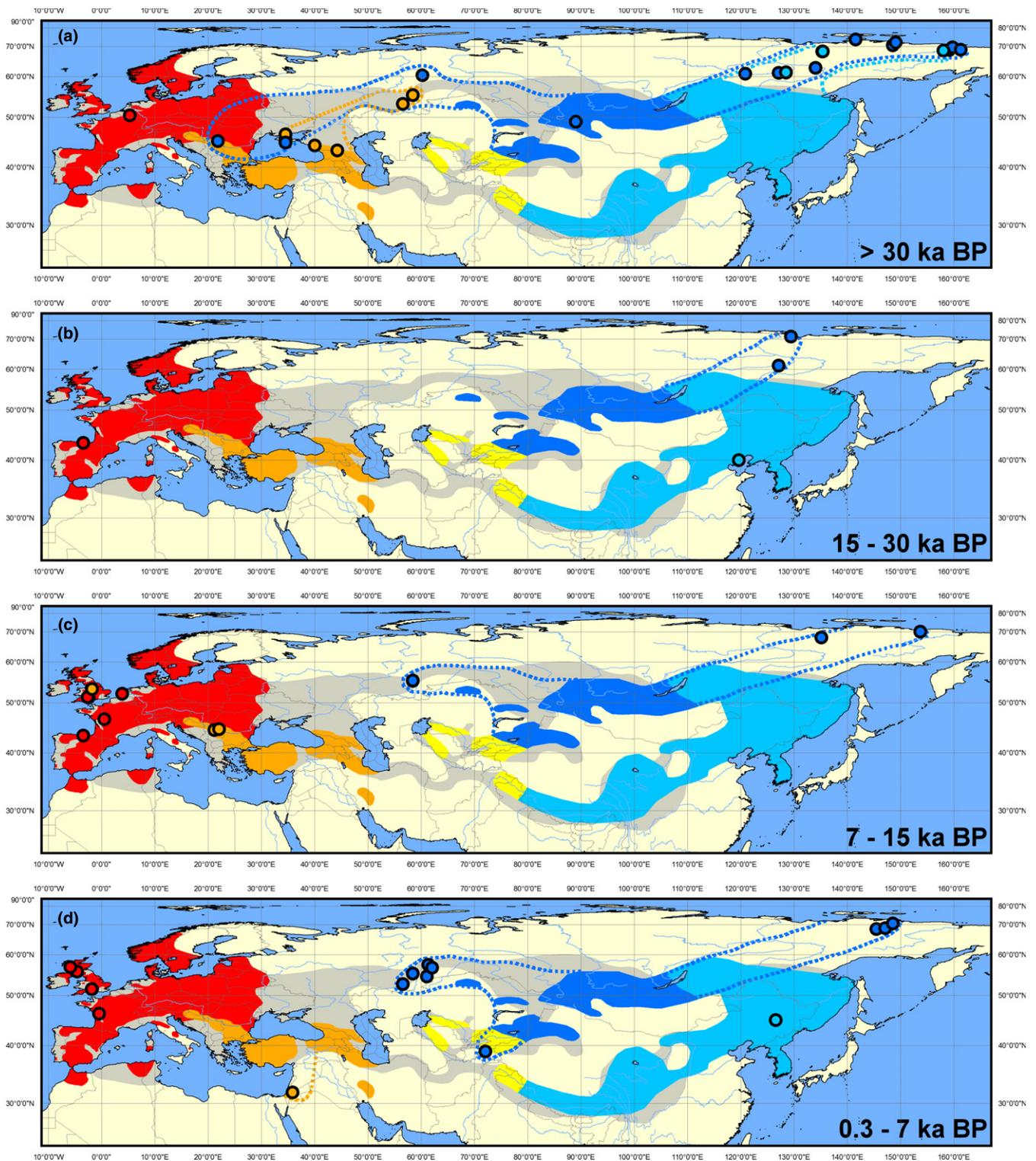


FIGURE 5 Ancient and modern biogeography of red deer in four time slices a–d. Underlying map of modern and historical distributions as in Figure 1, based on morphological and genetic data. Circles are ancient samples and are coloured according to inferred clade membership as in Figure 2 (For more details see Appendices S2 & S3). Where ancient populations extended beyond their current distributions their colours depart from those of Figures 2 & 3, which are based on location. For example ancient Romanian samples are red in Figures 2 & 3 (Europe) but blue here (wapitoid). Dashed lines show ancient expansions of clade distributions inferred from mtDNA and morphology

Considering the potentially long time to reciprocal monophyly, and the possibility of gene flow between subspecies, lack of mtDNA differentiation does not necessarily invalidate subspecies status

(Patten, 2015; Phillimore & Owens, 2006). Nuclear DNA data would provide further evidence in this regard, and morphology should also be taken into account. We suggest that subspecies may be identified

by either statistically robust morphological differentiation, or molecular differentiation, or both. One method cannot falsify robust results of another, but populations or individuals showing statistically robust differentiation *neither* in molecular *nor* in morphological markers cannot be supported as separate taxonomic units.

Within the western elaphoid clade (here *C. elaphus*), a series of haplogroups was identified by Skog et al. (2009) and Doan, Zachos, et al. (2017), and labelled A–D. These groups can be identified within our data set, which however reveals greater diversity (Appendix S5), as does the recent analysis of Doan, Mackiewicz, et al. (2017) who identify clades W1–W9. Clade A/W9 (various nominal subspecies of Western and Central European deer) is readily identifiable in our tree, as is clade B/W8 (*C. e. corsicanus* and *barbarus* from Corsica/Sardinia and North Africa). A small group of specimens, from the Czech Republic, Belarus and Karelia, forms a strongly-supported (0.95 PP) clade and may be equivalent to Clade C/W6 which is common in Eastern and Central Europe in the Skog et al. (2009) and Doan, Zachos, et al. (2017) data sets. As sister-groups to these clades, however, are a previously unrecognized, apparently extinct pair of haplogroups comprising ancient specimens (the latest at c. 16.5 cal kyr) from Western Europe (Spain and Belgium). The *maral* clade in our analysis, including modern Italian specimens, corresponds wholly or in part to clade D; the two poorly supported subclades may possibly equal W4 and W5 of Doan, Mackiewicz, et al. (2017). Interestingly, however, ancient Holocene Serbian specimens, in an area of SE Europe where modern deer have been considered “maraloid” in appearance (and are coloured orange in Figures 1 & 5), fall in the D haplogroup, but modern deer throughout this region are largely of the C haplogroup which extends even into the *maral* of western Anatolia (Doan, Zachos, et al., 2017; Skog et al., 2009), suggesting relatively recent interbreeding.

The greatest diversity in mtDNA is seen in *C. c. sibiricus/songarius*. Their haplotypes fall across both main wapitoid clades, suggesting that the Altai/Tien Shan was the area from which wapitoids dispersed (cf. Figure 1; also suggested by Mahmut et al. [2002] and Ludt et al. [2004]). One route would have been to the east and south-east, giving rise on the one hand to *C. c. xanthopygus* and *alashanicus*, on the other to the *C. c. macneilli/wallichi/kansuensis* group. The idea that these subspecies are “primitive” among wapitoids may be incorrect; their morphology may represent a secondary reduction rather than an ancestral form. In the case of *xanthopygus*, this would be consistent with their small size and paedomorphic form (Geist, 1999).

The second dispersal was north-east, crossing the Bering Strait to North America. This supports Ludt et al. (2004) and disagrees with Mahmut et al. (2002) and Polziehn and Strobeck (2002) who found an origin of N. American deer among the *xanthopygus* clade (which was poorly supported). The close relationship among the North American haplotypes supports a recent expansion into that continent (Meiri et al., 2014).

Our data indicate that ancient phylogeographical structure conforms in part to the modern situation, but some mitochondrial haplotypes are now extinct (Figures 2 & 3) and the geographical ranges of surviving lineages have shifted substantially (Figure 5).

Combining the different lines of evidence, we conclude the following for the recent biogeographical history of red deer:

- 1 Back to c. 50 kyr BP at least, populations are generally akin to modern representatives residing in the same area. This has been shown, on both morphology and mtDNA, back to pre-LGM times for Western/Central Europe (*C. e. elaphus/scoticus/hispanicus/hippelaphus/atlanticus*), Eastern Europe/SW Asia (*C. e. maral* and relatives), and central Siberia (*C. c. sibiricus/songarius*); and from the LGM onwards, for central-east China (*C. c. xanthopygus*, and the *alashanicus/macneilli/kansuensis* group).
- 2 Major extensions of range are, however, evident. Maral-like elaphoid deer extended eastwards to the Urals and southward to the Levant (Figure 5). Wapitoids extended northward to Yakutia and westward to the Urals and thence into SE Europe; these extensions occurred from pre-LGM up to historical times (not necessarily continuously) (Figure 5).
- 3 The range extensions greatly increased the areas over which the two main lineages were parapatric or even overlapped, compared to a very limited Central Asian area of proximity today.

Three alternative biogeographical models can be considered in the light of these range shifts:

1. The geographical and genetic division between wapitoid and elaphoid haplogroups remained sharp, but moved back and forth (generally eastwards and westwards) with climate change—in this case the pre-LGM elaphoids and wapitoids in the Urals and Crimea did not strictly coexist. Considering the alternating radiocarbon dates on specimens of the two groups (Appendix S1), this model would have required multiple movements.
2. Homogenization (lack of phylogeographical structure) prior to the LGM, contraction to refugia during the LGM, and establishment of phylogeographical structure (including contact zones) on expansion after the LGM (Hofreiter et al., 2004). The pre-LGM presence of both wapitoid and elaphoid haplotypes in the Urals and Eastern Europe conforms to such a model, as do rare Eastern European haplotypes in Western Europe pre- and post-LGM, and the clear phylogeographical structure at the present day. However, pre-LGM data from Western and Central Europe, and from Eastern Siberia and China, show only elaphoid and wapitoid haplotypes, respectively, suggesting (as far as sample sizes allow) that these areas remained relatively “pure.” The continuity persists at the subclade level, e.g. in the “maral” group in SE Europe and SW Asia, and the “Chinese” group of wapitoids. Some of these were likely refugial areas where the species persisted through its contraction phases, so any dissolution of mtDNA phylogeography occurred only in the more distal areas during expansion phases. Furthermore, a few “out of place” (relative to today) haplotypes occur even after the LGM, viz. “maral” haplotypes in W Europe (Meiri et al., 2013; Figure 2).
3. An intermediate model, whereby the boundaries between the haplogroups shifted, as in 1., but were not always sharp. The line

of sites from the Urals through the Crimea to Romania would then reflect an overlap zone between elaphoid and wapitoid populations (Figure 5). This model seems most conformable with the distributional data, and has been supported by approximate Bayesian computation analysis of the Crimean population (Doan, Mackiewicz, et al., 2017). We do not have direct genetic or morphological evidence to test for hybridization, but all the models may in future be testable using nuclear DNA data.

Our geographical sampling in the period around the LGM (c. 25–15 kyr) is too poor to accurately map refugial contraction, although there is evidence that in Europe *C. elaphus* contracted into Mediterranean refugia (Meiri et al., 2013; Sommer et al., 2008). There are wapitoid samples from NE China and NE Siberia dating close to the LGM (c. 21 kyr) (Figure 5c), indicating that a broad range was maintained. Perhaps the absence of LGM red deer at the well-sampled Ural sites, compared to its presence in all other time-intervals (Figure 5), indicates a period of range contraction away from the putative hybrid zone. Fossil and historical data clearly indicate that the post-glacial expansion of red deer re-occupied most of its pre-LGM range, the modern, fragmented situation representing a recent anthropogenic contraction.

In sum, the past 50,000 years have seen substantial range expansions and contractions of red deer populations, extensive contact between them with potential for hybridization, and regional or total loss of genetic diversity that has sharpened the divisions we see today. Nonetheless, the overall picture is that most lineages have maintained their genetic and morphological integrity and their core geographical distributions. Widespread homogenization alternating with contraction to small refugia is not supported by the red deer data. An important implication in terms of speciation, and the role of subspecies in that process, is that the differentiation of *C. elaphus* and *C. canadensis* to full species has developed over a long period, in spite of major environmental and population changes (Lister, 2004).

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DATA ACCESSIBILITY

DNA sequences have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>) with accession numbers: MG020561–MG020634.

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BIOSKETCH

The team is interested in processes of species-level evolution of large mammals such red deer (*Cervus elaphus/Cervus canadensis*) and moose (*Alces alces*). We study evolutionary divergence, migration and expansion of these species since the Late Pleistocene—from around 60,000 years ago to the present.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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