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Phylogeography and mitochondrial diversity of extirpated brown bear (*Ursus arctos*) populations in the contiguous United States and Mexico

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Abstract

The fossil record indicates that the brown bear (*Ursus arctos*) colonized North America from Asia over 50 000 years ago. The species historically occupied the western United States and northern Mexico but has been extirpated from over 99% of this range in the last two centuries. To evaluate colonization hypotheses, subspecific classifications, and historical patterns and levels of genetic diversity in this region, we sequenced 229 nucleotides of the mitochondrial DNA control region in 108 museum specimens. The work was set in a global context by synthesizing all previous brown bear control region sequences from around the world. In mid-latitude North America a single moderately diverse clade is observed, represented by 23 haplotypes with up to 3.5% divergence. Only eight of 23 haplotypes (35%) are observed in the extensively sampled extant populations suggesting a substantial loss of genetic variability. The restriction of all haplotypes from mid-latitude North America to a single clade suggests that this region was founded by bears with a similar maternal ancestry. However, the levels and distributions of diversity also suggest that the colonizing population was not a small founder event, and that expansion occurred long enough ago for local mutations to accrue. Our data are consistent with recent genetic evidence that brown bears were south of the ice prior to the last glacial maximum. There is no support for previous subspecies designations, although bears of the southwestern United States may have had a distinctive, but recent, pattern of ancestry.

Keywords: colonization, control region, grizzly bear, phylogeography, *Ursus arctos*

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Introduction

The brown bear (*Ursus arctos*) is distributed from western Europe across Asia through western North America. The fossil record indicates that it evolved in Asia (Kurtén 1968) or possibly Europe (Mazza & Rustioni 1994) over half a million years ago, and immigrated into the Beringia region of North America just 50 000–70 000 BP (Kurtén 1968, Kurtén & Anderson 1980). Until recently, the fossil record suggested that brown bears failed to reach mid-latitude North America until 11 000–13 000 BP. However, DNA sequence data of a brown bear specimen from southern Canada was dated to ~25 000 BP (Matheus *et al*. 2004) indicating that the brown bear was in southern Canada and potentially the lower 48 states prior to the recession of the Pleistocene ice-sheets.

Given their relatively recent arrival in North America, it is perhaps surprising that brown bears display enough colour and morphological variation to have stimulated extensive early taxonomic subdivision. Merriam (1918) named 96 subspecies in North America, 29 south of the Canadian border. By contrast, a continuous cline of increasing condylobasal length moving north and west into Alaska led Rausch (1963) to suggest a single mainland *U. arctos* subspecies in North America (*U. a. horribilis*). In mid-latitude North America, Rausch examined very few skulls from outside Yellowstone, but he did remark that 'rather distinctive cranial characters formerly existed in what is now California'. Hall (1984) revisited the subject and examined a larger sample of skulls from the contiguous United

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States. In mid-latitude North America, he delineated three *U. arctos* subspecies: *U. a. stikeenensis* along the Pacific coast south to Siskiyoo Mountains of Oregon, east to the Cascades; *U. a. californicus* in California; and *U. a. horribilis* in the interior.

More recently, genetic structure in brown bears has been studied in Europe, Japan, and across the extant parts of its range in North America. Sequence variation at mitochondrial DNA (mtDNA) shows strong geographical partitioning. The majority of Europe, is occupied by one clade (Clade 1) and western Asia and eastern Europe by another (Clade 3a), with documented overlap only in Romania and Scandinavia (Taberlet & Bouvet 1994; Kohn *et al*. 1995). In North America, where occupation is much more recent and a major ice-age must have driven a shifting distributional history, there is also a remarkable level of mtDNA genetic structure (Talbot & Shields 1996; Waits *et al*. 1998). Clade 3a is found in most of Alaska while a different clade (3b) is observed in eastern Alaska and western Canada. Geographical overlap between these clades has been documented only in the Arctic National Wildlife Refuge of Alaska. Two other spatially discrete clades are observed: Clade 2 is confined to a set of island in southeast Alaska (Admiralty, Chicagoff and Baranoff or the ABC islands) and Clade 4 is observed in extant populations of southern Canada and northern contiguous United States (Waits *et al*. 1998). Talbot & Shields (1996) explicitly compared patterns of mtDNA variation with prior subspecies designations in Alaska, but found poor concordance.

In an attempt to understand what lies behind this striking genetic pattern in North America, Leonard *et al*. (2000) and Barnes *et al*. (2002) sequenced mtDNA in over 30 permafrost samples from Alaska and northwestern Canada dated to between 10 000 and > 50 000 BP. The permafrost samples suggest a dynamic distributional and demographic history where regions occupied by one clade were formerly occupied by another. Despite this instability, it appears that replacing populations have generally been large enough to harbour considerable genetic diversity and have been monophyletic. Hence, the current distribution of clades does not appear to be simply the result of a set of founder events. Barnes *et al*. (2002) argue that it may have arisen from strong climatic regionalism in Beringia over tens of thousands of years. A potential exception to this pattern occurs near the Alaska/Yukon border where Clades 2 and 4 are found within 1000 years of one another ~35 000 BP. This raises the possibility that the ancestors of bears now found on the ABC islands and near the southern Canada/US border were in the same mixed population tens of thousands of years ago (Leonard *et al*. 2000).

In this study, we report on the mtDNA genetic diversity and structure of historical brown bear populations south of the Canadian border. In this region, an estimated 100 000 brown bears once roamed from the Pacific Coast to the Great Plains and South into Mexico, but now brown bears are extirpated from over 99% of this area (Allendorf & Servheen 1986). Currently, brown bear populations in this region are protected under the US Endangered Species Act and around 1000 bears remain in the states of Idaho, Montana and Wyoming (Servheen 1999). This research addresses four main questions about these extirpated populations: (i) Is there evidence for a substantial loss of genetic diversity due to severe range contraction? (ii) Did historical populations contain haplotypes only from the currently observed Clade 4 or is there evidence for maternal ancestry from other clades? (iii) Is there evidence of a rapid demographic expansion or does the DNA suggest an extended, more demographically stable history? (iv) Is there any concordance between subspecies designations and mtDNA structure? To address these questions, we sequenced a 229-base pair (bp) segment of the mtDNA control region for 108 historical museum specimens and compared our results with contemporary samples collected in North America, Europe and Asia.

Materials and methods

Samples and methods

Bone samples from bears of mid-latitude North America were taken from major museum collections in the US and British Columbia (BC) and DNA was extracted as described in Miller & Waits (2003; see Supplementary material for sample details). A 229-bp segment of the hypervariable mtDNA control region was sequenced in two overlapping sections. Polymerase chain reaction (PCR) was conducted as follows: in 15 μL total volumes with 2–4 μL of DNA template, 1.5 U of Ampli*taq* gold polymerase, 1× Ampli*taq* buffer, 2.5 μM $MgCl_2$, 0.1 μM of each dNTP, primer concentrations of 1.0 μM and PCR profile of 10 min at 95 °C, 45 cycles with 30 s at 95 °C, 30 s at 44 °C, 45 s at 72 °C, and a final 2-min extension at 72 °C. Primers were designed from the sequence in Paetkau & Strobeck (1994). The initial PCR was conducted with these terminal primers: (H)5′-CCTAAGACTAAGGAAGAAG-3′ and (L)5′-CTTATATGCATGGGGGCACG-3′ for segment one and (H)5′-CATCGCAGTATGTCCTCG-3′ and (L)5′-TACTCGCAAGGATTGCTGG-3′ for segment two. For samples that did not yield robust agarose bands, nested PCR was conducted using these primers: (H)5′-AGGAAGAAGCAACAGCTCC-3, and (L)5′-GGGCACGCCATTAATGCACG-3′ for segment one and (H)5′-CGCCAGTATGTCCTCGAATAC-3′ and (L)5′,_ AAGGATTGCTGGTTTCTCG-3′ for segment two. PCR products were purified using polyethylene glycol precipitation and sequenced in 10 μL total volumes with BigDye chemistry (Applied Biosystems): 2 μL BigDye, 1 μL DMSO, 2 μL primer, 1–4 μL PCR product, 0–4 μL H_2O (= 4 μL

product); Thermocycle: 96 °C for 3 min, 25 cycles of 95 °C for 30 s, 48 °C for 45 s, 57 °C for 2 min). Products were purified in Sephadex G-50 (Sigma) and run on an ABI 377 automated sequencer using 4% Long Ranger gels. Editing and alignment were done using SEQUENCHER (Perkin Elmer). Specimen details (museum, sample location and year, age, DNA sequence) are given in the Supplementary material. Sequences were deposited in GenBank and can be accessed by numbers DQ914292–DQ914411 and EF033706–EF034026.

Historical DNA precautions

To avoid contamination and authenticate results, extractions and first generation PCR setup occurred in a separate building where amplified mammalian DNA has never been present. No materials (clothing included) were allowed to move from the PCR/gel building back to the extraction/setup facility. One out of every 10 extractions was a negative control and one out of every 16 first generation PCRs was a negative control. For the nested PCRs where contamination was an increased concern, one in every eight reactions was a negative control. For each sample, both segments were sequenced at least twice from independent PCR products. Samples that did not yield consistent sequence were removed from the data set. Haplotypes observed only once in the entire data set or only once in a geographical region distant from other observations of the same haplotype were sequenced three times from three independent PCR amplifications.

Analysis

In the analysis, historical samples were combined with contemporary samples from Waits *et al*. (1998). Samples were grouped into populations based on spatial proximity and unifying ecological features such as mountain ranges. Samples from all of California were treated as one population because many of them lacked specific locations and because California is distant from all other sampled locals. For most populations where all samples were historical and none contemporary, or vice versa, all samples were included. In the Yellowstone and the Selkirk/Cabinet populations, both contemporary and historical samples existed. Assuming haplotypes unique to the contemporary sample were historically present but at low frequencies, one observation of each such haplotype was appended to the historical dataset and the remainder of the contemporary samples were excluded.

The dataset was analysed using nested clade analysis (NCA; Templeton 1998) through the programs TCS (Clement *et al*. 2000) and GEODIS (Posada *et al*. 2000). The analysis of mid-latitude North America samples were placed into a global context by gathering together all published control region sequences in brown bears and adding 21 unpublished samples from our laboratory and two from Pierre Taberlet (University of Grenoble, France) from previously unsampled regions (e.g. the Russian Far East, Pakistan, and Iran). A Bayesian phylogenetic analysis was performed using the program MRBAYES (Huelsenbeck 2000), employing the full GTR + I + Γ model, assuming uniform priors, and using the American black bear, *Ursus americanus*, as the outgroup. Genetic diversity in mid-latitude North America was compared to other regions in the world within populations and regions using statistics calculated in program ARLEQUIN 2.0 (Schneider *et al*. 1999). At the population level, populations with sample size < 5 were excluded and pairwise comparisons were performed using Welch's approximate *t*-test for uneven variance and sample size (Zar 1999). From California, only samples of known southern coastal origin were included. Because at the regional level the sampling unit is complicated by differing numbers and spread of populations sampled and differing within population sample sizes, only qualitative comparisons were performed.

Results

There is an extensive amount of diversity and phylogeographical structure in the global brown bear population. The Bayesian phylogenetic analysis, along with the minimum spanning network, suggests that the 85 known haplotypes represent at least eight divergent clades/lineages as well as several subclades (Figs 1 and 2). Some clades and subclades have very restricted geographical ranges (e.g. Clades 2 and 3d) while others are distributed across thousands of kilometres (e.g. Clade 3a is distributed from Scandinavia to Alaska).

In southern BC and the contiguous US, 108 historical sequences were obtained and combined with 80 contemporary samples to yield $n = 188$. Among these samples, 23 haplotypes were observed, all belonging to Clade 4 with 99% posterior probability (Fig. 1). Fifteen haplotypes are unique to the historical sample, three to the contemporary sample, and four are found in both. In Clade 4, 20 segregating sites are observed; all but one are transitions. At the population level, mid-latitude North America has moderately high levels of genetic diversity compared to other well-sampled regions in the world (Table 1a). The diversity among haplotypes in this region is also moderately high compared with within-clade levels of diversity from other regions (Table 1b).

Of the 23 Clade 4 haplotypes, five have widespread geographical distributions (37, 38, 111, 120 and to a lesser extent 115; Fig. 3) while the other 18 are either observed in only a single population (39, 52, 55, 60, 101, 103, 105, 108, 112, 113, 114, 117, 118 and 124) or are restricted to geographical regions (40, 51, 102, 119). By far the most abundant haplotype, both numerically and geographically, is

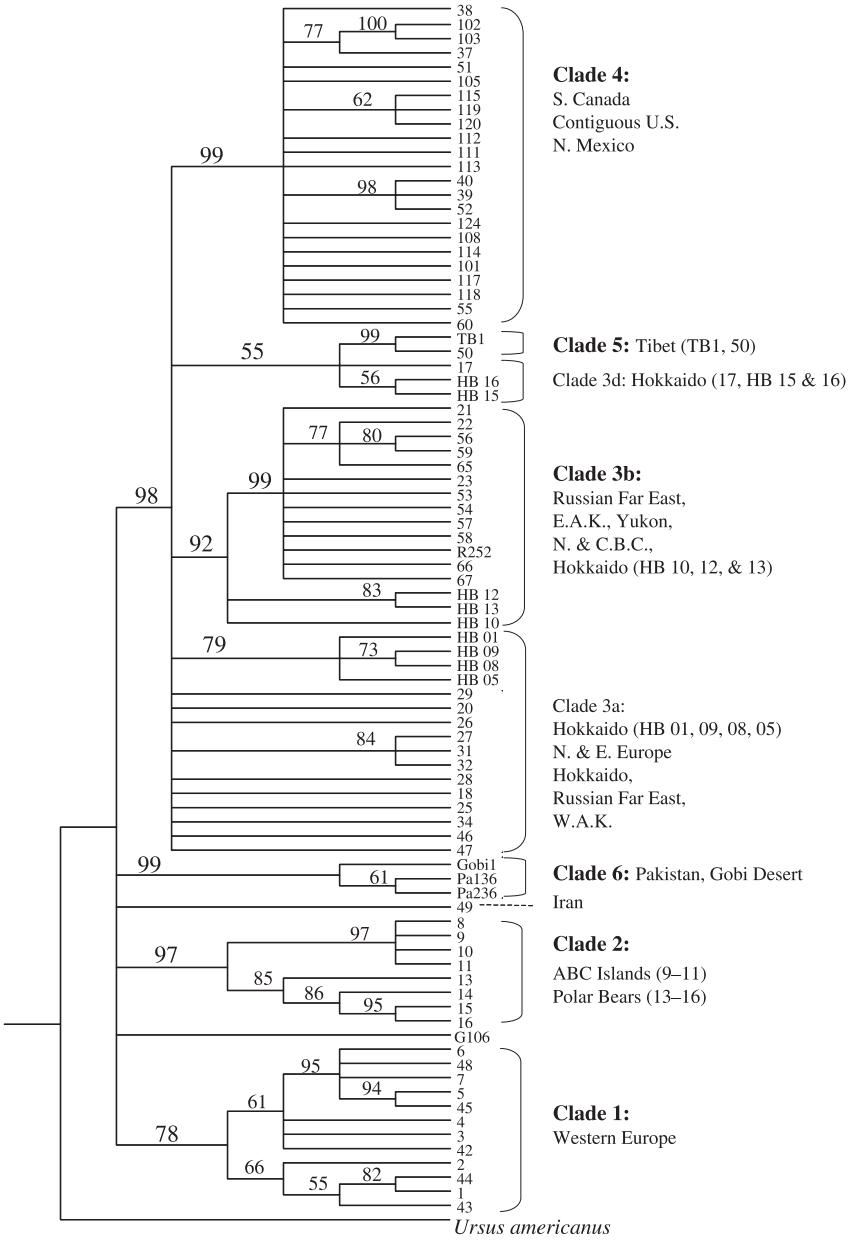

Fig. 1 Majority-rule (50%) consensus tree based on Bayesian phylogenetic analysis of 80 brown bear haplotypes from around the world and four polar bear haplotypes. Values above lines are posterior probabilities. Previously defined Clade 3a is not bolded to emphasize it does not form a clade in this analysis.

the central haplotype in the network: 38 (Fig. 3). Most populations are characterized by one or more private or regional haplotypes and one or more geographically widespread haplotypes (usually 38). Globally, this appears to be a recurring pattern: 78% of the known haplotypes have been observed in only one population. In addition to haplotype 38, the two other widespread haplotypes (29 from Clade 3a and six from Clade 1) are centrally located in the haplotype network (see online supplemental material for detailed data by population and comprehensive minimum spanning network). Of the five historical samples obtained from central BC, all belong to Clade 3b with 99% posterior probability (Figs 1 and 3).

The sequences display extensive homoplasy as evidenced by the five closed loops in the spanning haplotype network (Fig. 3b). The nested clade analysis was severely weakened because these loops made it impossible to resolve the interior-tip (ancestral-descendant) status and hence to draw inferences. Among the clades where polarity could be determined, the following clades (identified here by haplotypes) showed no evidence of genetic structure: (119–120) (111–113) (38-51-124) (39-40-52) (55–60) and (105–112). Clades suggesting restricted maternal gene flow were: (102–103) (117-38-51-124-114) (115-10-40-39-52). Clade (37-102-103) indicated range expansion (see Supplementary material for detailed NCA results).

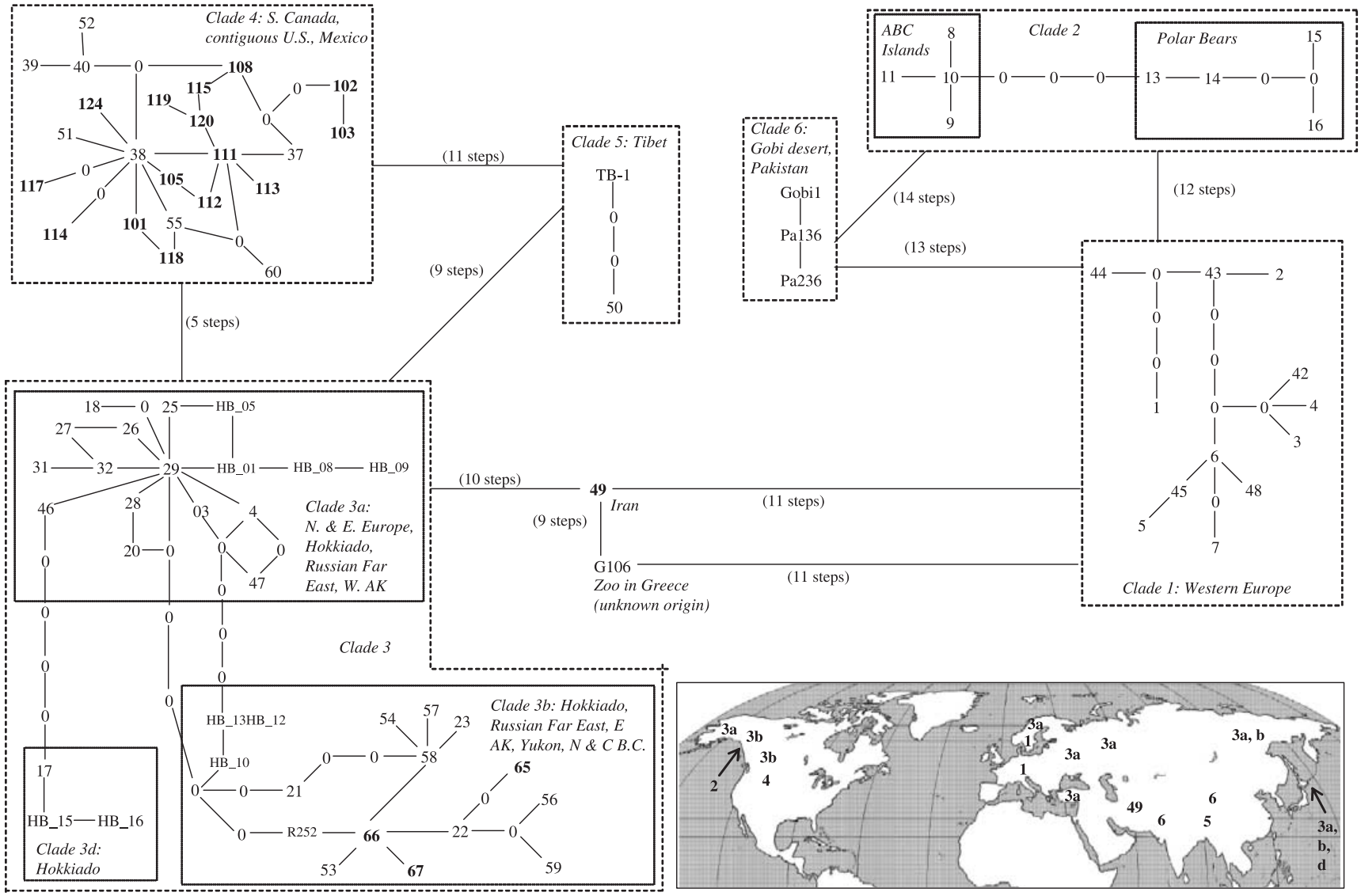

Fig. 2 (a) World *Ursus arctos* mtDNA control region minimum spanning network. Each line represents one mutational step unless otherwise labelled. Missing haplotypes indicated by '0.' Sequences published here are in bold. (b) Approximate geographical distribution of clades and subclades. See Tables S1 and S3 of Supplementary material for breakdown of haplotype counts by population and publication information (including equivalent haplotype names).

Table 1 Comparison of mtDNA diversity in mid-latitude North America to other well sampled areas at the level of the population (a) and the region (b). In (a): populations with $n < 5$ excluded; mixed clade populations (ANWR, Romania) excluded, but several regions (Alaska/Canada, Europe/Western Asia, and Japan) include populations of different clades; Japan populations defined as eastern, central and western monophyletic groups; Fairbanks permafrost samples treated as a population though samples span almost 3000 years. In (b): all haplotypes of specified region and clade included

(a) Within-population diversity

Region	# pops	Mean sample size per pop	Mean # haplos per pop*	Mean haplo diversity per pop	Mean nucleotide div
Mid-latitude N. Am.	11	12.9 (± 3.2)	2.5 (± 0.3)	0.38 (± 0.08)	3.8×10^{-3} ($\pm 9.9 \times 10^{-4}$)
Alaska & Canada	6	15.3 (± 2.7)	2.3 (± 0.6)	0.32 (± 0.13)	2.6×10^{-3} ($\pm 1.1 \times 10^{-3}$)
Europe/W. Asia	4	17.3 (± 3.9)	1.3 (± 0.3)†	0.05 (± 0.05)†	5.8×10^{-4} ($\pm 5.8 \times 10^{-4}$)‡
Hokkaido, Japan	3	19.0 (± 6.8)	3.3 (± 0.3)	0.49 (± 0.09)	3.6×10^{-3} ($\pm 9.9 \times 10^{-4}$)
Fairbanks (AK) 11 900–15 800 BP	1	12	6	0.80	1.1×10^{-2}

(b) Within-region (and within-clade) diversity§

Region	Clade	# pops	# haplos	# seg. sites	Mean pairwise diff (%)	Max pairwise diff (%)
Mid-latitude N. Am.	4	26	23	20	1.6	3.5
Europe	1	13	12	17	2.2	4.4
E. Europe/W. Asia	3a	7	6	5	0.9	1.3
ABC Islands	2	1	4	3	0.7	0.9
W. Alaska	3b	9	7	5	0.9	1.7
E. Alaska/Canada	3a	7	12	10	1.3	2.2

*Unadjusted means shown. However, statistical comparison made by standardizing # haplotypes at population (sub)sample size 5 (using equation from Comps *et al*. 2001).
†Diversity in mid-latitude North America > this region at $P = 0.005$ level.
‡Diversity in mid-latitude North America > this region at $P = 0.02$ level.
§No statistical analysis performed.

Discussion

At a global scale, the complex phylogeographical pattern observed in brown bears reflects a dynamic and geographically expansive evolutionary history. Despite limited geographical coverage and much smaller sample sizes in Asia, four divergent clades/lineages are observed (Clades 3, 5, 6 and haplotype 49). This is consistent with the palaeontological hypothesis that the species originated in Asia and has been there for hundreds of thousands of years (Kurtén 1968). Within Asia, it is interesting that bears of the Gobi desert are genetically distinct from neighbouring populations in Tibet, but share ancestry with bears of Pakistan (Fig. 2). Also, the lineage from Iran is divergent from bears to the west in Turkey and those to the east in Pakistan. In North America, the observation of Clades 3a and 3b in Alaska as well as in Asia agrees with an Asian origin of these clades. Given the relatively recent arrival of the species in North America, it is surprising that two clades (2 and 4) have been observed only there. This may be the result of insufficient sampling in Asia.

In the mid-latitudes of North America where we conducted extensive historical sampling in this study, only Clade 4 is observed. No correspondence is observed here between mtDNA and the geographical pattern of skull morphology that led to subspecies designations (Rausch 1963; Hall 1984). It is highly unlikely that subspecies reflect separate colonization events. Rather, the presence of lineages 38 and 120 in California and the Rocky mountains suggests that bears in these regions recently shared common ancestors (Fig. 3). Three samples from the North Cascades (potentially of the putative *Ursus arctos sitkeenensis* subspecies) carry a unique haplotype (114), but it is only two mutational differences from the widespread 38 (Fig. 3).

The lack of concordance between mtDNA and subspecies designations is similar to results obtained for brown bears in Alaska (Talbot & Shields 1996). Furthermore, evidence from nuclear microsatellite DNA in southeast Alaska indicates that male brown bears facilitate gene flow across mtDNA clade boundaries (Paetkau *et al*. 1998). These findings, along with ours, indicate that major genetic discontinuities probably did not and do not exist in North

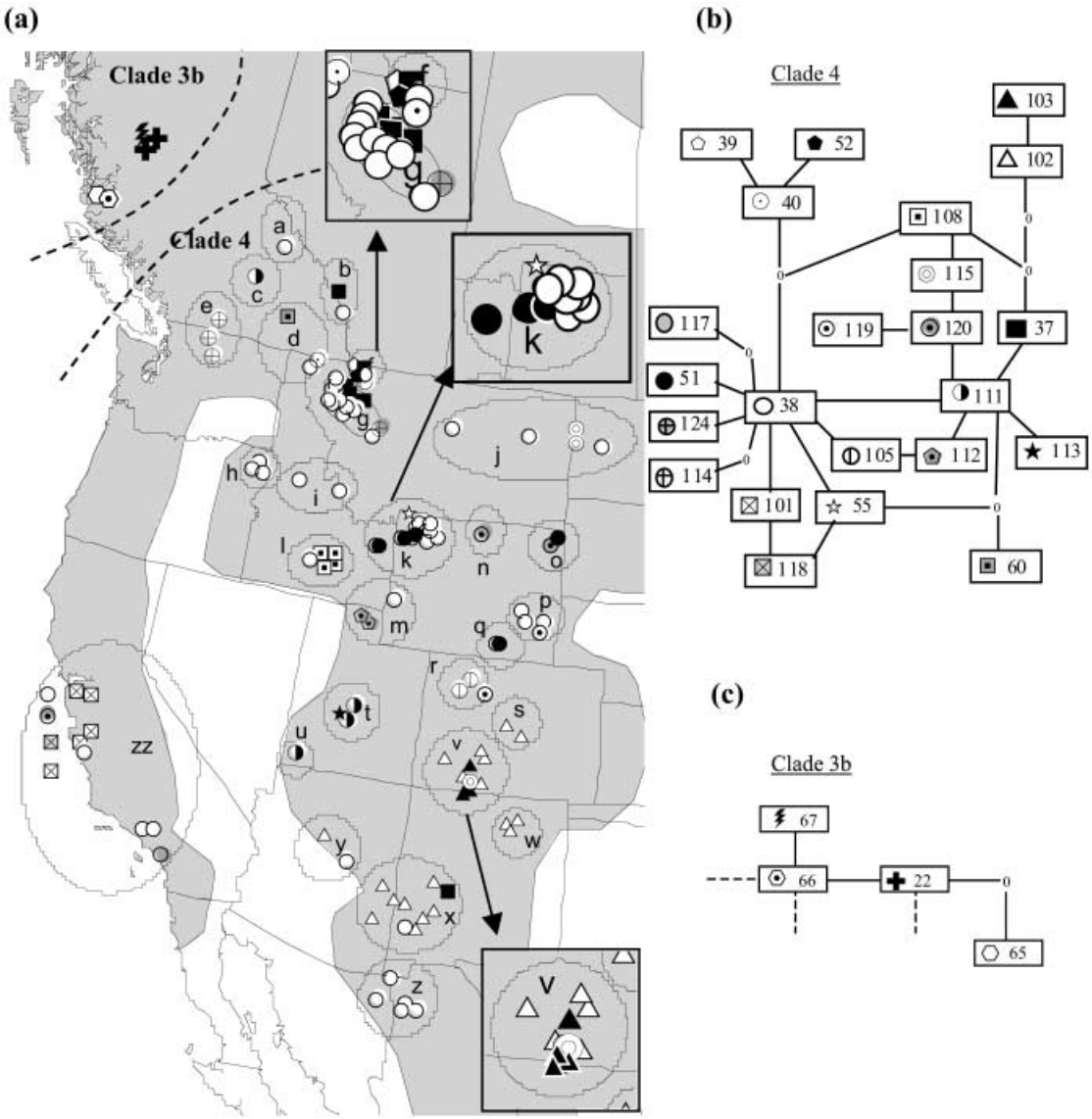

Fig. 3 Distribution of haplotypes (symbols) in mid-latitude North America (a) and the spanning networks that relates them (b & c). (a) Grey areas map approximate historical range; populations delineated by labelled ellipses; inlay boxes show detail of populations g, k and v. Haplotypes counts in populations a, b and f (too numerous to display) are as follows. In a: haplotype 37 (n = 10); in b: 37 (n = 1), 38 (n = 29); in f: 37 (n = 19), 38 (n = 3), 39 (n = 3), 40 (n = 11), 52 (n = 1). See Supplementary material table for counts by population. (b) Spanning network for Clade 4 haplotypes; lines indicate one mutational step, '0's represent unobserved haplotypes. (c) Small portion of Clade 3a spanning network containing four observed haplotypes. Dashed lines indicate connections to rest of network (see Fig. 2 for full network).

American brown bears. It should be pointed out, however, that a lack of major genetic discontinuities does not necessarily imply that there are no adaptive differences between geographically disparate populations.

The permafrost data from the Yukon (Leonard *et al*. 2000) suggest the possibility that the ancestors to mid-latitude North America brown bears were part of a mixed clade population. If this were the case, we would expect to either find multiple clades in mid-latitude North America, or evidence that the population had passed through a founder event and thereby been reduced to a single clade. However, we observe only Clade 4 across the entire sampled region. We were unable to formally test for a founder event because the extensive homoplasy and geographical subdivision grossly violate assumptions of available coalescent models. Nevertheless, the comparatively high levels of mtDNA diversity within populations and within the region (Table 1) do not favour a recent founder event. At the regional level, this may be partially an artefact of the large number of populations sampled and their geographical spread (Table 1b). But at the population level, diversity is comparatively high despite a relatively small mean sample size (Table 1a).

Another way to examine data is through the lens of haplotype networks as is done in NCA. If a diverse population expands geographically and demographically, then we expect a diverse array of haplotypes to be carried with the expansion to disparate locations. Furthermore, we would expect that, by chance, some of these would be tip haplotypes (Templeton 1998). Among the nine haplotype we can definitely call tips, none are widespread. The widespread haplotypes (37, 38, 111, 120, 115) are in the core of the nested clade network (Fig. 3). These results do not favour a recent expansion from a highly diverse colonizing population. Still, if the five widespread haplotypes evidence a minimum of five colonizing matralines, then a severe founder event is unlikely.

With time, we expect that mutations will produce new tip haplotypes and these haplotypes are expected to have small distributions until spread by gene flow (Templeton 1998). This matches the pattern we generally observed suggesting a moderate proportion of the Clade 4 haplotypes and diversity may have arisen since colonization. Taken together, the data suggest that an expansion occurred from a population that was not extremely small, long enough ago for local mutations to occur. With the recent discovery that Clade 4 brown bears have been in mid-latitude North America tens of thousand of years ago (Matheus *et al.* 2004), this scenario becomes much more plausible. There is one exception to this pattern and it occurs in southern Colorado, New Mexico, and eastern Arizona. Here there is the predominance of haplotypes 102 and 103 which are three and four mutations away from the other Clade 4 haplotypes (Fig. 3). Based on the clustering of haplotypes 102 and 103 and the widespread distribution of their putative ancestral haplotype 37, NCA suggests there is evidence for a distinct colonization event into this area. Finally, it is also important to note that confidence in these conclusions must be tempered by the fact that only a single maker was studied and our analysis has not accounted for all stochasticity underlying the genetic processes (e.g. Excoffier & Schneider 1999; Knowles & Maddison 2002).

As an interesting historical aside from this region, the last population to be extirpated in the contiguous United States occupied the San Juan Mountains in southwestern Colorado (*v* in Fig. 3). The last known bear in that ecosystem was a very old female killed by Ed Weismann, purportedly in self-defence in 1979 (Petersen 1995). The most recent confirmed record before this was nearly three decades previous, in 1952. The history of this bear appeared even more peculiar when a stable-isotope analysis of diet by Jacoby *et al.* (1999) revealed that the bear had an anomalously high percentage of meat in her diet. Any doubt that this bear (DMNH 7079) was truly a Colorado grizzly (and probably the last) is erased by the fact that she carried the San Juan private allele 103. Since that time, interests and attempts to document grizzly bears in the San Juans have continued (Petersen 1995), without any verified success. If any grizzly bear genetic material is obtained from that region in the future, it will be possible to verify its authenticity because of the unique haplotypes historically found there.

Using museum specimens, Leonard *et al.* (2005) studied levels of mtDNA variation in grey wolves in North America and found that most of the diversity resided in the extirpated wolves from south of the Canadian border. They suggested that high levels of genetic diversity in the south were related to this region acting as a refugium for wolves. Brown bears underwent a similar range collapse in the past 200 years and also appear to have suffered a substantial loss of genetic variability. Only eight of 23 (35%) of known Clade 4 haplotypes are from extant populations. In contrast to wolves, however, the mtDNA suggests that at the continental scale, most of the diversity persists. In mid-latitude North America, diversity has been reduced, but extirpated populations were closely related to surviving bears.

While the loss of genetic variability is regrettable, we view it as positive that no major evolutionary legacy has been lost. In our opinion, the more serious losses are ecological and cultural. As Aldo Leopold remarked (1949), 'Relegating grizzlies to Alaska is about like relegating happiness to heaven; one may never get there'. Still, restoration of extirpated populations may some day be feasible when the political and cultural landscapes have changed. The current analysis indicates that the bears of southern Canada, and the greater Glacier and Yellowstone National Park ecosystems are closely related to the extirpated populations.

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Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3105/MEC3105sm.htm

Table S1. Total (and contemporary) Clade 4 haplotype counts used in the analysis. Population letters correspond to Figure 3 in the study. Contemporary counts are not necessarily total observed (see text). v* are Colorado specimens without specified locations.

Table S2. Haplotypes of historical specimens divided by population. Populations delineated in Figure 2 in the study. Museum codes: UBC, University of British Columbia; MDP, Montana Department of Fish, Wildlife and Parks; USNM, Smithsonian in Washington D.C.; UMZM, University of Montana Zoological Museum; AMNH, American Museum of Natural History in

New York; DMNH, Denver Museum of Natural History; BMP, Berkeley Museum of Paleontology; ANS, Academy of Natural Sciences, Philadelphia.

Table S3. World control region haplotypes with names of equivalent haplotype(s) from previous publications and their counts by region/population. Clade 4 haplotypes given in Table S1. Haplotypes defined by 229 base pairs analyzed in this study. Counts superscripted to indicate study in which the haplotype was observed. Equivalent names also superscripted to indicate in which study that name was used. Studies identified by following superscripts: [a]Taberlet and Bouvet 1994, [b]Kohn *et al.* 1995, [c]Masuda *et al.* 1998, [d]Matsuhashi *et al.* 1999, [e]Waits *et al.* 1998, [f]Waits unpublished, [g]Taberlet unpublished, [h]this study.

Table S4. Results of nested clade analysis. Tip clades are indicated by bold when they can be determined; [S] and [L] indicate significantly small and large distances, respectively. Dc refers to clade distance, Dn to nested clade distance, and I–T to interior/tip contrast. Inf stands for inference, IBD for isolation by distance.

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