The evolutionary history of extinct and living lions

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Lions are one of the world's most iconic megafauna, yet little is known about their temporal and spatial demographic history and population differentiation. We analyzed a genomic dataset of 20 specimens: two *ca.* 30,000-y-old cave lions (*Panthera leo spelaea*), 12 historic lions (*Panthera leo leo/Panthera leo melanochaita*) that lived between the 15th and 20th centuries outside the current geographic distribution of lions, and 6 present-day lions from Africa and India. We found that cave and modern lions shared an ancestor *ca.* 500,000 y ago and that the 2 lineages likely did not hybridize following their divergence. Within modern lions, we found 2 main lineages that diverged *ca.* 70,000 y ago, with clear evidence of subsequent gene flow. Our data also reveal a nearly complete absence of genetic diversity within Indian lions, probably due to well-documented extremely low effective population sizes in the recent past. Our results contribute toward the understanding of the evolutionary history of lions and complement conservation efforts to protect the diversity of this vulnerable species.

lion | genomics | evolution

Until recently, the lion (*Panthera leo*), was one of the most widely distributed terrestrial mammals. As an apex predator, lions have important ecological impacts and have featured prominently in human iconography (1). During the Pleistocene, lions ranged over an enormous geographic expanse. This included modern lions (*Panthera leo leo*) in Eurasia, the cave lion (*Panthera leo spelaea*) in Eurasia, Alaska, and Yukon, as well as the American lion (*Panthera leo atrox*) in North America. At present, their range is mostly restricted to Sub-Saharan Africa, along with one small, isolated population of Asiatic lions in the Kathiawar Peninsula of Gujarat State in India. The global decline of lion populations started with the extinction of the cave and American lions in the Late Pleistocene, *ca.* 14,000 y ago (2). More recently, modern lion populations have disappeared from southwestern Eurasia (19th and 20th century) and North Africa (20th century) (Fig. 1*A*), likely as a result of anthropogenic

factors (3, 4). In the last 150 y, this decline has resulted in the extinction in the wild of the Barbary lion in North Africa, the Cape lion in South Africa, lion populations in the Middle East, and has led to increased fragmentation and decline of all of the remaining populations.

Although the global genetic structure of lions, including the relationship between the extant and extinct lineages, has been explored in previous studies, these inferences were based on mitochondrial DNA (mtDNA) data (5–8), or a limited number of mitochondrial and autosomal markers (9, 10). Here, we expand on these prior findings with whole-genome resequencing data from a set of modern, historic, and Pleistocene lions, including representatives from both their current and former distributions. Particularly, we aimed to answer: 1) What phylogenetic relationships are found among modern and cave lions? 2) Is there

Significance

Lions were once the most globally widespread mammal species, with distinct populations in Africa, Eurasia, and America. We generated a genomic dataset that included 2 extinct Pleistocene cave lions, 12 lions from historically extinct populations in Africa and the Middle East, and 6 modern lions from Africa and India. Our analyses show the Pleistocene cave lion as maximally distinct with no evidence of hybridization with other lion groups based on the level of population structure and admixture. We also confirm long-term divisions between other extant lion populations and assess genetic diversity within individual samples. Our work provides views on the complex nature of the global lion species-complex and its evolution and provides conservation data for modern lion regional populations.

any modern lion population genetically closer to the extinct cave lion? 3) When did the different modern lion lineages start to diverge? And 4) how does their past genetic diversity compare to that found today?

Results and Discussion

Lion Dataset and Genome-Wide Phylogeny. We generated whole-genome sequences from 20 ancient and modern individuals. These included 2 cave lion specimens from Siberia and Yukon that have both been radiocarbon dated to *ca.* 30,000 ^{14}C years before present, which were sequenced to an average depth of coverage of 5.3-fold and 0.6-fold, respectively (*SI Appendix,* Table S1). Our modern lions are represented by 12 historical *Panthera leo* samples that were collected between the 15th century and 1959, whose genome coverages range from 0.16-fold to 16.2-fold (*SI Appendix,* Table S1). The geographic distribution of these samples broadly covers the historical range of modern lions, including regions where they are currently extinct (North Africa, the Cape Province of South Africa, and Western Asia) (Fig. 1*A*). We completed our dataset with 6 wild-born present-day samples from Eastern and Southern Africa (*n* = 4) and India

(*n* = 2), as well as 2 previously published whole-genome sequences of Sub-Saharan African lions collected from a zoo (11).

To obtain an overview of the relationships among samples with special references to previous results, we built a phylogenetic tree based on the genome-wide pairwise divergences between individuals (Fig. 1*B*). In concordance with previous analyses based on mtDNA (5–8), cave lions were monophyletic and a clear outgroup to all modern lions. Within modern lions, we detected two lineages consisting of 1) a northern lineage comprised by Asiatic, North African, and West African lions and 2) a southern lineage comprised by Central, East, and South African lions (Fig. 1*B*). This partition within modern lions is largely consistent with patterns detected using mtDNA (12–14) and combined mtDNA/autosomal markers (9, 10). However, our genome-wide dataset revealed some important differences and details. First, mtDNA data have consistently clustered Central African lions with the northern group (a pattern that is maintained in our mtDNA phylogeny, *SI Appendix,* Fig. S1). In contrast, our analyses of whole genome sequencing (WGS) data grouped Central African lions with populations of the southern lineage (Fig. 1*B*). In addition, an analysis of local genealogies across the genome also supported this topology (*SI Appendix,* Fig. S19), as well as the phylogenetic signal in regions of low recombination (*SI Appendix,* Fig. S21), loci that are particularly useful to generate phylogenies in highly admixed lineages, such as cat species (15).

Similarly, mitochondrial data from this study (*SI Appendix,* Fig. S1) and previous studies (12, 14) suggested that extinct North African lions shared a more recent common ancestor with Asiatic, rather than West African lions, which is incongruent with genome-wide data strongly linking North African lions with West African lions (Fig. 1*B*). Such inconsistencies between mtDNA and nuclear DNA datasets are not unusual in the Felidae (9, 16), and may reflect the stochastic sorting of a single nonrecombining marker such as mtDNA, and/or a pattern of sex-biased population connectivity.

Divergence between Cave and Modern Lions. We next leveraged on the power of whole-genome data to estimate when the major lion clades diverged from each other. Previous studies based on partial fragments of the mitochondrial genome have estimated
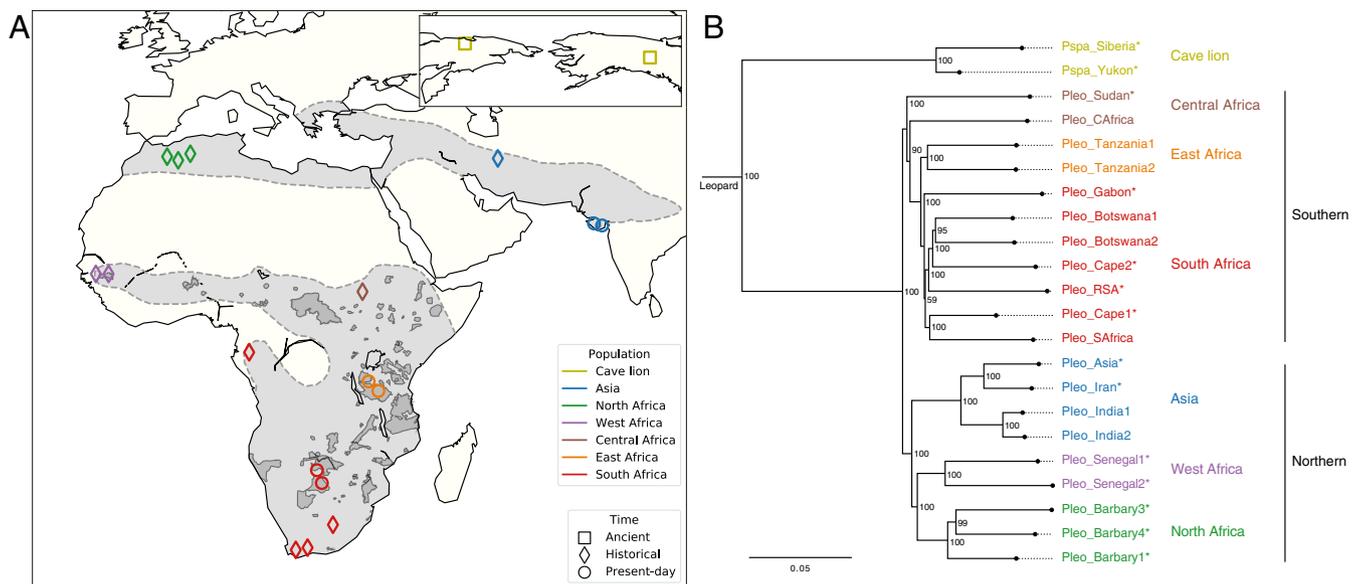

Fig. 1. (*A*) Map indicating approximate sites of origin for the lion samples. Individuals with country of origin are placed in the centroid coordinates. Random jitter is applied to help visualize samples sharing an origin. The dashed line and light-gray shading indicate the approximate historical distribution of modern lions, while dark-gray areas show their present-day distribution. (*B*) Neighbor-joining tree from pairwise genetic divergence of lion genome sequences rooted with leopard. Ancient and historical samples are highlighted with an asterisk. Support values from 100 bootstrap replicates are given inside each node.

that cave and modern lions diverged *ca.* 500,000 y ago, using the appearance in the fossil record of the ancestral cave lion *Panthera fossilis* (5, 6). More recently, the divergence time was estimated to be 1.89 million years ago using full mitochondrial sequences and multiple fossil constraints (8). To investigate these discrepancies among estimates, and to fully resolve the position of cave lions in the phylogeny, we applied three independent methods that leverage the power of whole-genome sequences and do not rely on fossil record calibrations to estimate the divergence time between cave and modern lions.

First, we investigated the split time between the ancestral populations by estimating the probability F ($A_{derived}|B_{heterozygous}$) (17) of an individual A (such as the Siberian or Yukon cave lion) carrying a derived allele discovered as a heterozygote in a modern lion individual B. This summary statistic was then used to estimate the divergence time of the cave lion lineage given a model of population history in modern lions inferred using the pairwise sequential Markovian coalescent (PSMC) (18). We estimated that F(Cave lion|Modern lion) averaged at ∼0.15 (Fig. 2*A*), meaning that cave lions carry the derived allele in ∼15% of the heterozygous sites detected in modern lions (14.7 to 16.4%; *SI Appendix*, Table S2). From a simulation of the expected distribution pattern of F(Cave lion|Modern lion), given the population history of modern lions, a mutation rate (μ) of 4.5×10^{-9} per generation (11), and a generation time of 5 y (19), we estimated that both lineages diverged *ca.* 470,000 y ago (Fig. 2*A*; 392,000 to 529,000 y ago, *SI Appendix*, Table S3).

We further explored the divergence time between cave and modern lions by exploiting the fact that the Siberian cave lion

was a male (*SI Appendix*, Table S5). Male X chromosomes can be used to construct synthetic pseudodiploid genomes and estimate rates of coalescence between their ancestral populations. Since the effective population size (Ne) is inversely correlated with the amount of coalescing events occurring at a particular point in time, the Ne inferred from a pseudodiploid chromosome of two diverged populations should suddenly increase around the time of their divergence, as no coalescent events can happen after this estimated time assuming reproductive isolation occurred after the split. Indeed, we inferred from the PSMC of cave and each modern lion combined X chromosomes that there was a sharp increase in Ne to an unmeasurably large size *ca.* 495,000 y ago (Fig. 2*B*; 460,000 to 578,000 y ago, *SI Appendix*, Table S6). However, we caution that there may be added uncertainty around this estimate due to the low depth of coverage in the X-chromosome of the Siberian cave lion (1.96-fold, *SI Appendix*, Table S5).

We also assessed estimates of sequence divergence (d_{xy}), which should be directly related to the split time (T) and the Ne of the common ancestor (Ne$_{anc}$) ($d_{xy} = 2T\mu + 4N_{anc}\mu$). We found that the mismatch rate between the Siberian cave lion and modern lions using only transversions was an average $d_{xy} = 0.00067$ (*SI Appendix*, Fig. S6). Assuming that $\mu = 4.5 \times 10^{-9}$ per generation (11), a transition/transversion ratio of 1.9 (SI section 4), a branch shortening in the Siberian cave lion of 6,000 generations (*SI Appendix*, Table S1) and Ne$_{anc}$ of 55,000 individuals (Fig. 3*A*), we obtained a split time of *ca.* 108,000 generations [540,000 y assuming a generation time of 5 y (19)]. Given the general congruence among our estimates, we conclude that the
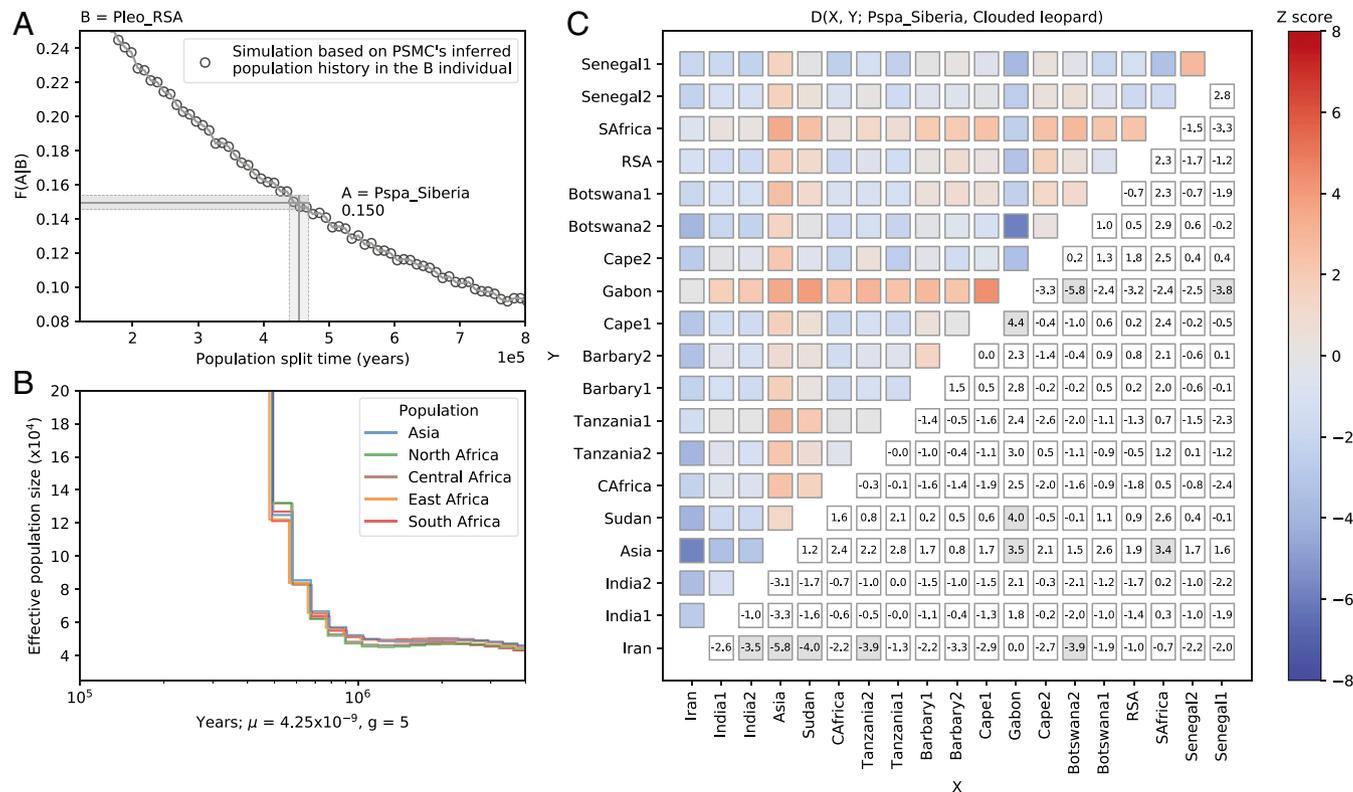
Fig. 2. (*A*) The probability F (A|B) of observing a derived allele in population A (Siberian cave lion) at a heterozygous site in population B (South African modern lion) is obtained by simulating the history of population B as inferred using the PSMC method. The vertical dotted lines indicate the split time range that encompasses the confidence interval of the observed F (A|B). (*B*) Population size history inferred using the PSMC method in pseudodiploid male X chromosomes of the Siberian cave lions and all other male modern lions with sufficient depth of coverage, assuming a male mutation bias of 1.4. × axis is in logarithmic scale. (*C*) All possible *D*-statistics tests with the population history {[(X:modern lion 1, Y:modern lion 2), Siberian cave lion], Clouded leopard}. Color of the cells above the diagonal represents the Z score of the test, with the values shown below the diagonal. Cells with |Z| > 3.3 are colored in gray (P ∼ 0.001).
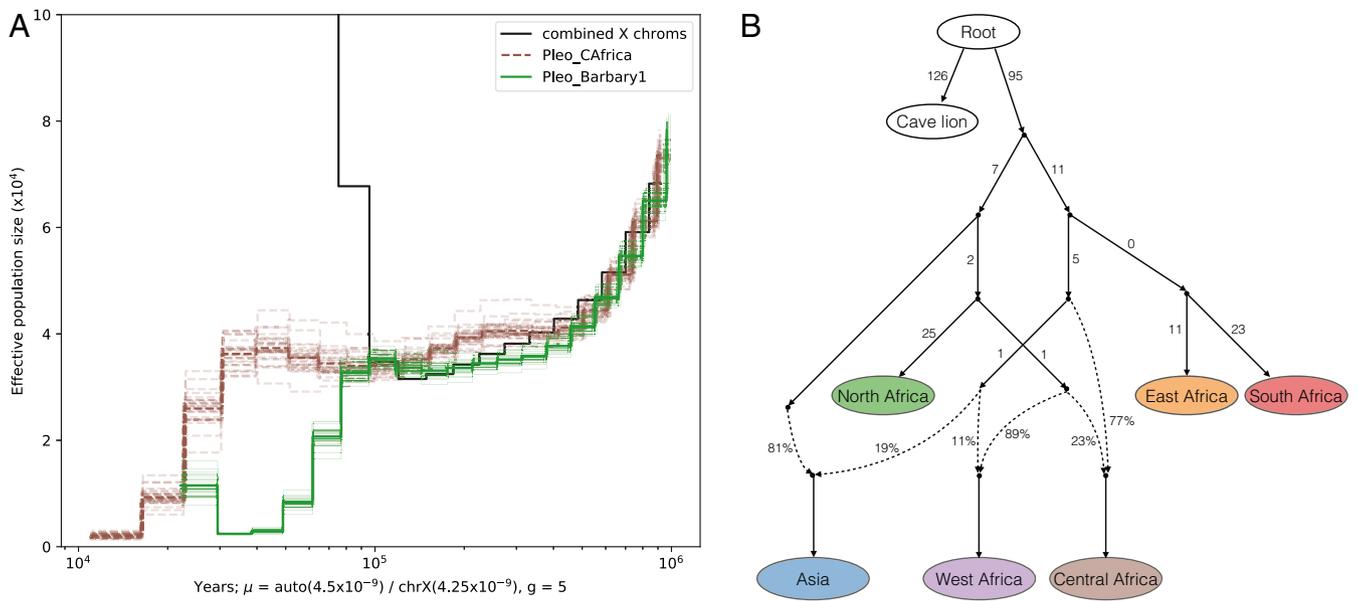
Fig. 3. (*A*) Population history of the northern (green) and southern (brown) modern lion lineages as inferred by the PSMC. The population history curve of the pseudodiploid chromosome X of the two individuals is shown in black, and the Ne was scaled by 0.75 to match the Ne in the autosomes. (*B*) Model of the phylogenetic relationships among lions augmented with admixture events. Branch lengths are given in drift units per 1,000. Discontinuous lines show admixture events between lineages, with percentages representing admixture proportions.

most likely split time between cave and modern lions is *ca.* 500,000 y ago. This estimate is also remarkably consistent with the early Middle Pleistocene appearance of *P. fossilis* in the European fossil record (5).

Gene Flow between Cave and Modern Lions. We next explored for evidence of gene flow between the cave and modern lions, which may have had a possible contact zone in southwestern Eurasia during the Pleistocene (20). To formally test relative relatedness of extant modern lion populations with cave lions, we employed the *D*-statistic (17, 21). Under the null hypothesis (*D* = 0, no gene flow between populations), all modern lions should be symmetrically related to cave lions under the following population history: {[(modern lion 1, modern lion 2), cave lion], clouded leopard}. We computed all possible combinations of this history, iterating over all modern lions and the two cave lion individuals. We found that the majority of tests involving the Siberian cave lion supported the null hypothesis (although only a few of these tests are marginally significant |Z| > 3.3, Fig. 2*C*), suggesting little or no gene flow between any extant modern lion lineage and Siberian cave lions. This is concordant with our estimates of F (A|B) and d$_{xy}$, which produced similar values across modern lion populations (*SI Appendix*, Table S3 and Fig. S6).

In contrast, estimated *D*-statistics with the Yukon cave lion suggested that it shared more alleles with South African modern lions than with other modern lion populations (*SI Appendix*, Fig. S7*B*). This result was unexpected given the results of the Siberian cave lion tests and the large geographic distance between South Africa and far northwestern North America. Nonetheless, we note that the current location of populations does not necessarily represent ancestral distribution patterns of lions hundreds to thousands of generations ago. Alternative complex scenarios not involving gene flow could also accommodate this observation (*SI Appendix*, section 9). We were, however, able to reproduce this result when we used a subset of the data so that the Siberian cave lion had a similar depth of coverage as the Yukon individual (*SI Appendix*, Fig. S7*D*). Thus, we believe the results are likely an artifact derived from the combination of: 1) the low depth of coverage in the Yukon cave lion (0.6-fold, *SI Appendix*, Table

S1), and/or 2) a bias in the calling of single nucleotide polymorphisms (SNPs) driven by the southern Africa descent of the reference individual (22, 23) (*SI Appendix*, section 9). We therefore conclude that there is no robust evidence for gene flow between the cave lion populations represented by our two samples and any of the modern lion lineages tested.

This observation is in stark contrast with the mounting evidence of interspecific hybridization in big cats (15, 24, 25). Although we cannot exclude the possibility of gene flow between the ancestors of all modern lions and cave lions, we hypothesize that the lack of admixture could have a plausible biological basis. For instance, cave lions and modern lions may never have been sympatric. Also, even if they had been, they might have been behaviorally or ecologically incompatible. For example, it has been suggested that male cave lions did not have the characteristic mane of male modern lions (1). Perhaps the possible lack of this notable secondary sexual character in male cave lions induced or strengthened behavioral (sexual) reproductive isolation between these forms. Other behavioral and ecological differences may have existed between the two lineages, including group-living and pride composition, which could also have played a role in reproductive isolation. For example, analyses of mtDNA from American and cave lions were consistent with a degree of reproductive isolation (6), suggesting that some form of competition may have also existed between these sister taxa.

Population History of Modern Lions. We also examined the population history of modern lions by performing principal component and population clustering analyses (*SI Appendix*, section 6). Results from both analyses highlight the distinctiveness of the northern and southern lineages (*SI Appendix*, Figs. S4 and S5), as well as the geographic population subdivision observed in our genome-wide phylogeny (Fig. 1*B*). To estimate the divergence date between modern lion populations, we performed identical analyses to those applied in our cave vs. modern lion split time estimation (F (A|B) and PSMC in pseudodiploid male X chromosomes). The deepest divergence within modern lions was between the northern and southern lineages (Fig. 1*B*), which shared an ancestor *ca.* 70,000 y ago (52,000 to 98,000 y ago; *SI*

Appendix, Table S3 and Fig. S9). This date is consistent with previous estimates based on mtDNA sequence variation (5, 14), although slightly younger than prior estimates based on autosomal markers (9). Interestingly, through a PSMC analysis of the individuals from the northern and southern groups, we inferred a sudden decline in Ne in the northern genetic lineage at roughly the same time as the split between the 2 groups (*ca.* 70,000 y ago, Fig. 3*A*). This severe population bottleneck in the northern genetic lineage suggests that regions north of the Sahara were populated by only a few migrants from the southern lineage at some point in the Late Pleistocene (1, 9, 14).

However, a closer inspection of the divergence within modern lions revealed that a phylogenetic tree such as the one in Fig. 1*B* does not capture the full complexity of their evolutionary history. For instance, while the Central African lions in our dataset belong to the southern lineage (Fig. 1*B*), they consistently show more recent split times to northern lions than the rest of southern populations (*SI Appendix*, Table S3 and Fig. S9). This is concordant with the mixed affiliation of Central African lions in the autosomal and mtDNA phylogenies (Fig. 1*B* and *SI Appendix*, Fig. S1), and strongly suggests that Central African lions carry substantial amounts of both northern and southern ancestry. In fact, a recent study using whole-genome data has suggested that Central African lions belong to the northern clade, further highlighting the complex phylogenetic position of this population (26).

To test for evidence of admixture, we computed *D*-statistics among all modern lion populations and integrated the observed signals into a single historical model using *qpGraph* (27). These results support the hypothesis that Central African lions share significantly more alleles with Asiatic lions than Eastern and Southern African populations do (|Z| > 5; *SI Appendix*, Fig. S10), and harbor an estimated 23% of northern-related ancestry (Fig. 3*B*; 22.96 ± 0.17% *SI Appendix*, section 10). In addition, we found that West African lions share more alleles with the southern lineage than North African lions do (|Z| > 5; *SI Appendix*, Fig. S11), and that *ca.* 11.4% of their genome is composed by southern-related ancestry (Fig. 3*B*; 11.38 ± 0.14% *SI Appendix*, section 10). This gene flow among populations is illustrated by one of the Senegalese lions, which carries a large amount of "southern alleles," and probably had an ancestor with mixed ancestry in the recent past (*SI Appendix*, Fig. S11). Altogether, these signals support a scenario in which west-central Africa was a "melting pot" of lion ancestries, where the southern and northern lion lineages plausibly overlapped and admixed after their earlier isolation ~70,000 y ago.

Interestingly, we also detected a greater extent of allele sharing between Asiatic lions and the southern lineage compared to North African lions (|Z| > 5; *SI Appendix*, Fig. S12), with as much as 18.5% of the Asiatic lions ancestry coming from a southern population (Fig. 3*B*; 18.56 ± 0.13% *SI Appendix*, section 10). This finding is surprising given the current large geographic distance between these populations. However, migration corridors between Sub-Saharan African and the Near East may have existed in the past, for instance through the Nile basin in the early Holocene (12). Under this hypothesis, we speculate that North African lions were isolated from such secondary contact with southern populations due to the significant geographical barriers represented by the Atlas Mountains and the Sahara desert. Another (nonexclusive) possibility that could mirror the effect of gene flow between Asiatic lions and the southern lineage could be admixture between North African lions and an extinct "ghost" lion population. However, further sampling of North African lions would be required to comprehensively test this hypothesis.

Inbreeding in Lions. Our sampling scheme allowed us to explore how lion genetic diversity has changed through time and space, a

particularly powerful approach to characterize population histories and quantify genetic threats in endangered species (28). To do so, we estimated autosomal heterozygosity and produced diploid genotypes for the 16 lions with a depth of sequencing coverage above fourfold (*SI Appendix*, Table S1). In order to avoid biases due to postmortem DNA damage (29, 30) (*SI Appendix*, Fig. S3), we restricted the heterozygosity calculation to transversions, a strategy that accurately recapitulates the underlying diversity using all polymorphisms (*SI Appendix*, Fig. S16). To identify runs of homozygosity (ROHs), we also scanned the genomes of the 16 individuals in sequence windows of 500 kbp with a slide of 200 kbp, and devised a method to collapse neighboring autozygous segments into continuous ROHs (*SI Appendix*, Fig. S14 and section 11).

The average autosomal heterozygosity of the Siberian cave lion was within the range observed in modern lions (*SI Appendix*, Fig. S8). The proportion of genome sequence in ROHs was among the lowest seen in our dataset (9.6%; Fig. 4*B* and *SI Appendix*, Table S7). These results are perhaps surprising, given that previous studies based on mtDNA suggested that there was a strong population bottleneck in cave lions between 47,000 and 18,000 y ago (6, 7), a time interval encompassing the age of our Siberian specimen (30,870 ± 240 y; *SI Appendix*, Table S1). Nonetheless, previous studies (6, 7) may have underestimated the amount of distinct cave lion lineages existing at that time, something that could explain our findings if the Siberian cave
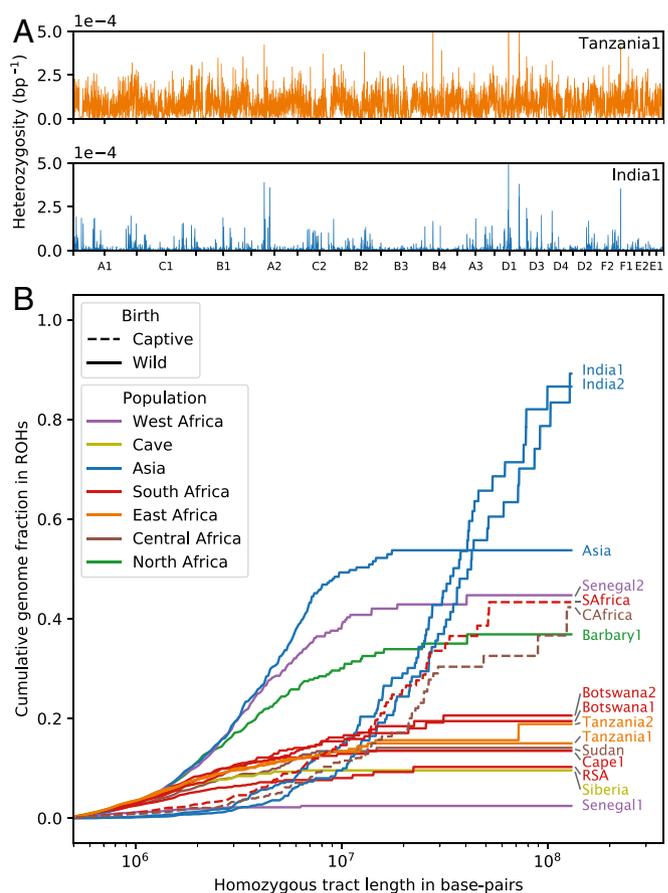

Fig. 4. (*A*) Heterozygosity in sequence windows of 500 kg-base pairs in a Tanzanian (orange) and Indian (blue) modern lions across the domestic cat assembly (all chromosomes are concatenated in the x axis). (*B*) Cumulative proportion of the genome contained in ROH below the length displayed on the x axis.

lion sampled here belonged to a population that was not subjected to strong population bottlenecks.

As expected from the results of our PSMC analyses of modern lions (Fig. 3*A*), we found that the mean autosomal heterozygosity per base pair in the southern lineage (7.8 to 11.6×10^{-4}; *SI Appendix*, Table S7) is higher than in the northern lineage (0.7 to 7.5×10^{-4}; *SI Appendix*, Table S7), with the exception of the admixed Senegalese lion (12.6×10^{-4}; *SI Appendix*, Table S7). Similarly, the northern populations carry more, and on average longer, ROHs than the southern populations (*SI Appendix*, Table S7), although lions born in captivity were an exception in that they displayed hallmarks of recent inbreeding (excess of ROHs of 10 to 100 mega base pairs in length, Fig. 4*B*). Altogether, this is consistent with a population history of consecutive bottlenecks in the northern lineage as their ancestors migrated away from Sub-Saharan Africa and persisted in more isolated smaller populations.

In addition (and nonexclusively), smaller population sizes in northern lions may also be due to sustained anthropogenic pressure, as there is evidence of a possible correlation between the level of inbreeding and the temporal extent of range overlap with large human civilizations (e.g., Indus Valley and Mesopotamia in Asia; ancient Egypt and the Greco-Roman empires in North Africa). Moreover, microsatellite data have indicated that genetic diversity of lions in some countries in southern Africa has significantly decreased over the 20th century, coinciding with the rise of a European colonial presence (31). Indeed, our genome-wide data also suggested recent increases in rates of inbreeding in the southern lineage (*SI Appendix*, Fig. S15). The four present-day wild-born individuals had on average a 49% increased fraction of the genome in homozygosity compared with the three historical samples (*SI Appendix*, Table S7). Nonetheless, these results must be interpreted with caution, since the historical specimens sampled here may not be direct ancestors of the contemporary individuals, and the number of samples analyzed here are too low to confidently rule out that elevated sampling numbers may affect the observations.

As expected given previous studies (32–34), the most extreme reduction of genomic diversity was found in present-day Indian lions, which had a 16-fold reduction in heterozygosity compared to lions in southern Africa (Fig. 4*A*). In contrast, it appears that lions in North Africa maintained a heterozygosity comparable to the present-day southern lions even within *ca.* 100 y from their extinction. We found that *ca.* 90% of the Indian lion genomes fall in ROHs, with a considerable amount of homozygous tracts extending for more than 50 mega base pairs (Fig. 4*B*). Furthermore, both Indian lions are nearly identical, differing at fewer than three sites every 10 kg base pairs. This remarkable absence of genomic diversity in Indian lions is consistent with their recorded strong population decline after the 18th century, mostly mediated by the advance of agriculture, the increased use of firearms, and other familiar companions of human population (4).

These factors brought the Indian lion population nearly to extinction, with individual counts as low as 20 in the Kathiawar Peninsula by the beginning of the 20th century (35). Interestingly, the historical Asiatic lion genome shows higher heterozygosity (53% of the genome in ROHs, Pleo_Asia in Fig. 4*B*), and ROHs tend to be shorter than those found in present-day Indian lions (*SI Appendix*, Table S7), consistent with older population bottlenecks around 1,000 to 4,000 y ago (34). It is tempting to speculate that our historical sample predates the extreme bottleneck suffered by Asiatic lions by the beginning of the 20th century. However, as no accurate geographical information exists for the origin of this museum sample, interpretations about the timing and place of the lion population decline in Asia remain elusive until further sampling is performed.

Similar footprints of extensive inbreeding have been reported in isolated carnivore populations (36, 37). Inbreeding depression can compromise the survivability of populations through an increase of strongly deleterious mutations in homozygosity (38). To test whether small population size and inbreeding have led to an accumulation of deleterious mutations in Indian lions, we assessed the effects of DNA sequence variants using SIFT (The Sorting Tolerant From Intolerant algorithm) (39), which estimates whether missense mutations are likely to be damaging by assessing evolutionary constraint in homologous protein alignments. We found that Indian lions carry on average 12.7% more deleterious mutations in homozygosity (*SI Appendix*, Fig. S17 and Table S8), implying a substantial genetic load, particularly if deleterious variants are recessive. These findings are consistent with reports of reduced sperm mobility, low levels of testosterone, and cranial defects in Indian lions due to extensive inbreeding (33).

Additionally, to more directly assess the efficacy of selection, we examined the ratio of homozygosity between missense deleterious mutations and synonymous mutations. This ratio is predicted to be elevated in small populations, since deleterious alleles can increase in frequency under strong drift and weakened selection (40). Indeed, we found that this ratio is significantly higher in Indian lions than in African lions (*SI Appendix*, Fig. S18), consistent with a relaxed efficacy of selection.

Implications for Conservation. Historically, up to 11 subspecies of modern lions have been recognized (41). In 2017, the number was reduced to two in light of the results of molecular studies (42): 1) *P. leo*; in Asia, West Africa, and Central Africa and 2) *P. leo melanochaita*; in East and South Africa. Here, we show that although Central African lions cluster with *P. leo leo* in mtDNA-based phylogenies (*SI Appendix*, Fig. S1), their genome-wide ancestry shows higher affinity with *P. leo melanochaita* (Fig. 1*B* and *SI Appendix*, Fig. S19). Therefore, our results suggest that the taxonomic position of Central African lions may require revision. We caution, however, that our data are based on genome-wide data from a single wild-born Central African lion (*SI Appendix*, Table S1), and a recent study using whole-genome and microsatellite data suggests that Central African lions from the Democratic Republic of Congo (DRC) and Cameroon preferentially cluster with *P. leo leo* (26). In addition, gene flow in Central and West Africa may have been common in the past (Fig. 3*B*): both lineages probably lived in sympatry for long periods of time and their genetic divergence is not high. In any case, further studies should increase sampling from West and Central Africa to fully resolve this issue.

Our results also provide insights into the now extirpated Cape and Barbary lion populations, both of which have been argued to represent distinct groups. Based on its morphology (the big black mane of males) the Cape lion was considered a unique lion population/subspecies exclusively found in the southern part of the Republic of South Africa (43). However, more recently (44), evidence based on mtDNA data suggested that the Cape lion might not have been phylogenetically unique. Our genome-wide data support this finding, and place Cape lions within the genetic diversity found in South African lions (Fig. 1*A*). In addition, the restoration of the extinct North African Barbary lion has attracted the attention of conservationists, both inside and outside North Africa (45). Although circumstantial evidence suggested that North African lions could have survived in captivity, the most likely descendants of wild Barbary lions from the Moroccan Royal Menagerie have appeared to be of Central African maternal descent (44). Studies based on mtDNA argued that the North African lion could be restored using the most closely related extant population, the Indian lions (14). However, we show that, while Indian and North African lions are closely related based on mtDNA (*SI Appendix*, Fig. S1), genome-wide data reveal West African lions to be the most-closely related lineage (Fig. 1*B*). Thus, we conclude that any scope for restoration of the North African lion should consider the West African as a better "donor" population than the Indian lion.

Lastly, our results may be useful in light of conserving the remaining wild lions. For example, in the context of Africa, we hope that future studies may be able to build on to our data to explore how diversity has changed in the populations through time; for example, through quantifying genomic erosion (28). Furthermore, they may be relevant to the Indian subcontinent, where today lions are only found around the Gir Forest on the Kathiawar Peninsula of Gujarat. First, consistent with previous publications (34, 46), we found no evidence to support the recent claim that the remaining population is not indigenous to the region, but instead were introduced from outside of India (47) as our Indian lions are clearly genetically distinct to the other sampled populations (Fig. 1*B*). Secondly, although conservation efforts are contributing to increasing population size after centuries of decline, their remarkable lack of genomic diversity suggests that they could be extremely susceptible to inbreeding depression and genetic erosion, as well as future pathogen outbreaks. Given that our data indicate they diverged from other extant populations in the northern lion clade *ca.* 30,000 y ago (*SI Appendix*, Fig. S9), one future action to consider would be boosting their genetic diversity through outbreeding with such lions. However, in this regard, we are fully aware that this strategy would be both politically challenging, and, in light of recent observations on the effect of genetic introductions in the Isle Royale wolves (37), not guaranteed to be beneficial, so such decisions should not be taken lightly.

Materials and Methods

Summary of Methods. We generated a resequenced genomic dataset using Illumina and BGISeq sequencing technology, that included 2 extinct Pleistocene cave lions, 12 lions from historically extinct populations in Africa and the Middle East, and 6 modern lions from Africa and India. All historic and ancient DNA manipulation was performed in a dedicated ancient DNA laboratory at the University of Copenhagen. The age of both cave lion samples was determined through radiocarbon dating. Post sequencing, the resequenced data were initially processed using established protocols for ancient DNA data, including sequence trimming, mapping to the domestic cat (felCat8) and lion reference genomes (NCBI Bioproject PRJNA615082, accession JAAVKH000000000) (48), assessment of ancient DNA damage,

molecular sexing of the individuals, and calling of polymorphisms. The processed dataset was then used to explore phylogenetic relationships using both the nuclear and mtDNA components of the dataset, as well as analyze population structure, population split times, explore for geneflow between the modern and cave lions, assess inbreeding levels and genetic load, and generate local genealogies. Full methodological details are provided in the *SI Appendix*.

Data Availability. The sequencing data for all resequenced specimens is available from the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) as BioProject PRJNA611920 (49). The lion reference genome against which this data was mapped (BioProject PRJNA615082) is available from NCBI GenBank as accession no. JAAVKH000000000 (48). The accession numbers for all of the data are listed in *SI Appendix*, Table S1.

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