

High genetic diversity of Tibetan Mastiffs revealed by mtDNA sequences

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Many studies have been reported the origin and evolution of domesticated animals, particularly dogs, however, few have systematically examined the Tibetan Mastiff (TM), a dog that lives primarily in the Qing-Tibet Plateau. Here, we study the origin and evolution of TM based on the population comparison of mtDNA D-loop sequences from TM and other dogs across the world. Intriguingly, non-simultaneous arrivals of Tibetan Mastiffs ancestors to Tibet occurred, which may result from continuous Tibetans' settlement on Qing-Tibet Plateau supported by archaeological and genetic evidence. High genetic and haplotype diversity and robust haplotypes sharing with other dogs unique to East Asia are observed in Tibetan Mastiff, supporting that it is a very archaic breed derived probably from East Asia.

Tibetan Mastiff, genetic diversity, mitochondrial

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The domestic dog is the mammal that has enjoyed the closest relationship with humans over many centuries and has evolved many mutually beneficial associations, e.g. sharing living space and food sources, playing roles in herding, hunting, accompanying, and obedience. Dog domestication has been traced back to at least 16300 years ago from a wild ancestral gray wolf from either East Asia or Middle East Asia [1–4]. Following human migrations, it rapidly spread throughout the Eurasian continent after domestication, and then into Africa, Oceania and the Americas [5–7]. Due to the intimate association between dogs and humans, hypothetically we should be able to explore human migration history by following the history of dogs, although to date, there is little genetic evidence supporting parallel migrations of humans and dogs.

Due to extreme environmental conditions, the Qing-Tibet Plateau is one of the most challenging areas for settlement by both humans and dogs. Mitochondrial genomic evidence revealed successful late Paleolithic settlement and subse-

quent Neolithic arrivals of Tibetans to the plateau [8]. Since the Tibetan Mastiff (TM) performs as an indispensable role as a protector of the livestock of Tibetans, could a better understanding of the TM, and its arrival in the plateau, provide insight into the Tibetan settlement of the plateau, and also who is the ancestor of the TM?

The available earliest written accounts using “Mastiff” to name a large dog dated back more than 2000 years [9]. A scenario has been proposed according to the geographic distribution and historic records where the TM traces its ancestry to the Qiang dogs, which were kept by the Di-Qiang minority an ancient tribe that resided in northwest China about 3000 years ago [9]. Further, it was proposed that the TM was isolated in the Himalayan area until they were enrolled into the Mongolian army of Genghis Khan which then spread quickly across Eurasia [9–11] in the early 13th century. It is commonly theorized that a consequence of this expansion was that the early TM contributed to the germplasm of many famous large breeds such as the St. Bernard, English Shepherd, Pyrenean Mountain dog, and other mastiff-like dogs [11,12].

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The primary origin of the TM has been questioned ever since the arrival of Marco Polo to China in the 13th century [13]. Recent mtDNA evidence revealed that the TM shares a common ancestor with other dogs deriving from the wild gray wolves [2,12,14]. The TM and gray wolf are estimated to have diverged 58000 years ago, with the TM exhibiting a much closer phylogenetic relationship with the Old English Sheepdog, Leonberger, and Saint Bernard, than with other large dog breeds [12,14]. This conclusion, however, is limited as only a single individual was used for each breed, which could not accurately apportion mitochondrial haplotype diversity in dogs according to breeds [15]. High genetic diversity has been observed within TM from microsatellite [16], RAPD [17], and several protein polymorphism analyses [18], which suggests that a single individual could not represent the diversity of the TM population. A single TM individual would probably fail in deciphering the true evolutionary history of the TM and its genetic contribution to other large dog breeds of the world.

To examine the origin and evolution of the TM at a fine scale, here we collected 47 individuals from independent maternal lineages from Tibet, and surrounding areas, and analyzed 582 bp of mitochondrial D-loop sequence in combination with 439 published domestic dog haplotypes covering almost all phylogenetic clades, 95 gray wolf haplotypes, and 24 coyote haplotypes that were used as an out-group (download from the non-redundant NCBI database after a BLASTN search [19], Table S1). Genetic polymorphism data was calculated using DnaSP v5 [20] and the phylogenetic tree was constructed using the Neighbor-Joining (NJ) method [21] implemented in MEGA 4 [22]. Median-joining networks, for the inference of haplotype genealogy, were constructed by Network [23].

Among the TM sampled, 14 mtDNA haplotypes including two new ones (which we named A184 and A185) were identified. With published data a total of 15 haplotypes were found in 50 TMs (47 individuals that we sequenced and three individuals from previous studies [2,14]). Intriguingly, phylogenetic analysis indicated that all 15 haplotypes were grouped into haplogroup A, the oldest clade of dog haplogroups [3] (Figures 1 and S1), a pattern very different from that of other ancient dogs or breeds collected from the Qing-Tibet Plateau, which share similar proportions of individuals belonging to clades A, B, and C [2,3,24]. Although the Himalayas and Hengduan Mountains form natural barriers isolating and separating the Qing-Tibet Plateau from either Indian subcontinent or East Asia, a large haplotype diversity was seen in TM, with TMs differing with up to nine substitutions, a diversity similar to the maximum of 11 substitutions seen in clade A in a previous report [3]. In contrast, in Australian dingoes, which are domesticated dogs that have become feral, 20 haplotypes were identified from 211 individuals that differed at most by only two substitutions [7]. The discovery rate for TM haplotypes (15 haplotypes observed in 50 individuals) was slightly higher

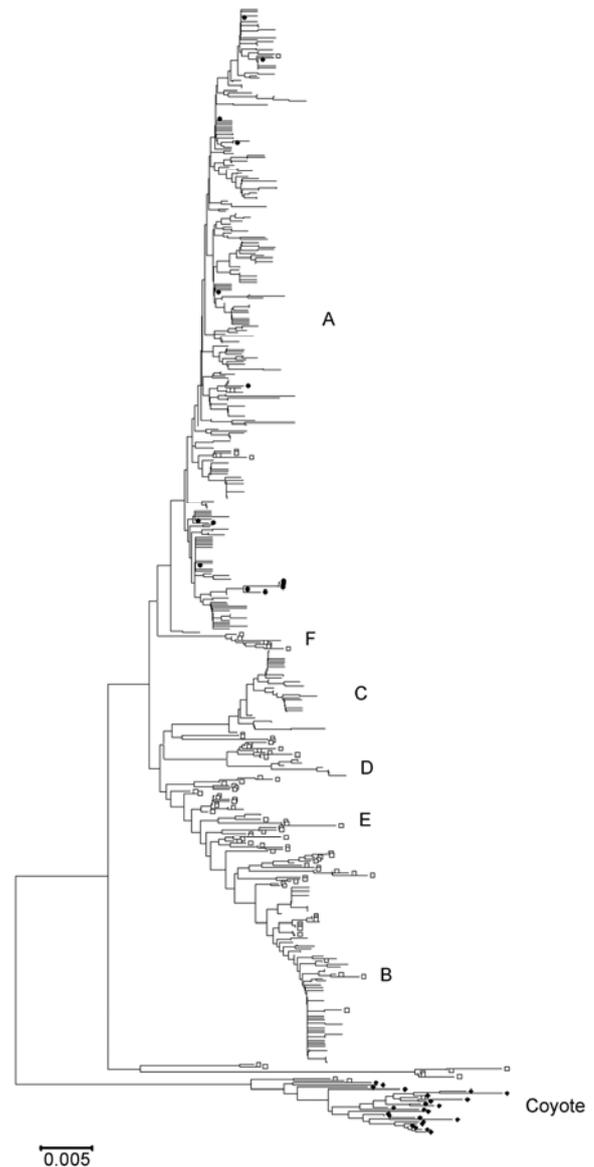


Figure 1 Phylogenetic tree of dog, wolf, and coyote mtDNA haplotypes. Unlabeled leaves denote mtDNA types from domestic dogs, dots denote haplotypes found in the Tibetan Mastiff, and squares denote wolf haplotypes. The tree is rooted with the coyote sequences, denoted by black diamonds. Dog clades A–F are indicated with letters.

than that for dogs from East Asia (121 haplotypes found among 730 dogs), the only region that has near complete representation across clade A [2,3]. The genetic diversity, with a mean pairwise sequence distance of 3.85 (SD=0.21) in TM, is also higher than the previously reported values of 2.28 (SD=0.23) for dogs of clade A in Southwest Asia, 2.97 (SD=0.08) for dogs in Europe, and 3.39 (SD=0.13) for dogs of East Asia [3]. To avoid an ascertainment bias due to population size, we re-sampled the TM haplotypes and haplotypes from East Asian dogs (based on the sample information from Pang et al. [2] on 106 A clade haplotypes from 576 individuals) 1000 times with replacement. Although the mean number of TM haplotypes was lower than

that from East Asia with the simulated data (7.80 vs. 11.06, sample size=15), the haplotype diversity of TMs remained higher than that from East Asia (0.14 vs. 0.07, sample size=50). In addition, the mean pairwise distance of 3.80 in TM was very close to that of 3.85 from East Asian dogs in the simulated data (resampled 100 times, size=50)(see Table S2). Together, these results supported the hypothesis that

the TM is an ancient dog with high genetic diversity, which could have helped enable them to avoid extinction during their adaption to the extreme environment of the Qing-Tibet Plateau.

To better understand the origin and evolution of TM, we reconstructed the phylogenetic tree and the median-joining network of individuals of only haplogroup A (Figure 2). A

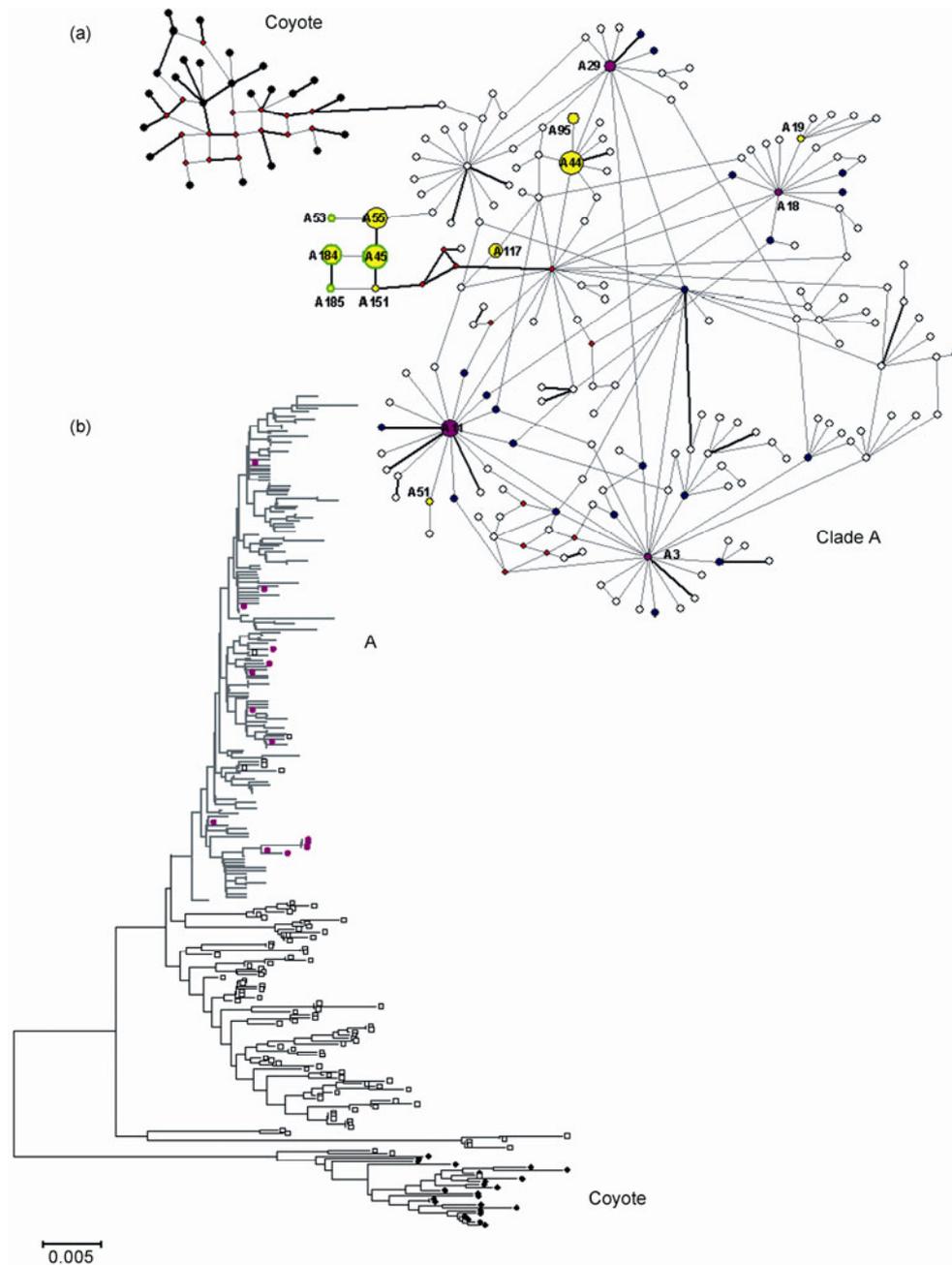


Figure 2 Phylogenetic analysis of the dog control region sequences that belong to haplotypes A. (a) Median-joining network showing the genetic relationships among mtDNA dog control region haplotypes of phylogenetic clade A, ignoring wolves. Haplotypes (circles) and empty nodes (red solid dots) are separated by one mutation (gray line) or at least two mutations (black line), including indels. Black circles are haplotypes of the coyote that was used as the outgroup. Yellow circles, are haplotypes found in the Tibetan Mastiff. Yellow circles with green outline, are haplotypes unique to the Qing-Tibet Plateau. Purple circles are haplotypes found in both the Tibetan Mastiff and other large dog breeds. Blue circles are haplotypes derived from the purple haplotypes and are found in large breeds. The sizes of the yellow and purple circles are proportional to frequencies of the haplotypes among Tibetan Mastiffs. (b) Phylogenetic tree constructed by only haplotypes in clade A, rooted by the coyote sequences. Purple dots denote mtDNA haplotypes found in the Tibetan Mastiff.

total of nine TM haplotypes (A11, A29, A19, A18, A3, A51, A117, A95, A44) were found to be interspersed across clade A, and covered the central nodes in a star-like shape. Of these nine, five (A11, A29, A19, A18, A3) were universally occurring haplotypes, representing the major haplotypes shared by dogs across the Old World (Figure 2). These five major haplotypes, and their derived haplotypes, are found in many large breeds (with average heights over 20-inch, Table S3) (Figure 2), implying a potential genetic link between TM and other large dog breeds. The other six of the 15 haplotypes (A184, A185, A45, A151, A55, A53) were clustered in specific subclades, where four (A184, A185, A45, A53) were restricted to the Qing-Tibet Plateau, and three (A184, A185, A45) were unique to the TM. When TM were compared with other dogs across the World, using the sample information from the study by Pang et al. [2], five TM haplotypes (A51, A95, A117, A55, A44) were shared with dogs only located to East Asia, and one (A151) shared with dogs from India, suggesting a probable spread to the Qing-Tibet plateau from the east of Himalayas rather than from the west. Coincident with the non-star-like pattern in the network, Fu's F_s test [25] for the TM also showed no significant signal for population expansion ($P > 0.1$). Haplotypes A184, A185, A45, A151 differed merely by indels, and these four haplotypes along with A53 were derived from haplotype A55, a common haplotype that is shared by individuals across East Asia. The unique characters of A55 subclade, including its robust differences, and prevalent frequencies among TMs, strongly suggest that the A55 subclade had a different history of origin from the other nine haplotypes, potentially indicating an independent arrival to the Qing-Tibet Plateau. It is impossible to identify the exact arrival date of the TM to the Qing-Tibet Plateau by dating the the MRCA for these major haplotypes, as previous research into dog mtDNA has revealed that a large number of female lineages contributed to the initial domestication [2]. Nonetheless, the distributions of both the dispersed (A11, A29, A19, A18, A3, A51, A117, A95, A44) and clustered (A184, A185, A45, A151, A55, A53) haplotypes in the phylogeny tree and the pair of patterns of sharing major haplotypes with other dogs and specific derived haplotypes unique to the Qing-Tibet Plateau in the network, imply a non-simultaneous independent arrivals of Tibetan Mastiffs ancestors to Tibet, in line with the continuous migrations of humans to the Qing-Tibet Plateau [8].

More intriguingly, three TM haplotypes showed a genetic link between dogs of the Qing-Tibet Plateau and Japan (A29, A11, A19), similar to the genetic link between Tibetans and Japanese [8,26]. Based on the rare derived haplotypes from the three major haplotypes in Tibet, it is prudent to conclude a Holocene origin for the Tibetans-Japanese linkage, while the hypothesis of Pleistocene migration to-

wards both the Qing-Tibet Plateau and eastern seacoast to Japan needs more evidence.

Ever since the domestication of the gray wolves, dogs have accompanied human migrations to spread across the World. Therefore, by understanding the spread of dogs, we better understand human history. Our data suggest a probable history of the TM, with settlement of the Qing-Tibet Plateau with humans. In conclusion, a better understanding of domestic animals will facilitate a better understand of our history.

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Supporting Information

Table S1 MtDNA D-loop haplotypes analyzed in this study

Table S2 Genetic distance of TM and dogs from East Asia corrected by resampling

Table S3 Haplotypes observed in large breeds and TMs, or haplotypes observed in large breeds but derived from TM haplotypes

Figure S1 Compressed phylogenetic tree of all dog, wolf, and coyote mtDNA haplotypes within each dog clade A–F and coyote clade. Unlabeled leaves denote mtDNA types for domestic dogs, black dots denote haplotypes found in Tibetan Mastiff, and squares denote wolf haplotypes. The tree is rooted to coyote sequences, denoted by black diamond. Dog clades A–F are indicated with letters.

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