Table 1 Frequencies of *CTSBJ12* alleles among scrapie-positive sheep and scrapie-negative flock mates.

CTSBJ12 allele	Scrapie-positive (n = 137)	Scrapie-negative (n = 137)	Scrapie-positive (classical) (n = 88)	Scrapie-negative (classical) (n = 88)	Scrapie-positive (atypical) (n = 42)	Scrapie-negative (atypical) (n = 42)
133	0.004	0.000	0.000	0.000	0.012	0.000
139	0.000	0.007	0.000	0.011	0.000	0.000
141	0.209	0.212	0.216	0.187	0.202	0.250
143	0.132	0.135	0.153	0.136	0.083	0.119
145	0.033	0.040	0.040	0.051	0.024	0.012
147	0.179	0.201	0.176	0.182	0.167	0.238
149	0.230	0.226	0.188	0.239	0.298	0.202
151	0.201	0.172	0.204	0.182	0.214	0.179
153	0.012	0.007	0.023	0.012	0.000	0.000

ovine scrapie cases with unusual (atypical) diagnostic features compared with anterior (classical) scrapie cases have been observed in the European sheep population.[2,3] Therefore, the type of scrapie (classical/atypical) was determined in most of the scrapie-positive sheep using published methods.[3] In 11 flocks, positive sheep (n = 88) were diagnosed with classical scrapie while in 37 flocks, all affected sheep (n = 42) showed an atypical scrapie type. It was not possible to determine the type of scrapie in some sheep because of advanced dissolution of the brain samples.

Genotyping methods: DNA extraction and determination of *PRNP* haplotypes have been described previously.[4] Primers for markers on sheep chromosome 13 (OAR13), including *CTSBJ12* (DQ399872), *HUJ616*[5] and *URB58*[6], were obtained from the Australian Sheep Gene Mapping web site (http://rubens.its.unimelb.edu.au/~jillm/jill.htm). Scrapie-positive sheep and scrapie-negative flock mates had previously been screened for *PRNP* haplotypes at codons 136, 154 and 171 (data not presented), which are known to be associated with scrapie susceptibility.[7]

Statistical methods: Linkage between *PRNP* and the three microsatellites on OAR13 was analysed using CRI-MAP version 2.4.[8] The ovine *PRNP* gene was assigned to the OAR13 linkage group: *HUJ616* – (5.7 cM) – *URB58*– (3.1 cM) – *PRNP*– (19.0 cM) – *CTSBJ12*. Using two-point analyses of *PRNP* with each of the three microsatellites, the following distances and LOD scores were found: *HUJ616* (8.0 cM, 27.06), *URB58* (3.0 cM, 45.81) and *CTSBJ12* (18.0 cM, 14.76). The numbers of informative meioses were 279, 266, 226 and 299 for *CTSBJ12*, *HUJ616*, *PRNP* and *URB58* respectively.

To remove the effect of *PRNP* haplotype in the analysis of *CTSBJ12* with scrapie susceptibility, scrapie-positive sheep were matched with scrapie-negative flock mates according to *PNRP* haplotypes. Chi-square and Fisher's exact test (in the case of low numbers of observations within subclasses) were used to test for significant differences between *CTSBJ12* allele frequencies in scrapie-positive sheep and in scrapie-negative flock mates (Table 1). No significant difference in *CTSBJ12* allele frequency was observed between groups of scrapie-positive sheep and scrapie-negative flock mates, even when differentiation of classical and atypical scrapie cases was taken into consideration.

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Mitochondrial DNA diversity and population structure of four Chinese donkey breeds

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Source/description: The origin and evolution of Chinese domestic donkeys (*Equus asinus*) is still uncertain. Although previous genetic studies revealed two mitochondrial DNA (mtDNA) types in a few Chinese donkey breeds,[1,2] it remains unclear whether Chinese donkeys originated from previously reported African

[1]These authors contributed equally to this work.

maternal sources because there is no comparison between the two Chinese mtDNA types and the well-characterized Nubian and Somali lineages.[3] Herein, we sequenced a 440-bp fragment of the mtDNA control region (CR) of 146 individuals from four Chinese donkey breeds and compared our sequences with published sequences from donkeys from the Old World[3] so as to understand the origin of Chinese donkeys.

Sequencing: The ASS-F and ASS-R primers[3] were used to amplify the mtDNA CR fragment. A total of 146 individuals from Yunnan ($n = 53$), Xizang ($n = 16$), Xinjiang ($n = 52$) and Guangzhong ($n = 25$) were sequenced (Table 1). Polymerase chain reaction (PCR) products were purified using the Watson PCR Purification Kit (Watson BioTechnologies Inc., Shanghai, China) and were directly sequenced. Sequences were edited and aligned by DNASTAR 5.0 package (DNASTAR Inc., Madison, WI, USA) and deposited into GenBank (DQ448878–DQ449023).

mtDNA variation: There were 37 haplotypes defined by 37 polymorphic sites (five transversions) among 146 Chinese donkey sequences (Fig. S1). Comparing these haplotypes with previously well-defined Nubian and Somali lineages,[3] a median-joining network[4] drawn by Network 4.1 (http://www.fluxus-engineering.com) clearly showed that 15 haplotypes were shared by China and other worldwide regions, and all Chinese donkey haplotypes were divided into two star-like Nubian and Somali lineages with three (d7, d12 and d25) and two (d3 and d11) central founding haplotypes respectively (Fig. 1). Given that all five founding haplotypes found in donkeys from China are also present in other worldwide geographical regions including northeast Africa, these results provide further support that Chinese donkeys were derived from African donkeys and that northeast Africa is the most probable location for donkey domestication.[3]

Table 1 Sample size (N), number of haplotypes (n), haplotype diversity (h) and observed frequency and nucleotide diversity values (π) of Nubian and Somali mtDNA lineages in four Chinese breeds of donkeys.

Breed	N	n	h (±SE)	Nubian (%)	Somali (%)	Nubian π (SE)	Somali π (SE)
Yunnan	53	16	0.9347 ± 0.0115	64.2	35.8	0.00612 ± 0.00371	0.00468 ± 0.00306
Xizang	16	6	0.7667 ± 0.0839	81.25	18.75	0.00274 ± 0.00210	0.00303 ± 0.00311
Xinjiang	52	21	0.9163 ± 0.0199	46.2	53.8	0.00450 ± 0.00293	0.00363 ± 0.00247
Guanzhong	25	13	0.9333 ± 0.0280	72.0	28.0	0.00625 ± 0.00389	0.00476 ± 0.00345

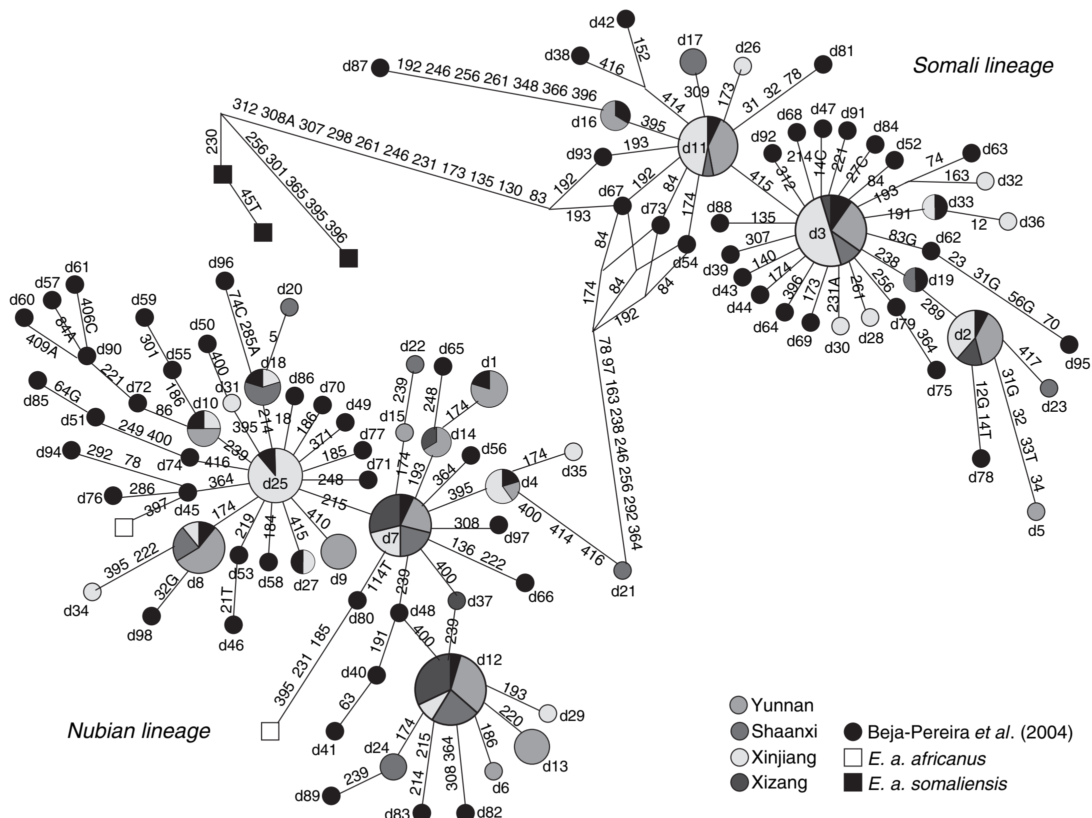

Figure 1 Median-joining network of 98 haplotypes of domestic donkeys and five sequences of African wild asses. The circle area is proportional to haplotype frequency. Suffixes A, C, G and T indicate transversions. Nucleotide positions 1–440 correspond to nucleotide positions 15 407–15 846 of *Equus asinus* (X97337).

Diversity measures calculated by ARLEQUIN 2.0 (http://anthropologei.unige.ch/arlequin) showed higher levels of mitochondrial diversity detected in Guangzhong and Yunnan breeds (Table 1). Due to the high degree of divergence between the Nubian and Somali lineages,[3] we performed analysis of molecular variance[5] using ARLEQUIN 2.0 on Chinese donkey breeds according to lineage. For the Nubian and Somali lineages, only 8.81% ($P < 0.01$) and 2.34% ($P = 0.22$) of the genetic variance could be attributed to differences among breeds. This suggested weak population substructures among the four Chinese donkey breeds.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com:

Figure S1 Sequence variation of 37 haplotypes found in the mtDNA CR region of Chinese donkeys.

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Comparative mapping of mink chromosome 8p: *in situ* hybridization of seven cattle BAC clones

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Source/description: We performed fluorescent *in situ* hybridization (FISH) of seven cattle BAC clones with American mink (*Mustela vison*) chromosomes. These clones were previously mapped and ordered on cattle chromosome 19 (BTA19) and anchored to human chromosome 17 (HSA17) and mouse chromosome 11 (MMU11).

FISH conditions: Seven cattle BAC clones from the CHORI-240 bovine BAC library were selected for FISH with mink chromosomes (Table 1). Culturing of BACs, DNA extraction and labelling were performed as described earlier.[1] The hybridization of probes with mink chromosomes from mink fibroblasts was performed according to standard procedures[2] for 48 h with modifications. The hybridization signals were analysed using an Axioskop 2 epifluorescence microscope (Carl Zeiss, Göttingen, Germany) equipped with a Paco CCD camera (CV M300; JAI Corporation, Yokohama, Japan), a CHROMA filter set and the ISIS4 image-processing package (MetaSystemsGroup, Inc., Watertown, MA, USA). FISH signals were assigned to mink chromosome regions defined by DAPI banding according to mink chromosome nomenclature.[3] At least 20 metaphases were analysed for the regional assignment of BACs on mink chromosomes.

Comparative analysis: All bovine BAC clones were assigned to mink chromosome 8p (MVI8p). Chromosomal and subchromosomal localizations of cattle BACs on mink chromosomes are presented in Table 1 and Fig. 1a. Comparison of the order of the BACs in MVI8p and HSA17 revealed three evolutionary breakpoints between the orthologous mink and human chromosomes. Two evolutionary breakpoints were found when comparing the MVI8p and BTA19 cytogenetic maps.[1] No difference in the order of the BACs in MVI8p and MMU11 was noticed. The mink–mouse homologous synteny blocks (HSBs) were defined and visualized next to HSBs from six mammalian genomes (Fig. 1b) using the Evolution Highway tool (http://evolutionhighway.ncsa.uiuc.edu/).

Conclusions: We have generated the first comparatively anchored, ordered cytogenetic map of MVI8p. Our analysis shows

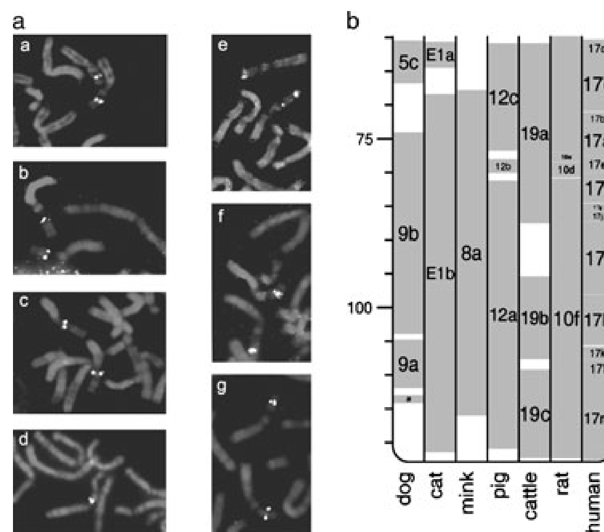

Figure 1 (a) Fluorescent *in situ* hybridization (FISH) of seven BAC clones with mink chromosomes. Images (a–g) are FISH results for BAC clones assigned to MVI8p: (a) 67N13; (b) 403K17; (c) 459E01; (d) 45D09; (e) 207O05; (f) 233H17; (g) 253B15. The clone 207O05 has the strongest signal on MVI8p26(pter) and a weak signal on MVI8p21. The weak signal overlaps with the position of the nuclear organizer region in MVI8 and was noticed in only 30% of all analysed cells, whereas the signal on 8p26(pter) was found in 100% of cells. (b) Homologous synteny blocks from seven mammalian genomes visualized on MMU11.