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Late-Quaternary biogeographic scenarios for the brown bear (*Ursus arctos*), a wild mammal model species

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ABSTRACT

This review provides an up-to-date synthesis of the matrilineal phylogeography of a uniquely well-studied Holarctic mammal, the brown bear. We extend current knowledge by presenting a DNA sequence derived from one of the earliest known fossils of a polar bear (dated to 115 000 years before present), a species that shares a paraphyletic mitochondrial association with brown bears. A molecular clock analysis of 140 mitochondrial DNA sequences, including our new polar bear sequence, provides novel insights into the times of origin for different brown bear clades. We propose a number of regional biogeographic scenarios based on genetic data, divergence time estimates and paleontological records. The case of the brown bear provides an example for researchers working with less well-studied taxa: it shows clearly that phylogeographic models based on patterns of modern genetic variation alone can be substantially improved by including data on historical patterns of genetic diversity in the form of ancient DNA sequences derived from accurately dated samples and by using an approach to divergence-time estimation that suits the data under analysis. Using such approaches it has been possible to (i) establish that the processes shaping modern genetic diversity in brown bears acted recently, within the last three glacial cycles; (ii) distinguish among hypotheses concerning species' responses to climatic oscillations in accordance with the lack of phylogeographic structure that existed in brown bears prior to the last glacial maximum (LGM); (iii) reassess theories linking monophyletic brown bear populations to particular LGM refuge areas; and (iv) identify vicariance events and track analogous patterns of migration by brown bears out of Eurasia to North America and Japan.

1. Introduction

Patterns of genetic variation can be used to evaluate the importance of different historical processes affecting populations and species (i.e., phylogeography; Avise, 2000). Information about phylogeographic processes acting upon mammal species has been accumulated in large part through genetic studies of modern populations. However, this approach alone may be insufficient to reveal the influence of historical processes in taxa that have undergone recent demographic bottlenecks, because population reduction increases the loss of genetic diversity through stochastic drift. Analysis of modern samples also provides little information about the timing of evolutionary events (e.g., divergence between taxa), which can be essential for linking phylogeographic patterns with particular historical processes. Indeed, inferences made on the basis of modern samples alone have been characterised as 'time trapped' (Pääbo, 2000). In some cases, this trap can be circumvented using DNA from ancient subfossil material to provide direct information about historical phylogeographic patterns and to calibrate molecular clock analyses. However, few species offer

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a sufficient abundance of ancient samples for this approach to be possible. In this respect, the Quaternary phylogeography of the brown bear *Ursus arctos* is unusually well-studied, since genetic material from both modern and ancient samples has been extensively sampled across a wide geographic range.

Due to its large size and habit of using caves (especially as hibernacula) the brown bear has left a considerable trace in the fossil record (e.g. Sommer and Benecke, 2005; Østbye et al., 2006). Polar bears are believed to have diverged from brown bears in the late Pleistocene (see discussion below); and prior to this the brown/polar bear branch shared a common ancestor with cave bears *Ursus spelaeus*. Pioneering paleontological work placed the divergence between brown and cave bear lineages at approximately 1.2–1.7 million years ago (Kurtén, 1968). Evidence from more recently discovered sites (e.g., Vallonet, Atapuerca, Untermaßfeld, Cal Guardiola) has revealed apparently intermediate ancestral forms from the Epivillafranchian – 0.9–1.2 million years ago – providing broad support for Kurtén's (1968) estimate but raising the possibility of a slightly more recent divergence time (Mazza and Rustioni, 1994; García and Arsuaga, 2001; García, 2004; Madurell-Malapeira et al., 2009). Support for the approximate timing of this divergence event has also been provided by genetic studies (Loreille et al., 2001; Korsten et al., 2009).

Currently, the brown bear is one of the largest extant terrestrial carnivores and has a wide Holarctic distribution (Fig. 1a). The status of the brown bear as a useful animal model in Pleistocene biogeography appears to have come about due to general interest in the biogeography of large mammals and concerns about the conservation of particular local brown bear populations. In addition, the modern brown bear population is widely distributed, and the relative availability of subfossil genetic samples has made large-scale genetic analysis of ancient populations possible. While certain phylogeographic patterns exhibited by modern brown bear populations have been characterised as paradigmatic (Taberlet et al., 1998; Hewitt, 2000; Korsten et al., 2009), there has been little attempt to integrate ancient and modern genetic analysis with

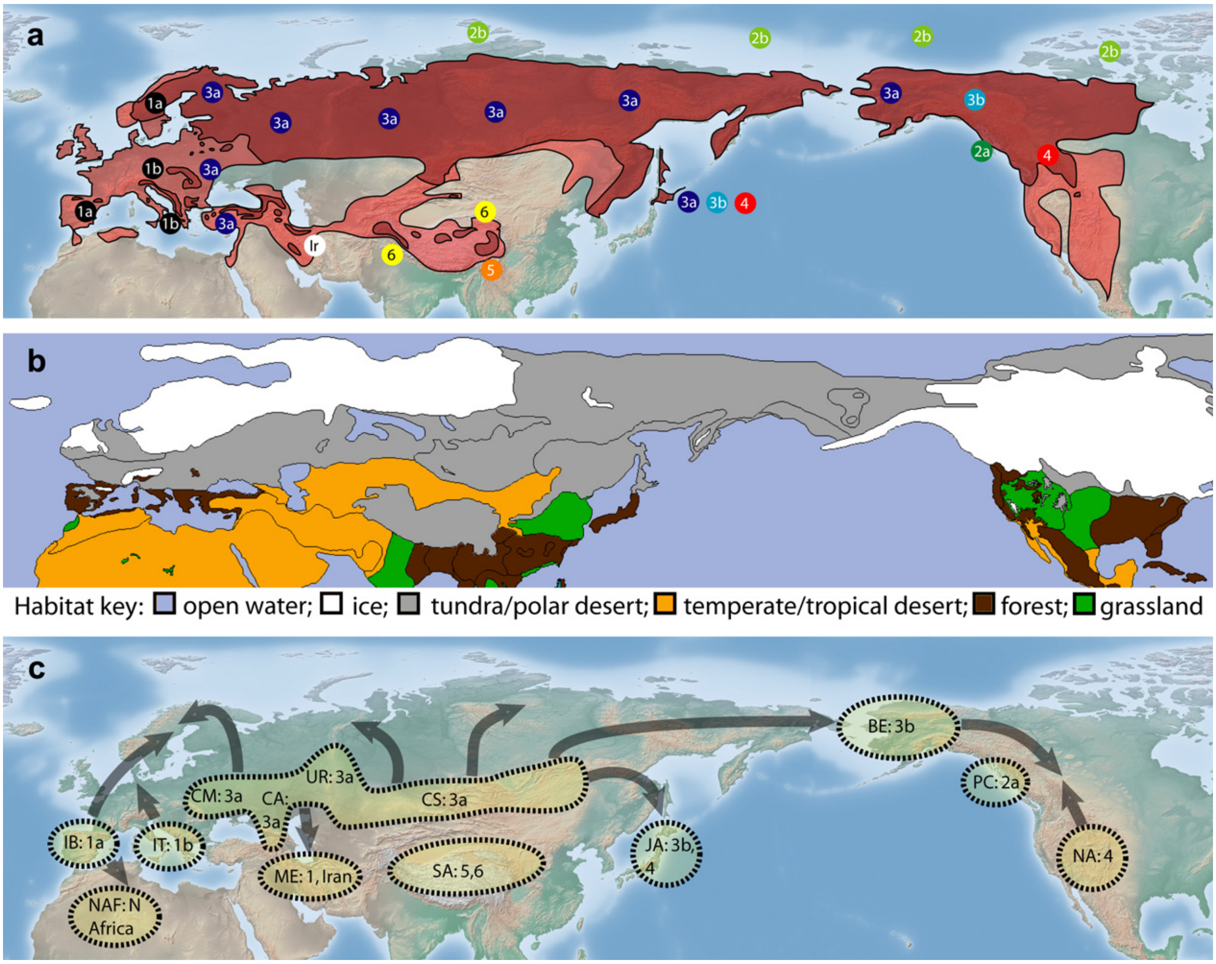

Fig. 1. a. The current (dark shade) and additional historical (light shade) distribution of brown bears. The approximate geographic distribution of extant matrilineal clades 1–6 and Iran [Ir] (following Leonard et al., 2000; also including data from Shields et al., 2000; Miller et al., 2006 and Korsten et al., 2009; see Fig. 3 and text for further details) is shown. Note that the distribution area of polar bears (clade 2b) is unshaded. b. Coverage of major biomes in the Northern hemisphere during the last glacial maximum (LGM; approximately 18 kyBP), adapted from Ray and Adams (2001). Note that the tundra zone extending from Europe through central and northeast Eurasia contained some areas with forest coverage (Crowley, 1995; Alfimov and Berman, 2001; Willis and van Andel, 2004; Kuzmin, 2008). c. Putative LGM refuge areas (approximate locations marked as filled areas with dashed outline) and post-LGM colonization routes (filled arrows) for brown bears. Refuge area locations: IB – Iberia; IT – Italo-Balkan peninsula; CM – Carpathian Mountains; CA – Caucasus; UR – Ural Mountains; CS – central Siberia; NAF – North Africa; ME – Middle East; SA – south Asia; JA – Japan; BE – Beringia; PC – Pacific coastal islands; NA – continental North America (Matheus et al., 2004). The clade(s) putatively occupying each area is given after the area identity. The scenario presented here includes several details that are particularly uncertain: (i) colonization of north-east Europe, northern Asia and western Alaska by subclade 3a may have been from a single or multiple refuge areas broadly corresponding to the areas CM, CA, UR or CS (a large single refuge area scenario is shown here); (ii) the contribution of populations in the different European peninsulas to the recolonization of Europe by clade 1 is uncertain (the model of Taberlet et al., 1998 is presented here; see text for more details); and (iii) the PC refuge area may not have been occupied by subclade 2a at the time of the LGM.

molecular-clock timing estimates and paleontological evidence to reveal wide-scale patterns. Such an approach has the potential to shed new light on established theories (Knowles, 2009); including, in the case of the brown bear, those relating to the colonization of North America and population responses to climate change. Moreover, at a time when technological advances are permitting the use of increasingly powerful molecular methods (e.g., high-throughput sequencing), a synthesis of results derived from traditional sequencing of short molecular markers provides a baseline against which to compare new data. More generally, insights gained from the brown bear can serve as a useful guide for studies of species where data are more limited; for example, where only modern DNA sequences are available.

In this review, we synthesise the results of 33 studies published between 1991 and 2010, which to the best of our knowledge represent all matrilineal phylogeographic studies from throughout the brown bear geographical range (Table S1). Furthermore, we present valuable new genetic data from one of the oldest known fossil polar bears (dating to 115 kyBP), a species that shares a paraphyletic association with brown bears on the basis of mitochondrial studies. In order to estimate the timing of evolutionary events within the brown bear lineage, we present a new molecular clock analysis including the new polar bear sequence and a combination of paleontological and ancient DNA calibrations. Using these timing estimates, along with patterns of modern and historical phylogeographic structure, and paleontological records, we propose regional biogeographic scenarios that illustrate different processes acting on brown bears during the late Quaternary.

2. Mitochondrial sequence analysis

Currently, three basic genetic marker systems are available for animal phylogeography studies: (i) mitochondrial DNA (mtDNA), which can be used to characterise the evolution of female lineages; (ii) autosomal markers such as microsatellites, which reflect the combined history of female and male lineages; and (iii) sex chromosome markers, such as male-specific Y-chromosome microsatellites in mammals, which can be used to characterise the evolution of male lineages. In common with many animal phylogeography studies, the majority of brown bear research has hitherto relied on analysis of mtDNA sequence data (Table S1). Nuclear microsatellites have for the most part only been used in population genetic studies covering relatively limited geographic areas (e.g., Taberlet et al., 1995, 1997; Paetkau et al., 1998; Cronin et al., 1999, 2005; Waits et al., 2000; Miller and Waits, 2003; Jackson et al., 2008); though a recent study revealed population genetic parameters in a population inhabiting a large part of northeastern Europe (Tammeleht et al., 2010). Nonetheless, current advances in sequencing technology are likely to make available a number of additional markers from the brown bear nuclear genome in the near future, including single nucleotide polymorphisms, insertional (viral) markers (e.g., Chessa et al., 2009) and Y-chromosome polymorphisms.

mtDNA is a useful genetic marker for phylogeographic studies because it is present in cells in high copy number (especially useful in ancient DNA analyses), does not usually recombine and is characterised by relatively rapid mutation rates and coalescence times. It is also maternally inherited, which makes it useful for studying wide-scale phylogeographic patterns in species such as the brown bear where females are more philopatric (i.e., they do not disperse far from their natal range) than males (McLellan and Hovey, 2001; Støen et al., 2006; Zedrosser et al., 2007). In such species, geographic structure in female-specific genetic markers is likely to change more slowly than that in autosomal biallelic genetic markers, and hence is more likely to retain the signatures of ancient phylogeographic processes. However, a maternally inherited marker might not represent the phylogeography of an entire species precisely because it does not reflect male-mediated gene-flow. It would undoubtedly be of great interest to investigate wide-scale patterns of genetic diversity using autosomal and Y-chromosome nuclear markers. However, the remainder of this review will consider only the maternal phylogeography of brown bears, based on analysis of mtDNA, since the characteristics of mtDNA and level of previous work using this marker mean that it currently provides a more comprehensive picture than other approaches.

Most phylogeographic studies of brown bears have used sequences from the hypervariable 5′ end of the non-coding control region (CR) within the mitochondrial genome (Table S1; Fig. 2). A rapid mutation rate makes the CR a useful marker for recent intraspecific phylogenies, but also leaves it prone to saturation (multiple substitutions at single sites) meaning that some characters may be homoplasious (i.e., identical by chance, not by common descent). Sections of the cytochrome b gene (cytb; Table S1; Fig. 2), which has a slower mutation rate, have also been used. With recent technological advances, it is becoming easier to sequence longer sections of DNA, which is important because this generally leads to improved phylogenetic resolution. The sequencing of entire mitochondrial genomes, from modern and ancient samples, is now a realistic aim for phylogeographic studies (Gilbert et al., 2008; Millar et al., 2008; Stiller et al., 2009; Ho and Gilbert, 2010), and recently a small number of brown and polar bear mitochondrial genome sequences have been published (Bon et al., 2008; Krause et al., 2008; Lindqvist et al., 2010). However, common sequences shared by different studies are short, and the most comprehensive global phylogenies attempted so far have been based on <229 bp of the CR (Fig. 2; Miller et al., 2006; Ho et al., 2008a; Korsten et al., 2009).

3. Sampling of modern brown bear mtDNA diversity

To provide an indication of the confidence with which conclusions about large-scale biogeographic scenarios can be drawn, it is informative to consider sampling effort throughout the brown bear range. Modern mtDNA sequences have been characterised from 22 out of the 48 countries in which the species currently occurs (Table S1; McLellan et al., 2008), with continental-scale variation existing in sampling effort. It is currently estimated that there are close to 200 000 brown bears worldwide, of which approximately 14 000 live in Europe, 110 000 in Asia (including the whole of Russia) and 58 000 in North America (McLellan et al., 2008). Meanwhile 596, 332, and 873 individual modern (defined as animals living within the last 30 years) brown bear individuals have been sampled in these respective regions (Table S1), which corresponds to 4.3%, 0.3% and 1.5% of all bears in the respective current populations, i.e., sampling rates differ between Europe and Asia by a factor of approximately 15. Small numbers of samples from Siberia and southern Asia have been analysed (Table S1), but given the current abundance of bears in the former area and the status of Asia as the putative origin of the species, these represent the geographic areas that are least well sampled.

4. Sampling and analysis of ancient brown bear DNA

To date, ancient DNA sampling has largely focused on putative glacial refuge areas in the south and west of Europe (44 individuals) and the north-west of North America (44 individuals), with further samples derived from North Africa and the Middle East (27 individuals; Table S1). In common with analysis of modern sequences, ancient DNA analysis has concentrated on the CR; however, due to the particular challenges posed by degradation of samples (Lindahl,

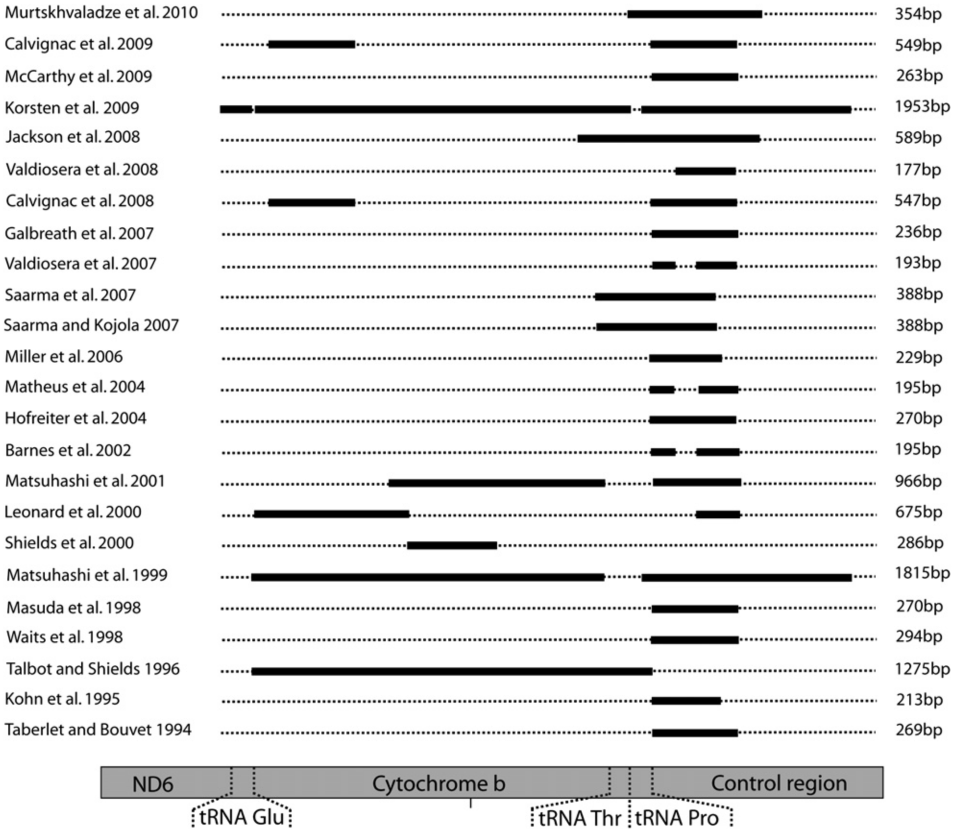

Fig. 2. Publicly available mitochondrial DNA sequences (dark bars) used in brown bear phylogeography studies. Where sequences of different lengths were used in individual studies, the longest are shown here. In addition to the sequences shown here, full mitochondrial genome sequences (c 17 000 bp) have been used in recent phylogeographic work by Lindqvist et al. (2010).

1993; Willerslev and Cooper, 2005; Rohland and Hofreiter, 2007), the resulting sequences have typically been slightly shorter than those derived from modern samples (Fig. 2; Table S1).

5. Global genetic diversity

An important aspect of phylogeography is the identification of monophyletic groups of individuals (i.e., clades) that share closer common ancestry with one another than with members of any other group. Among modern bear populations, a number of divergent and for the most part geographically distinct clades have been identified, largely on the basis of CR data but generally also supported by analysis of cytb (Fig. 3; node support values in Table 1). The nomenclature for the clades proposed by Leonard et al. (2000), and extended by later authors (Barnes et al., 2002; Miller et al., 2006; Calvignac et al., 2008, 2009) to include six numbered clades (1–6) and two clades named on the basis of their geographical provenance (Iran and North Africa), represents the most widely recognised terminology and will be followed in the remainder of this review.

The well-supported clades (i.e., concordant across studies and with high statistical support) fall into two broad clusters – clades 1 and 2 on one hand and clades 3, 4 and the Iranian clade on the other (Fig. 3; Table 1); however, the relationships of clades 5, 6 and the North African and Iranian clades with the aforementioned clades are not clear (though for a well-supported partial phylogeny containing the Iranian and North African clades, see Calvignac et al., 2009). Within some clades, a significant level of divergence has led to the identification of monophyletic subclades (Fig. 3). While modern populations of clades 1 (Europe), 4 (North America and Japan), 5 (Asia) and 6 (Asia) currently occur in relatively restricted geographical areas, clade 3 has a wide distribution encompassing Europe, Asia and North America (Fig. 1a).

A striking aspect of modern brown bear phylogeography is that the different clades and subclades are on the whole geographically separate from one another (Fig. 1a). Assessing the degree to which this pattern supports different hypotheses about brown bear biogeography has been an important component of many of the studies listed in Table S1, and is discussed below. Ancient DNA studies have tracked the historical occurrence of clades 1, 2, 3, and 4 in Europe, North America, North Africa and the Middle East, while revealing a divergent extinct clade in North Africa.

6. The phylogenetic placement of polar bears

Mitochondrial DNA analysis of North American ursids has revealed that polar and brown bears share a paraphyletic relationship (Cronin et al., 1991; Shields and Kocher, 1991; Talbot and Shields, 1996). Thus, brown bears of subclade 2a are more closely related to polar bears (subclade 2b) than to other brown bear clades (Fig. 3). Nonetheless, the polar bear possesses many derived characters that have been used to assign species status, and its range is generally separate from that of the brown bear (see Fig. 1a). This relationship represents an example of incomplete lineage sorting. Given enough time, and assuming that neither species becomes extinct, the brown bear and polar bear will eventually become mutually monophyletic.

Here, we present a mtDNA control region sequence (246 bp) derived from an ancient polar bear subfossil (a rib bone; from "Norcem" cave in Kjøpsvik, North Norway N 68°05′, E16°22′;

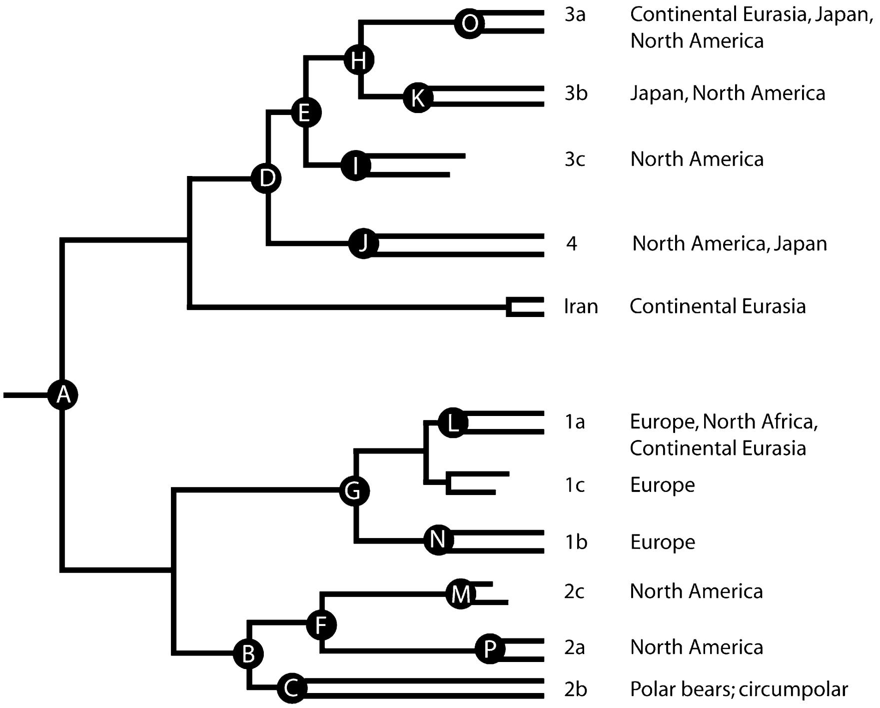

Fig. 3. Schematic representation of a phylogeny containing all well-resolved brown bear taxa, generalised from wide-scale phylogenies in Ho et al. (2008a) and Korsten et al. (2009), and the individual studies presented in Table S1 (statistical supports for nodes are shown in Table 1). Numerical clade nomenclature, following Leonard et al. (2000) is shown as is the approximate geographical distribution of each clade – see also Fig. 1a. Nodes for which timing estimates have been calculated are labelled A–P (see Table 1).

Collection number KJ91-2a, Osteology Collections, Bergen Museum, Norway). The rib bone was found along with a polar bear metatarsal and vertebra disk (within 2 m of each other in the same stratum; all specimens were identified by Dr R. Lie, Osteological Collections Curator, Bergen Museum). Initial radiocarbon dating of the specimen returned a date of 42 ± 1.5 kyBP. However, this date almost certainly reflected the upper temporal limit of the technique for several reasons. First, the fossil rib was situated below a clay layer from

Table 1

Posterior probability support for brown bear clades and times to most recent common ancestor (node ages), using different molecular clock calibrations. Nodes A–P correspond to those shown in Figs. 3 and 4. Support values for brown bear clades are shown from this analysis (control region; CR) and Korsten et al. (2009) (cytochrome b gene; cytb).

Node	Node description (MRCA of listed clades)	Support		Node age (kyBP)				
		CR	cytb	Multiple calibration^{g}	Multiple calibration^{h}	Internal calibration	External calibration	Imported substitution rate
A	All sampled brown bears	1.0	1.0	263 (162–400)	193 (114–325)	143 (100–200)	974 (410–1920)^{a}	850^{d+} 550–700^{e+}
B	Clade 2	1.0	–	160 (124–210)	102 (60–163)	77 (56–106)	415 (209–659)^{b}	146–185^{f+}
C	Clade 2b (polar bears)	1.0	1.0	146 (120–179)	49 (22–97)	37 (21–60)	208 (55–398)^{b}	–
D	clades 3 and 4	1.0	1.0	140 (87–213)	–	–	702 (290–1390)^{a} 300–500^{c+}	280–515^{f+}
E	Clade 3	0.86	1.0	114 (74–166)	100 (69–148)	79 (61–103)	525 (307–793)^{b}	–
F	Clades 2a and 2c	0.94	–	113 (66–161)	–	51 (26–85	–	–
G	Clade 1 (extant; Europe, Middle East)	1.0	1.0	100 (49–164)	81 (45–144)	60 (42–89)	394 (150–790)^{a} 415 (198–646)^{b}	–
H	Clades 3a and 3b	0.55	1.0	92 (51–133)	–	35 (11–75)	374 (89–741)^{b} 500–1400^{c+}	245–310^{f+}
I	Clade 3c (extinct; N America)	0.98	–	87 (59–121)	79 (58–112)	69 (57–87)	260 (147–394)^{b}	–
J	Clade 4 (extant; N. America; Eurasia)	0.81	–	87 (42–147)	74 (42–137)	54 (37–87)	458 (167–755)^{b}	–
K	Clade 3b (extant; N. America, Eurasia)	0.53	1.0	75 (43–113)	66 (40–104)	51 (32–77)	380 (168–620)^{b}	–
L	Clade 1a (extant; Europe)	0.82	–	66 (32–109)	–	–	–	–
M	Clade 2c (extinct; N America)	1.0	–	64 (48–87)	57 (48–76)	53 (48–64)	–	–
N	Clade 1b (extant; Europe)	0.80	–	63 (40–98)	–	–	–	–
O	Clade 3a (extant; N. America; Eurasia)	0.96	0.79	49 (18–86)	41 (17–72)	26 (13–46)	362 (67–735)^{b}	–
P	Clade 2a (extant; N. America)	0.97	1.0	45 (10–91)	33 (12–64)	24 (11–43)	72 (17–149)^{b}	–

Externally calibrated and imported-rate estimates are from: (a) Hofreiter et al. (2002), (b) Ho et al. (2008a), (c) Matsuhashi et al. (1999), (d) Taberlet and Bouvet (1994), (e) Talbot and Shields (1996); and (f) Waits et al. (1998). Ages are presented as means with 95% higher posterior density intervals in parentheses or a single or range of point estimates (+).

[g] Multiple-calibration estimates are those calculated in this study (incorporating the new polar bear sequences).

[h] Multiple-calibration estimates and internally calibrated estimates are taken from Korsten et al. (2009), except internally calibrated nodes H and P, which are from Ho et al. (2008a).

which other specimens (calcareous concretions that formed after the clay was deposited) have been dated to 70 ± 8.5 kyBP using $^{230}Th/^{234}U$ (Lauritzen et al., 1996; Nese and Lauritzen, 1996). This dating technique is a robust approach that requires 5–7 parallel dates from the same horizon (Hillaire-Marcel and Causse, 1989). Second, the rib was obtained from a depositional horizon with a magnetic signature close to reversal, which is compatible with a transitional field configuration, most likely representing the onset or termination of a paleomagnetic excursion. Given the minimum $^{230}Th/^{234}U$ estimate, the observed signature could correlate with the Norwegian-Greenland Sea event (70–76 kyBP; Bleil and Gard, 1989) or the Blake event (approximately 115 kyBP; Denham et al., 1977; Zhu et al., 1994). However, the thick layer (50 cm) of clay capping the bone layer and the fact that in this environment calcareous concretions tend to form during periods of climatic amelioration at the end of stadials are not consistent with the younger event. Thus, an age corresponding to the Blake event (approximately 115 kyBP) is taken as the best estimate for the specimen. It is notable that strata corresponding to the Blake event are known from other European caves (Bosák et al., 2004). DNA extraction and amplification details are presented in the supplementary material. The polar bear sequence has been submitted to GenBank (accession numbers HM584820-HM584821).

While this review was in preparation, a whole mt genome was generated from another ancient polar bear specimen (Lindqvist et al., 2010) of equivalent age (110–130 kyBP; Ingólfsson and Wiig, 2008) from Svalbard. The homologous region from the mt genome from the Svalbard specimen (Genbank accession GU573488) is similar but not identical to the one presented in this study, with 5/247 bp varying between the two sequences.

7. Estimating time-scales for population divergence

In order to infer phylogeographic processes from patterns of genetic diversity, it is essential to understand the time-scales during which evolutionary events may have occurred. With such information, it becomes possible to link genetic patterns with historical parameters, such as climatic fluctuations (Hundertmark et al., 2002; Shapiro et al., 2004; Saarma et al., 2007) or domestication (Ho et al., 2008b). Various attempts have been made to estimate the timing of splits between brown bear clades using different molecular clock techniques, which all rely on the accurate determination of a nucleotide substitution rate. In turn, this depends on appropriate calibration using an independent source of age information, such as the fossil record.

The importation or calculation of a nucleotide substitution rate has the potential to be the single most important source of error in molecular clock calculations. This is exemplified by the timing estimates made for the evolution of brown bear clades, which vary up to four or five-fold depending on the choice of imported (from other species), external (calculated using interspecific age information), internal (using intraspecific age information) or multiple (combination of interspecific and intraspecific age information) calibration points (Table 1, see Supplementary Material for discussion of calibration techniques applied to brown bear timing estimates). For datasets containing a mixture of closely and distantly related taxa, a multiple-calibration approach including a temporal range of age information appears to represent the most theoretically sound approach (Korsten et al., 2009). In this paper, we extend Korsten et al.'s (2009) multiple-calibration analysis to incorporate the two new ancient polar bear sequences (from this study and Lindqvist et al., 2010); resulting in a total of 140 sequences from clades 1, 2, 3 and 4. Otherwise, our analysis followed that of Korsten et al. (2009); see Supplementary Material for details and a BEAST input file. The ages of the most recent common ancestors (MRCAs) of brown bear clades are presented in Table 1. The ages of nodes are on average 40% older than the respective figures from the analysis of Korsten et al. (2009) (Table 1), suggesting that the addition of the ancient polar bear sequences had an important effect on calibration.

During the last million years, the earth's climate has oscillated between relatively long, cold glacial periods and shorter, warmer interglacials, with each cycle lasting approximately 100 ky (Petit et al., 1999; Jouzel et al., 2007). Previous estimates produced using imported substitution rates or external calibration suggested that divergences between extant brown bear clades started <9 glacial cycles before present (Table 1). By contrast, internal- and multiple-calibration estimates place all the divergences within the last three glacial cycles, with the majority occurring during the last glacial period (Fig. 4; Table 1). The discrepancies between estimates produced by different calibration approaches indicate the importance of appropriate calibration for phylogeographic inference. Moreover, the relatively recent timing estimates produced by the multiple-calibration approaches (theoretically the most suitable approach for this data set) make inference more straightforward, since the climate and land cover during the most recent glacial period is better understood than that of earlier periods (Fig. 1b; e.g., Ray and Adams, 2001; Svendsen et al., 2004). A further important conclusion is that the genetic diversity currently observed in brown bears arose far more recently than the split between the brown bear and its close relative, the cave bear (>1 million years ago; Loreille et al., 2001). This implies that considerable genetic variation remains unsampled or, more likely, has been lost, such that all modern clades descend from a relatively recent common ancestor. Widespread loss of genetic diversity during the late Pleistocene has been demonstrated for a range of other extant and extinct large mammals, including steppe bison (Shapiro et al., 2004), muskox (Macphee et al., 2006) and cave bears (Knapp et al., 2009), and a widespread extinction of megafauna occurred at this time (Barnosky et al., 2004).

Our analysis places the separation between polar bears and the most closely related brown bear clades at approximately 160 kyBP. This estimate is in good accordance with the mean estimate of Lindqvist et al. (2010) (152 kyBP), which was derived using longer sequences, but with only one of the ancient polar bear samples. This is indicative that partial CR sequences may provide reliable estimates. Our timing estimate is also concordant with the paleontological view that polar bears evolved from a brown bear population in the late Pleistocene (Kurtén, 1964). In combination with data on the morphology and stable isotope content of the ancient polar bear fossils (Lindqvist et al., 2010), these timing estimates indicate that the polar bear underwent rapid morphological evolution shortly after diverging from brown bears.

8. Regional biogeographic scenarios

The following sections combine information derived from modern and ancient mtDNA analysis, multiple-calibration estimates of divergence times and paleontological data to create regional biogeographic scenarios for brown bears during the mid-late Pleistocene and Holocene. Greatest detail is reserved for the two best-studied regions: North America and Europe.

8.1. Phylogeography of North American brown bears

Three genetically divergent clades have been identified among modern bears living in North America: 2, 3 and 4. Clades 2 and 3 are further divided into four modern subclades – 2a, 2b, 3a and 3b (Fig. 3; Talbot and Shields, 1996; Waits et al., 1998) – while bear remains corresponding to two extinct subclades, 2c and 3c (Fig. 3),

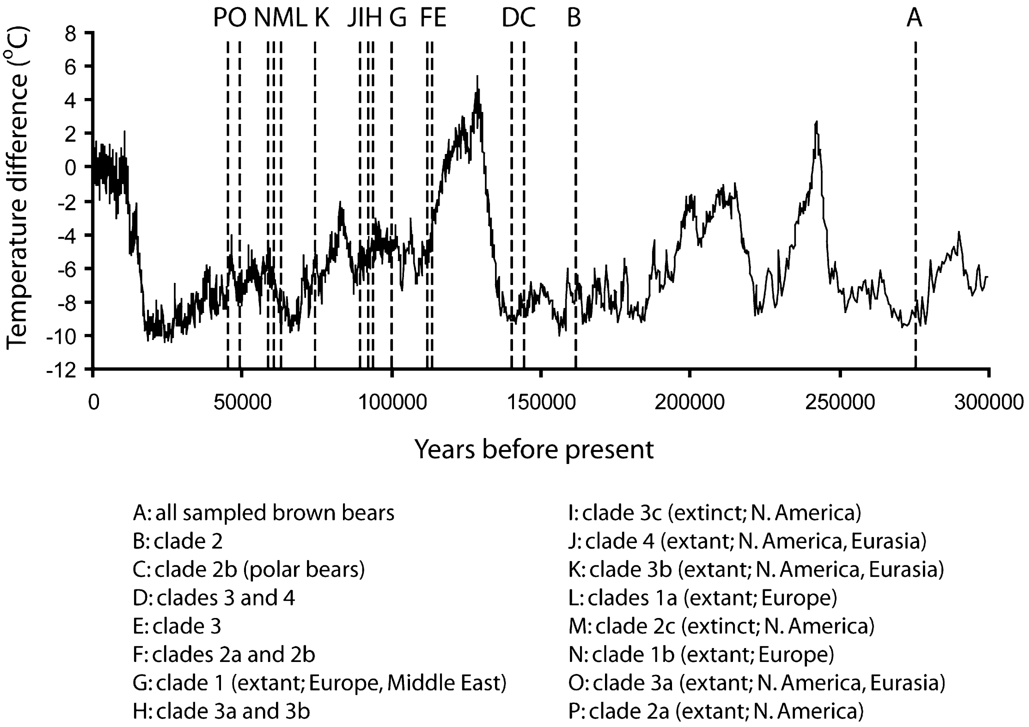

Fig. 4. Divergence of brown bear matrilineal clades in relation to climatic oscillations during the late Pleistocene and Holocene. Surface temperature estimates (°C) derived from isotope analysis of Antarctic ice cores covering the last 250 kyBP are presented relative to the 1950 temperature (Jouzel et al., 2007). Mean estimates of the time to most recent common ancestor (MRCA) for different brown bear clades, with labels corresponding to nodes in Fig. 3 and Table 1, are shown by dashed lines.

have been dated to >48 kyBP (Leonard et al., 2000; Barnes et al., 2002). The modern clades and subclades are geographically separate: subclade 3a is currently found in northwestern Alaska; subclade 3b in eastern Alaska and Canada; and Clade 4 in the northern contiguous United States and southern Canada (Fig. 5d); though subclades 3a and 3b form a narrow zone of overlap in the Arctic National Wildlife Refuge in Alaska (Talbot and Shields, 1996; Waits et al., 1998). Moreover, analysis of ancient samples has indicated

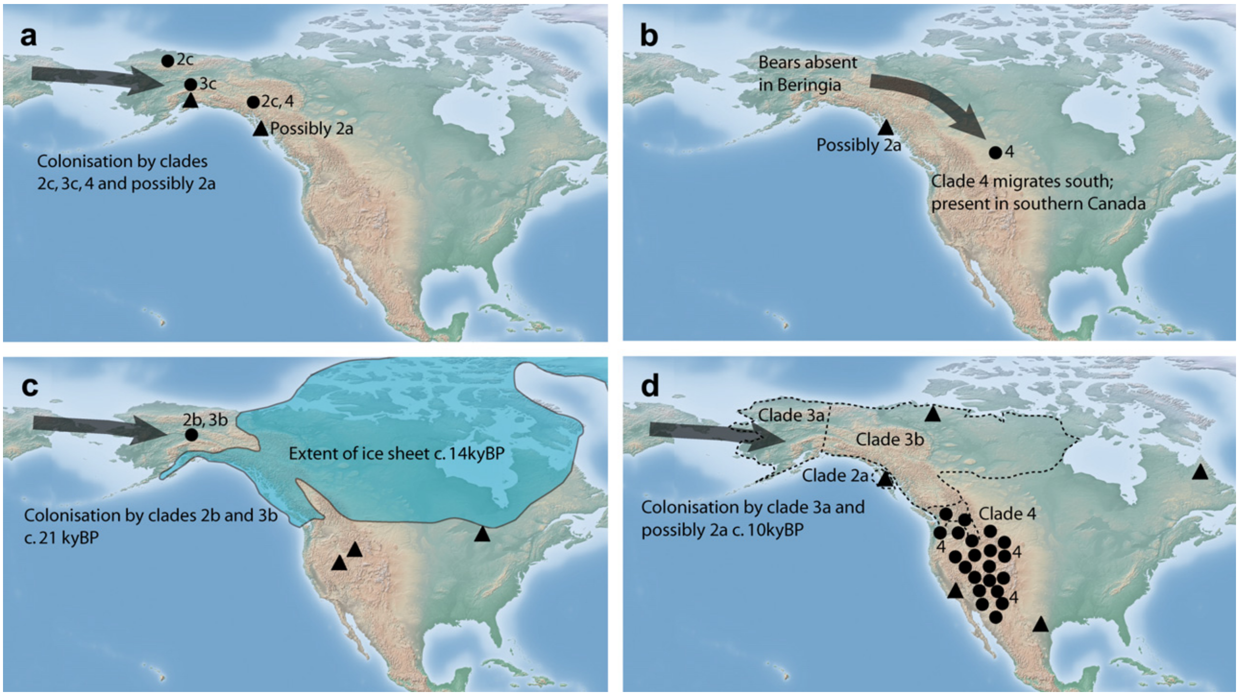

Fig. 5. Late-Quaternary phylogeography of North American brown bears: a) 70–33 kyBP; b) 33–21 kyBP; c) 21–10 kyBP; and d) 10 kyBP-present. Symbols represent radiocarbon-dated or known-age subfossil bear remains that have been subject to genetic analyses (circles with clade marked) or not (triangles); note that within each time period geographically proximate records are represented by a single symbol. Clade nomenclature follows Leonard et al. (2000). The distribution of modern brown bear clades (dashed lines) is taken from Waits et al. (1998), and the extent of glacial ice is reproduced from Mandryk et al. (2001). Sources of subfossil bear data are listed in the Supplementary Material.

that a degree of phylogeographic structure might have existed since the colonization of the continent, with only the distribution of clades changing over time (Barnes et al., 2002).

Climatic shifts, combined with strong environmental regionalism in Alaska and Canada (Kotilainen and Shackleton, 1995; Lister and Sher, 1995), may have contributed to the maintenance of marked division by causing isolation and extinction of local populations. Equally, a high level of natal female philopatry means that female bears do not rapidly invade areas that are already occupied (McLellan and Hovey, 2001). Thus, areas of overlap, or contact zones, can occur if female population density is low, while relatively high densities can prevent introgression of neighbouring mtDNA clades into territory occupied by other clades (Hewitt, 1988). However, the phylogeographic pattern of North American bears also provides an example of the importance of historical colonization and vicariance events in shaping modern genetic diversity.

8.1.1. *Beringia as an important refuge area and migration corridor*

The continents of Eurasia and North America are currently separated by the Bering Strait, a stretch of water approximately 85 km wide and for the most part 30–50 m deep. On numerous occasions during the Pleistocene, however, sea-level has decreased sufficiently for this water body to disappear: the region served as a land-bridge between Eurasia and North America during glaciations, when sea-level was typically 100–135 m lower than today (Hopkins, 1973; Clark and Mix, 2002). Hultén (1937) was the first to postulate that an ice-free area of land connecting Eurasia and North America could have served as a northern refugium for arctic and boreal biota during glacial periods. Subsequent paleoecological studies have confirmed that at the time of its last appearance, the land-bridge represented a cold, dry steppe environment that was nonetheless sufficiently productive to serve as an important refuge area and migration corridor for many taxa, including brown bears (Elias, 2001; Zazula et al., 2003; refuge area BE in Fig. 1c).

8.1.2. *First colonization of North America*

Fossil evidence suggests that the brown bear evolved in Eurasia (Kurtén, 1968; Mazza and Rustioni, 1994) and only entered North America through Beringia in the late Pleistocene, around 70 kyBP (Craighead and Mitchell, 1982). Following the first colonization (Fig. 5a), brown bears belonging to at least three different clades or subclades were present in eastern Beringia until 33 kyBP: 3c (present from >59 kyBP), 2c (>48 kyBP) and 4 (>36 kyBP) (Leonard et al., 2000; Barnes et al., 2002). Multiple-calibration estimates place the MRCAs of these subclades at 91 kyBP, 65 kyBP (Table 1) and 50 kyBP (for North American clade 4 only; not shown in Table 1), respectively. These new estimates are consistent with these clades forming as the result of colonization events at approximately the time indicated by paleontological evidence. Besides continental Beringia, fossil records indicate that brown bears also colonised the Pacific coastal islands off Alaska (PC in Fig. 1c) prior to 35 kyBP and remained there until at least 26 kyBP (Heaton and Grady, 1993, 2003; Heaton et al., 1996; Fig. 5a and b); however the genetic identity of these bears is unknown. Interestingly, no brown bear bones have been found in eastern Beringia dating to the period 33–21 kyBP (an absence perhaps attributable to competition with the giant short-faced bear *Arctodus simus*; Barnes et al., 2002), and examples of subclades 2c and 3c from after this period have not been recovered (Fig. 5b).

It was originally thought that brown bears did not migrate southwards from eastern Beringia before the postglacial period (i.e., until after 13 kyBP; Herrero, 1972; Kurtén and Anderson, 1980; Barnes et al., 2002), but recently a clade 4 individual dated to approximately 26 kyBP was recovered from near Edmonton (Canada), in mid-continent North America (Matheus et al., 2004; Fig. 5b). Thus, after entering North America, but prior to the disappearance of bears from Beringia, some populations clearly migrated south to the mid-territories of the continent (NA in Fig. 1c; Fig. 5b).

8.1.3. *Second colonization of North America*

Around the time of the last glacial maximum (LGM; 26–13 kyBP), the Laurentide and Cordilleran ice-sheets covered a large area between the mid-continent of North America and the current Alaska-Canada border (Mandryk et al., 2001; Fig. 5c), thereby restricting migrations from eastern Beringia southward and from the mid-continent northward. Around 21 kyBP, a second wave of brown bears, containing subclade 3b, entered Beringia from Eurasia (Barnes et al., 2002; Fig. 5c). The timing of this event is of interest since it coincides with the LGM and follows a period of 12 ky during which brown bears were absent in the Beringian fossil record. Assuming the fossil record accurately reflects the timing of this event, north-east Eurasia must also have been inhabited by brown bears at the time of or just prior to the LGM, as the immigrating bears are highly likely to have arrived from this direction. The relatively early MRCA of clade 3b (78 kyBP) supports Barnes et al.'s (2002) view that the members of this clade that colonized North America probably belonged to a relatively large, diverse population.

8.1.4. *Third colonization of North America*

The continental ice-sheets retreated at the end of the LGM (13–9 kyBP), coinciding with a rise in sea-level and the reappearance of the Bering Strait around 11–13 kyBP (Elias et al., 1996). This blocked further movements of terrestrial mammals between Eurasia and North America. However, before the Bering Strait became a definitive movement barrier, subclade 3a colonised Alaska (Figs. 1c and 5d). The recent arrival of this subclade in North America is evident from phylogenetic analyses showing Alaskan and Eurasian representatives of the subclade to be extremely similar (differing by 1 mutation in a 1942 bp section of mtDNA; Korsten et al., 2009) and the lack of subclade 3a bears in the earlier Alaskan fossil record (Leonard et al., 2000; Barnes et al., 2002).

The fossil record from the Alaskan coastal islands indicates an absence of brown bear remains dating to 26–12 kyBP, while bears occupying the area since 12 kyBP exclusively represent the previously unrecorded subclade 2a (Talbot and Shields, 1996; Barnes et al., 2002). If bears did not survive the LGM in coastal Alaska then it seems likely that subclade 2a entered North America at the same time as subclade 3a. However, it is plausible that the gap in the fossil record does not indicate a true absence, and that brown bears survived the LGM in refuge areas on the west-coast of the Alexander Archipelago (PC in Fig. 1c), an area that supported various other taxa during the LGM (Carrara et al., 2003, 2007; Kaufman and Manley, 2004). If this was the case then the most parsimonious phylogeographic scenario would involve the bears inhabiting the area prior to the LGM belonging to the clade currently present, i.e., subclade 2a, and this subclade actually constituting part of the first wave of colonization rather than the third (Fig. 5a). The estimated age of the MRCA for subclade 2a of 45 kyBP is consistent with such a scenario, but could also indicate a diverse founder population (see clade 3b above).

8.2. *Phylogeography of European bears*

Three mitochondrial subclades are currently present in Europe, and, in common with the pattern in North America, their distribution exhibits considerable geographic differentiation (Randi et al., 1994; Taberlet and Bouvet, 1994; Kohn et al., 1995): subclade 1a is found in northern (southern Scandinavia) and south-western Europe (Spain and France); subclade 1b in the south and east of Europe (Italy, Slovenia, Croatia, Bosnia, Greece, Bulgaria, Romania, Poland); and subclade 3a in the north-east and east of Europe (northern Scandinavia, Finland, Russia, Estonia, Slovakia and Romania) (Fig. 6d). Contact zones occur between 1a and 3a in Sweden (Taberlet et al., 1995) and between 1b and 3a in Romania (Kohn et al., 1995) (Fig. 6d). Broadly similar phylogeographic structure has been revealed in other mammalian taxa, including common shrew (*Sorex araneus*) (Fredga and Nawrin, 1977), bank vole (*Clethrionomys glareolus*, also *Myodes glareolus*) (Tegelström, 1987) and root vole (*Microtus oeconomus*) (Brunhoff et al., 2003). On this basis, the brown bear has been used to represent a common phylogeographic model (Hewitt, 1999; Hewitt, 2000, see also Korsten et al., 2009 for northeastern Europe and Asia).

8.2.1. Appearance of brown bears in Europe

Paleontological evidence regarding the appearance of brown bears in Europe is equivocal. Kurtén (1968) concluded that the species entered Europe during the mid-D-Holsteinian (approximately 230 kyBP), but more recently fossil remains of putative brown bears dating to approximately 500 kyBP have been identified (e.g., Moigne et al., 2006; Sardella et al., 2006). Meanwhile, bear remains from the early-Pleistocene (approximately 900 kyBP) that exhibit mixed brown and cave bear features are considered by some authors to represent early brown bear forms (Mazza and Rustioni, 1994), while others consider them to be ancestral or related species (Baryshnikov, 2007). Irrespective of exactly when brown bears appeared in Europe, it should be noted that the first bears were not necessarily ancestral to the Late Pleistocene and modern populations considered in the remainder of this section. Mean estimates for the time since MRCA of European brown bears – 175 kyBP (Saarma et al., 2007) and 263 kyBP (this study; for all studied bears) – are almost certainly more recent than the arrival of the species in Europe.

8.2.2. Late Pleistocene up to the LGM (approximately 130–25 kyBP)

While little is known about brown bear distribution and phylogeography prior to the late Pleistocene (before 130 kyBP), a greater number of fossil records from central and southern

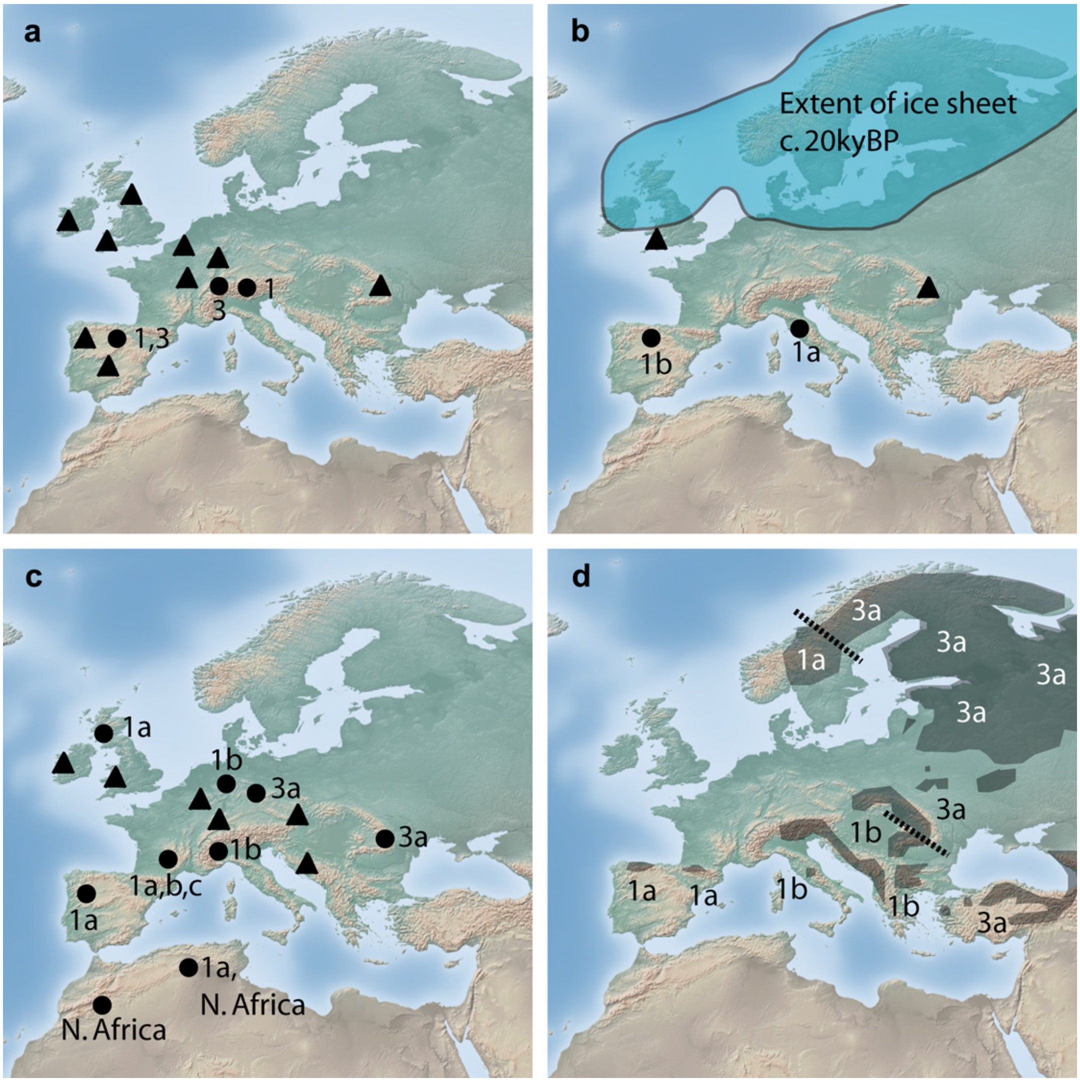

Fig. 6. Late-Quaternary phylogeography of European brown bears: a) >25 kyBP; b) 16–25 kyBP; c) 16–1 kyBP; d) contemporary distribution. Symbols represent radiocarbon-dated or known-age subfossil bear remains that have been subject to genetic analyses (circles with clade marked) or not (triangles); note that within each time period geographically proximate records are represented by a single symbol. Clade nomenclature follows Leonard et al. (2000). The distribution of modern brown bear populations (McLellan et al., 2008) is shaded, and the identity of corresponding clades is taken from Taberlet and Bouvet (1994), Randi et al. (1994), Kohn et al. (1995), Taberlet et al. (1995) and Saarma et al. (2007). The locations of known contact zones between modern clades are marked with dashed lines. The extent of glacial ice is reproduced from Svendsen et al. (2004). Sources of subfossil bear data are listed in the supplementary material.

Europe (Fig. 6a; Kurtén, 1968; Sommer and Benecke, 2005) are available from the late Pleistocene up to the LGM (approximately 130–25 kyBP). Analysis of late Pleistocene samples has revealed that lineages that cluster phylogenetically with modern clades 1 and 3 have been present in Europe for >100 ky (Fig. 6a; Hofreiter et al., 2004; Valdiosera et al., 2008). Moreover, these records reveal an intriguing phylogeographic pattern: both central Europe and Iberia were home to bears representing ancestral clades 1 and 3. The sympatric occurrence of these divergent clades suggests that the degree of phylogeographic structure currently observed in Europe did not exist prior to the LGM (though the various subfossils are not completely contemporaneous with each other).

Multiple-calibration timing estimates indicate that subclades 1a and 1b shared an MRCA during the late Pleistocene (106 kyBP). This figure has important implications for our understanding of the relationship between patterns of genetic diversity and the climate and in particular the expansion/contraction (EC) model (Hewitt, 1996). In the context of Europe, the EC model proposes that, during glacial cycles, species repeatedly recolonised mainland Europe from discrete Mediterranean refuge areas where a suitable climate persisted even during cold periods. In light of the fossil evidence indicating a lack of phylogeographic structure prior to the LGM, the most plausible explanation was a repeating cyclical process such that: (i) during each interglacial, some degree of mixing occurred between monophyletic populations originating from different refuge areas, resulting in genetically diverse northern populations; (ii) however, at the onset of glacial maxima, northern populations disappeared and the high densities of individual lineages occupying the cores of particular refuge areas prevented the introgression of mtDNA from other 'immigrating' clades (Hofreiter et al., 2004). While such a process may have played a role during the most recent glacial cycle, it is likely that early investigations substantially overestimated time-scales for the divergence of lineages. Multiple-calibration time estimation suggests that the MRCA of clade 1 was sufficiently recent that repeated restriction of subclades 1a and 1b into different refuge areas during multiple glacial cycles did not occur (Table 1). Rather, these two subclades descended from a common ancestral population that existed at the start of the most recent glacial period (Fig. 4).

8.2.3. *Last glacial maximum (LGM) and Holocene (25 kyBP until present)*

During the LGM, the Weichselian ice sheet covered northern Europe, permafrost and tundra covered much of central Europe, and the southern European peninsulas contained a mixture of forest and steppe (Figs. 1b and 6b; Svendsen et al., 2004). Thus, southern Europe is believed to have provided temperate species with suitable conditions even at the height of the LGM. The current distribution of genetic diversity among European brown bears, whereby clade 1 is divided into two allopatric subclades, 1a and 1b (Fig. 6d), suggest that the Iberian and Balkan/Italian peninsulas were used as LGM refuge areas by these respective subclades (Fig. 1c; Taberlet and Bouvet, 1994; Taberlet et al., 1998). In light of the current European distribution of subclade 3a (Fig. 6d), early studies similarly proposed that this subclade was restricted to a glacial refuge area located somewhere in south-east Europe or adjoining parts of Asia, possibly in the Caucasus (CA in Fig. 1c) or Carpathian mountains (CM in Fig. 1c; Sommer and Benecke, 2005; Saarma et al., 2007), where brown bear remains dating to the LGM have been recovered (David, 1999; David et al., 2003; Musil, 2003).

Paleoecological data and recent genetic studies have questioned this neat refugial model. First, it is becoming clear that relatively benign conditions existed north of the classical peninsula refuge areas during the LGM, and that a number of tree and mammal species were present in parts of central Europe during the LGM (Stewart and Lister, 2001; Willis and van Andel, 2004; Sommer and Nadachowski, 2006). While there are no clear-cut examples of LGM-dated (c.18–20 kyBP) brown bear remains from outside classical refuge areas, remains from Great Britain radiocarbon-dated to 17670 ± 140 and 24760 ± 260 kyBP (Aldhouse-Green and Pettitt, 1998; Fig. 6b) indicate that brown bears had a wide distribution during cold periods. Second, ancient DNA studies have shown some mixing of currently allopatric subclades 1a and 1b in southern refugia during the LGM, suggesting gene flow across southern Europe (Fig. 6b; Valdiosera et al., 2007, 2008).

These observations suggest that the simplest refuge models and versions of EC theory require some modifications or additions to explain both current and historical patterns of phylogeographic structure. Possible explanations could be that: (i) the monophyletic northern populations of the modern day originated from genetically diverse refuge populations, and many lineages were lost from rapidly increasing Holocene populations through leptokurtic expansion, i.e., the leading edge of the colonising populations became progressively less diverse as a result of a series of genetic bottlenecks (Hewitt, 1996, 2000); (ii) refugial populations may have displayed differential responses to changing conditions or there may have been population substructure within glacial refuge areas (Gómez and Lunt, 2007), such that bears from certain populations dominated the recolonization of northern latitudes; among beech (*Fagus sylvatica*) populations restricted to the south of Europe during the LGM, certain subpopulations expanded to recolonise much of the continent, while others continued to inhabit a small area or even declined in size (Magri, 2008); or (iii) the declining distribution of some subclades in the south and west of Europe during the Holocene and an associated loss of genetic diversity may be explained by stochastic processes acting on populations that were declining as a result of human activity (Irwin, 2002; Valdiosera et al., 2007).

9. Brown bear phylogeography in other regions

Brief summaries of brown bear phylogeography in other regions are presented here. Further discussion can be found in the Supplementary Material.

9.1. *Northern continental Eurasia*

Clade 3a is practically the only subclade reported in the vast area of northern continental Eurasia (Saarma et al., 2007; Korsten et al., 2009; Murtskhvaladze et al., 2010). This population underwent a severe bottleneck and is descended from a fairly recent common ancestor (Korsten et al., 2009; subclade 3a Table 1). However, it is not clear whether the ancestral population was limited to a single small LGM refuge area (e.g., CM, CA, UR or CS; as proposed by Korsten et al., 2009) or whether it was present in a larger area or in multiple areas, as indicated by the pre-LGM timing of the MRCA (Table 1) and LGM-dated fossil records from sites in the Carpathian and Ural mountains, and further east in Russia (Fig. 1c).

9.2. *Japan*

In Japan, brown bears are only present on Hokkaido, the second-largest and northernmost island. Phylogeographic investigation has revealed three well-supported distinct brown bear clades, corresponding to 3a, 3b and 4, which are also geographically separate from one another (Masuda et al., 1998; Matsuhashi et al., 1999; Korsten et al., 2009). Molecular date estimates (Table 1) and paleogeographic data are consistent with multiple colonizations: by 3a approximately 12–18 kyBP; and by 3b and 4 earlier in the most recent glacial period, perhaps coinciding with the colonizations of North America by these latter clades.

9.3. South Asia, Middle East and North Africa

While little is known about brown bear phylogeography in South Asia, recent work has provided important information about North African and Middle Eastern populations (Calvignac et al., 2008, 2009). In particular, it is clear that that these areas have historically been home to endemic divergent clades: 5, 6, North Africa and Iran. It is also clear that the population currently found in south-west Europe that is characterised by clade 1 previously inhabited a larger area in the Middle East and North Africa, even in recent historical times.

10. Implications for phylogeographic study and future approaches

Aside from *Homo sapiens*, the brown bear is arguably the best-studied extant mammal in terms of its Pleistocene–Holocene biogeography. Decades of research have revealed in considerable detail the spatial and temporal dynamics of different populations and genetic lineages. Complex patterns of migration, colonization and vicariance have been described, and inferred population responses to climate change have challenged the prevailing paradigm.

The case of the brown bear also provides a cautionary example for case studies on species where data are more limited. It is widely acknowledged that phylogeographic interpretation can benefit from the incorporation of information from climatology, paleontology and ancient DNA analysis in addition to the traditional approaches of population genetics and phylogenetics (e.g., Knowles, 2009). Indeed, it is clear that the interpretation of brown bear phylogeography is influenced considerably if any one of the following sources of data is not considered: modern DNA data; ancient DNA data; suitably calibrated molecular clock estimates; and paleontological data. This is not to say that every aspect of brown bear phylogeography is well understood. The use of longer, more informative mtDNA sequences, including full mitochondrial genome sequences, has the potential to greatly assist phylogeographic interpretation. The current use of a short section of CR for phylogenetic analysis means that time estimates may be biased due to homoplasy and are generally associated with considerable uncertainty. The use of coding regions and longer sequences from ancient samples would represent a considerable advance. While a reliance on mtDNA – which represents a single locus and can only inform about the female lineage – is widespread in animal phylogeography, it clearly limits the questions that can be posed and the interpretation of results. Moreover, there is ongoing debate surrounding the reasons for observed discrepancies between mitochondrial and nuclear phylogeographic studies (Zink and Barrowclough, 2008; Barrowclough and Zink, 2009; Edwards and Bensch, 2009). Given this, the use of autosomal and Y-chromosome markers over a wide geographic scale can complement and to an extent corroborate results obtained using mtDNA.

Phylogeographic inference is also hampered by uncertainty regarding the phylogenetic positions of certain mitochondrial lineages in south Asia, and, to a lesser extent, the Middle East and Japan. Meanwhile, certain geographical areas, including large parts of Asia, require more extensive sampling. The value of ancient DNA samples from putative refuge areas has been very well demonstrated in Beringia and Iberia (Leonard et al., 2000; Barnes et al., 2002; Valdiosera et al., 2008); similar studies elsewhere, especially in Asia – the putative origin of brown bears – could provide further important insights into the processes acting upon refugial populations.

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Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.quascirev.2010.11.023.

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