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DOG GENOMICS

Arctic-adapted dogs emerged at the Pleistocene–Holocene transition

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Although sled dogs are one of the most specialized groups of dogs, their origin and evolution has received much less attention than many other dog groups. We applied a genomic approach to investigate their spatiotemporal emergence by sequencing the genomes of 10 modern Greenland sled dogs, an ~9500-year-old Siberian dog associated with archaeological evidence for sled technology, and an ~33,000-year-old Siberian wolf. We found noteworthy genetic similarity between the ancient dog and modern sled dogs. We detected gene flow from Pleistocene Siberian wolves, but not modern American wolves, to present-day sled dogs. The results indicate that the major ancestry of modern sled dogs traces back to Siberia, where sled dog–specific haplotypes of genes that potentially relate to Arctic adaptation were established by 9500 years ago.

Despite decades of studies, consensus has yet to be reached on when and where dogs were first domesticated and when they were first deliberately used in many of the roles they exhibit today. In Siberia, late Upper Paleolithic artifacts of carved bone, antler, and ivory similar to tools used by modern Inuit for securing dog harness straps suggest ancient origins of dog sledding (1). Furthermore, archeological findings from Zhokhov Island provide evidence of sled technology and dogs by the Sumnagin Mesolithic culture ~9000 to 8000 years ago (1–3) (fig. S1), offering an opportunity to use genomics to further our understanding of early dog domestication and the origin of sled dogs.

We generated nuclear genomes from a dog mandible present at this site ("Zhokhov," 9.6× coverage), dated to 9524 calendar years before present (YBP) (Fig. 1A and fig. S2), and a Siberian Pleistocene wolf mandible

("Yana," 4.7× coverage), dated to 33,019.5 YBP (Fig. 1A and fig. S3). In addition, we sequenced 10 modern Greenland sled dog genomes, a dog best described as an indigenous landrace breed used for hunting and sledging by Inuit. Samples consisted of two individuals from each of five geographically diverse localities (Fig. 1A), thus providing a broad representation of the indigenous dog diversity.

We analyzed our data alongside genomes from 114 geographically and genetically diverse canids (table S1) using whole-genome pairwise distances, principal component analysis, TreeMix (4) admixture graphs, and D statistics (Fig. 1). Yana appeared alongside wolves (Fig. 1, B and C), whereas Zhokhov was found to be most closely related to dogs. Specifically, Zhokhov was most similar to modern sled dogs (Greenland sled dogs, Alaskan malamutes, and Alaskan and Siberian huskies) and American pre-European-contact dogs (PCDs), best illustrated by the ~2×

Port au Choix dog from Maritime Archaic cultural context ~4000 YBP (3). Unsupervised clustering analyses with NGSadmix software (4) (fig. S6) grouped modern domestic dogs into four clusters: African, European, Asian, and sled dogs including Zhokhov. These relationships were confirmed by an admixture graph in which Yana was more closely related to a Pleistocene wolf from Taimyr Peninsula than to modern wolves, whereas Zhokhov represents a lineage that diverged from the ancestor of present-day sled dogs (Fig. 1C and figs. S8 and S9). This suggests genetic continuity in Arctic dog breeds for at least the past ~9500 years, setting a lower bound on the origin of the sled dog lineage.

Next, D statistics indicated an excess of allele sharing between Yana-Taimyr wolves and PCDs-Zhokhov-sled dogs (Fig. 1D and fig. S14), corroborating previous reports (3, 5). This suggests that the admixture occurred between Pleistocene wolves and the ancestors of PCDs, Zhokhov, and sled dogs.

Previous studies have demonstrated an association between canine transmissible venereal tumors (CTVTs) and sled dogs, especially PCDs (3). Here, we evaluated the relationship among Zhokhov, two CTVT genomes (table S1), and dogs and wolves using f_3 statistics and phylogenetic analysis. Recent analyses of exome data suggested that CTVT expanded across Eurasia ~6000 years ago (6), thus reducing the likelihood that this transmissible cancer originated in the Americas. In our study, both the phylogenetic analysis (fig. S9) and f_3 statistics (fig. S10) placed the CTVT genomes closer to PCDs than to sled dogs or Zhokhov. These results suggest that the basal dog lineage that led to PCDs (3) occurred in Eurasia ~6000 years ago and/or there were multiple introductions of PCD-like dogs to the Americas.

We used NGSadmix, admixture analyses, and D statistics (figs. S6 to S8 and S11 to S15) to evaluate gene flow and shared ancestry between Zhokhov and modern dogs and wolves. We found no significant gene flow between any sled dog (including Zhokhov) and modern American–Arctic wolf populations compared

with the Eurasian wolf (fig. S15), suggesting that gene flow from modern wolves has not contributed to the sled dog gene pool within the past 9500 years. This result was surprising given genetic evidence for postdomestication admixture between other wolves and dog breeds (5, 7). Furthermore, ethnographic evidence from Greenland indicates that, at least historically, dog-wolf matings were not uncommon (8). If true, then the lack of gene flow from modern American-Arctic wolves into sled dogs implies selection against hybrids.

The clustering and admixture results show gene flow between some sled dogs and other modern dog breeds (Fig. 1C and figs. S6 to S8). We further explored this by comparing pairs of sled dogs with Zhokhov using D statistics

(Fig. 2A). Although pairs of Greenland sled dogs are symmetrically related to Zhokhov ($D\sim0$), indicating a lack of admixture, comparisons involving non-Greenland sled dogs were not always consistent with the null hypothesis of no admixture. D-statistics and admixture analyses (Fig. 2B and fig. S13) indicated that non-Greenland sled dogs carry ancestry from non-sled dogs and that Greenland sled dogs are the least admixed. These results imply that Greenland sled dogs have largely been kept isolated from contact with other dog breeds, and that their lineage traces more genomics ancestry to Zhokhov-like dogs relative to other dog breeds. Isolation of Greenland sled dogs was supported by inference of their historical effective population size (fig. S16), which

showed that these dogs had a relatively stable population size until a severe bottleneck ~850 years ago. The timing of the bottleneck is consistent with the colonization of Greenland by Inuit (9), suggesting isolation in Greenland ever since.

Numerous generations of sled dogs living in the Arctic environment and being used as draft animals may have provided a unique selection pressure to these dogs. To detect putative signals of positive selection, we used population branch statistics (PBS) (10) to scan for genomic regions highly differentiated in modern sled dogs relative to non-sled dogs (hereafter referred to as "other dogs") and wolves. We computed these statistics on modern genomes of 17 sled dogs, 61 other dogs,
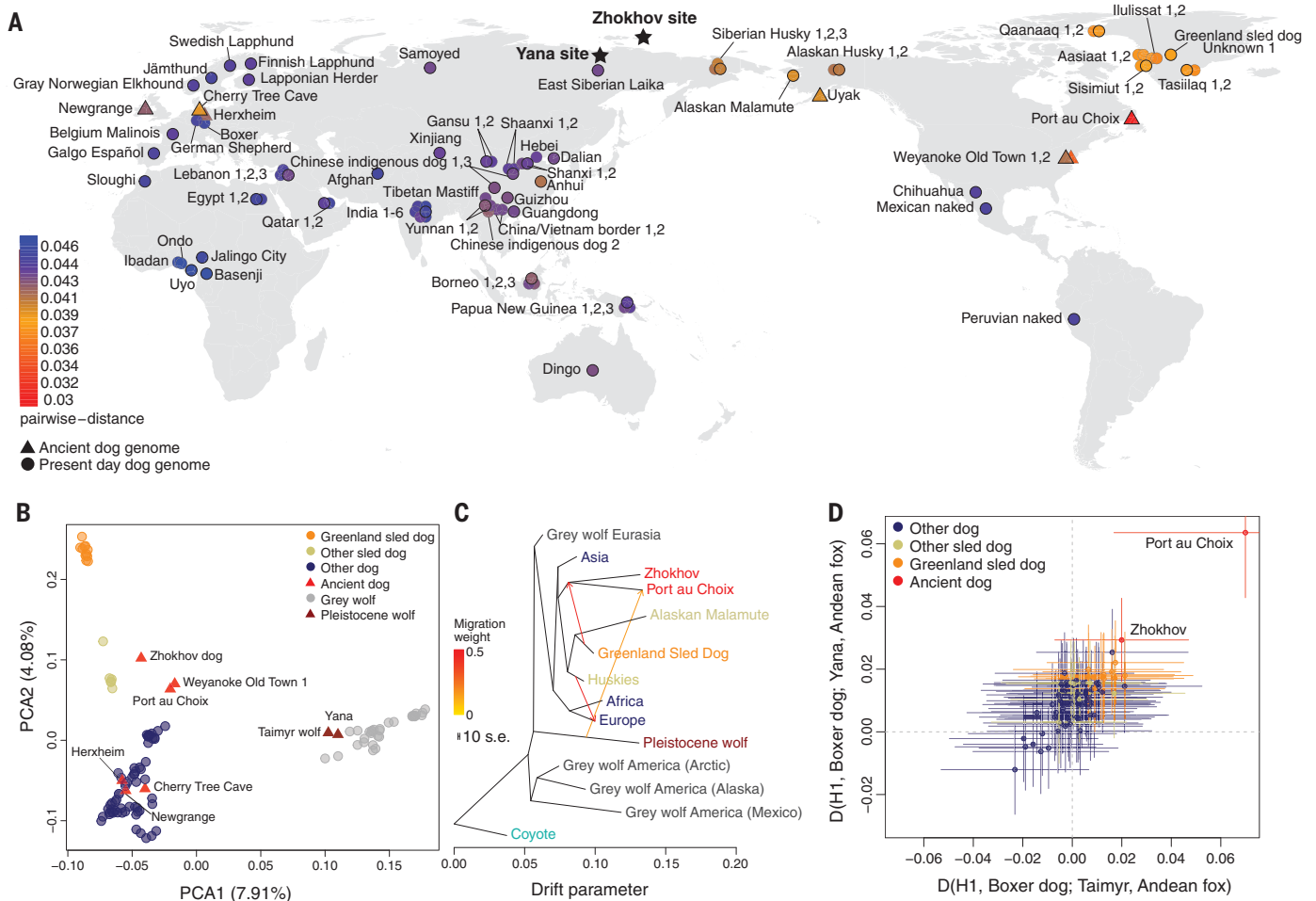

Fig. 1. Geographic location of the samples and overall genetic affinities. (**A**) Identity by state pairwise distances between Zhokhov and present-day dogs (table S1) of geographic affiliation of dogs and archaeological sites. Color scale indicates genetic distance between Zhokhov and each sample. Circles and triangles represent modern and ancient dogs, respectively. Stars show Zhokhov and Yana sites. (**B**) Principal component analysis (PCA) using whole-genome data (2,200,623 transversion sites) on all samples. (**C**) TreeMix admixture graph built using whole-genome data (766,082 transversion sites) on a dataset consisting of 66 canids merged into 15 groups according to their geographic location and admixture profile (table S1 and fig. S6). Colors indicate main groups as in (B). Arrows show inferred admixture edges colored by migration weight. (**D**) D statistic of the form D(H1, boxer dog; Taimyr or Yana, Andean fox) testing for Pleistocene wolf gene flow in ancient and modern dogs and whether samples share more alleles with Taimyr (x-axis) or Yana (y-axis) wolves when compared with the boxer dog. Color indicates the type of sample in H1. Points show the D statistic, and horizontal and vertical lines show 3 SEs for the test with the Taimyr (x-axis) and Yana (y-axis), respectively. The results obtained from both ancient wolves fall along the diagonal, suggesting that they are symmetrically related to all dogs.

and 30 wolves (table S1). A sliding window analysis revealed several genomic regions with high PBS values, hinting at selection in sled dogs (Fig. 3A). We took an outlier approach and focused on the most extreme values of the empirical distribution (above the 99.95th percentile). For each of these outlier regions (table S4), we identified overlapping genes and compared haplotypes across samples.

Enrichment analysis (4) on genomic regions with high PBS values (above the 99.95th percentile) identified three gene ontology (GO) terms that were overrepresented (table S6): γ-aminobutyric acid secretion (GO: 0014051, $p = 0.119$), calcium ion import (GO: 0070509, $p = 0.119$), and calcium ion transmembrane transport (GO: 0070588, $p = 0.382$). To investigate further, we focused on eight genomic regions that are highly differentiated in sled dogs and three regions where other dogs differ from sled dogs and wolves (Fig. 3A and fig. S18), and validated the autosomal regions with a cross-population composite likelihood ratio statistic (5) (fig. S21). In the differentiated regions, we focused on two sets of genes: those in which Zhokhov carries the same haplotype as modern sled dogs and those involved in adaptation to different diets.

$TRPC4$ is highly differentiated in sled dogs, and the putatively selected haplotype bears a marked similarity to Zhokhov (Fig. 3, A and B). $TRPC4$ is a transient receptor potential (TRP) channel protein that plays an important role in vasorelaxation and lung microvascular permeability (11). It is also involved in a temperature sensitivity pathway (12, 13), where it interacts with $TRPV2$, which is also highly differentiated in sled dogs (99.8th PBS percentile; table S4 and fig. S19A) and codes for temperature and potentially pain receptors (14). Several related thermo-TRP sensors in the same pathway, calcium ion transmembrane transport, have been previously reported to be under selection in cold-adapted woolly mammoths (15), which suggests convergent evolution in Arctic adaptation.

Another highly differentiated gene in sled dogs is $CACNA1A$ (Fig. 3, A and C), a calcium channel subunit that plays an essential role in skeletal muscle contraction (16). Further, $CACNA1A$ has been reported to be under positive selection in humans, specifically the Bajau sea nomads (17), where it is involved in hypoxia adaptation (18), indicating a possible role in managing exercise-induced hypoxia in sled dogs. We hypothesize that the $TRPC4$, $TRPV2$, and $CACNA1A$ genes are involved in

functions beneficial to physical activity in the Arctic. If so, given that the differentiated haplotypes are also found in Zhokhov (Fig. 3, A and B, and fig. S19A), any advantages that they confer would have been important to dogs in the Arctic ~9500 YBP.

Most domestic dogs are adapted to starch-rich diets through marked increases in $AMY2B$ copy numbers and strong positive selection for a dog-specific $MGAM$ haplotype (19). Consistent with previous findings (20), we observed that sled dogs carry substantially fewer $AMY2B$ copies than other dog breeds (fig. S20). We also found that $MGAM$ and $AMY2B$ are the regions of the genome with the lowest PBS, suggesting high differentiation of other dogs relative to sled dogs and wolves (Fig. 3A). Because negative PBS can arise under different demographic scenarios, we confirmed these observations by computing PBS with other dogs as the focal population (fig. S18). Indeed, modern sled dogs and Zhokhov are among the only dogs in our dataset that carry the ancestral $MGAM$ haplotype found at high frequency in wolves (Fig. 3C and fig. S18). Therefore, our observations suggest that sled dogs do not carry the genetic adaptations to starch-rich diets seen in other dog breeds.
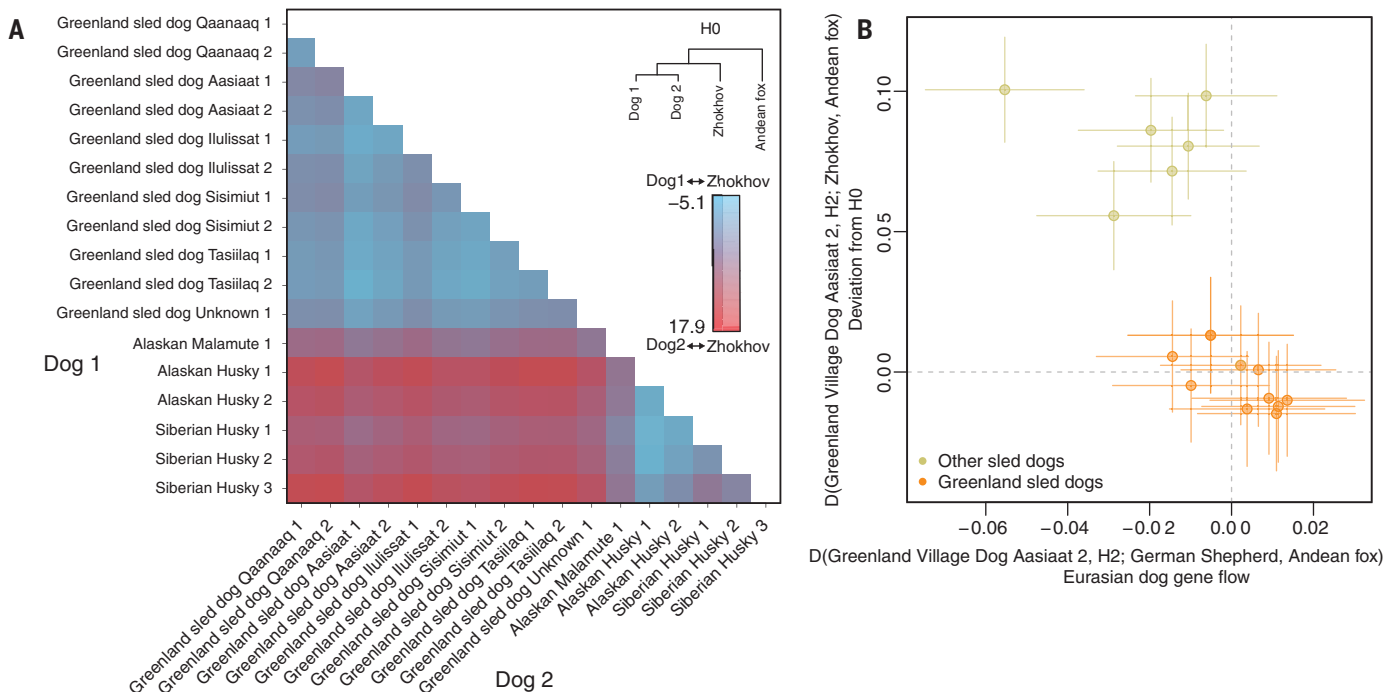
Fig. 2. Relationships between Zhokhov and present-day sled dogs. (**A**) D statistics testing the relationships between pairs of sled dogs and Zhokhov. Cell colors indicate the Z scores obtained from the test D(dog1, dog2; Zhokhov, Andean fox), where dog1 and dog2 are all possible pairs of sled dogs. Comparisons involving pairs of Greenland sled dogs and non-Greenland sled dogs resulted in significant deviations from H0 ($|Z| > 3$). (**B**) D statistics showing that sled dogs that are significantly further from Zhokhov compared with Greenland sled dog Aasiaat 2 [y-axis: D(Greenland sled dog Aasiaat 2, H2; Zhokhov, Andean fox)] also show evidence of significant gene flow from other dogs [x-axis: D(Greenland sled dog Aasiaat 2, H2; German shepherd dog, Andean fox)]. Points indicate the D statistic, and horizontal and vertical lines indicate 3 SEs for the x-axis and y-axis, respectively. We consider the test to be significant for gene flow when these lines do not overlap with the dotted line ($|Z| > 3$).

Fig. 3. Adaptation.
(**A**) Manhattan plot of the PBS values (*y*-axis) in windows of 100 kg base pairs (kb) using a 20-kb slide across chromosomes (*x*-axis). Data points between the 20th and 80th percentile of the empirical distribution are not plotted and dashed red lines show the 99.95th and 0.05th percentiles. Names of genes within the highest peaks are shown, with asterisks representing no overlap with genes. We note that other genes not displayed in the figure can overlap the outlier regions; a full list can be found in tables S4 and 5.
(**B** to **D**) Haplotype structures for TRPC4 (B), CACNA1A (C), and MGAM (D). Rows represent individuals, and columns represent polymorphic positions in the dog genome. Cells are colored by genotype:

Dark gray indicates that the alternative allele is homozygous, light gray that it is heterozygous, and white that the reference allele is homozygous. The row height for ancient individuals was increased to facilitate visualization. Zhokhov is highlighted with a red asterisk. SDs, sled dogs.
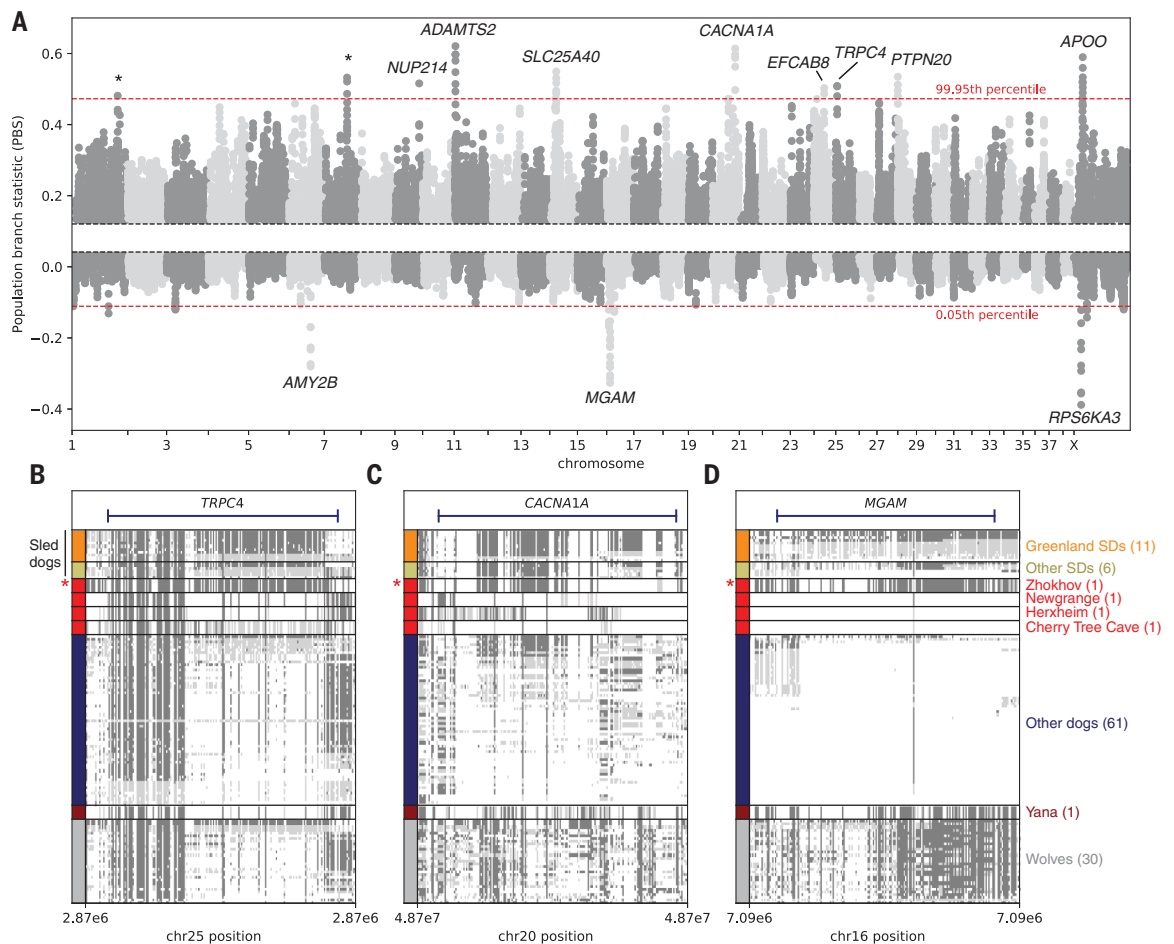
By contrast, sled dogs harbor specific haplotypes of genes involved in coping with a high intake of fatty acids. *SLC25A40*, a mitochondrial carrier protein involved in clearing triglycerides from the blood (*21*), and *APOO*, an apolipoprotein gene involved in regulating high levels of fat and fatty acid metabolism (*22*), are both highly differentiated in sled dogs (Figs. 3A). The derived haplotypes of both genes are absent in Zhokhov, indicating that the haplotypes are specific to modern sled dogs and postdate their common ancestors with Zhokhov (fig. S19, B and E). As another example of convergent evolution, another gene of the apolipoprotein family, *APOB*, is reported to be under selection in polar bears, possibly as a result of adaptation to fat-rich diets and clearance of cholesterol from the blood (*23*). Overall, similar adaptations to high intake of fatty acids have been described in Inuit and other Arctic human populations (*24*, *25*), so our observations suggest that sled dogs adapted to a fat-rich and starch-poor diet, echoing the dietary adaptations of the Arctic human cultures with whom they coexisted.

Bone composition of polar bears and reindeer consumed at the Zhokhov site indicate an extensive hunting range and transport of large body parts back to camp (*26*). Further, abundant obsidian tools found at the site reveal movement of obsidian from ~1500 km away (*3*). Together, these findings indicate substantial long-distance travel and transportation of resources, in which dog sledding would have been highly advantageous—if not necessary. Putative sled remains and our genomic analyses of a 9500-year-old dog from the Zhokhov site indicate that the traditions and key genomic variations that define modern sled dogs were established in the northeast Asian Arctic >9500 years ago. Our results imply that the combination of these dogs with the innovation of sled technology facilitated human subsistence since the earliest Holocene in the Arctic.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S21
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Science

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Sled dog arctic adaptations go far back
    Dogs have been used for sledding in the Arctic as far back as ~9500 years ago. However, the relationships among the earliest sled dogs, other dog populations, and wolves are unknown. Sinding *et al.* sequenced an ancient sled dog, 10 modern sled dogs, and an ancient wolf and analyzed their genetic relationships with other modern dogs. This analysis indicates that sled dogs represent an ancient lineage going back at least 9500 years and that wolves bred with the ancestors of sled dogs and precontact American dogs. However, gene flow between sled dogs and wolves likely stopped before ~9500 years ago.
    Science, this issue p. 1495

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