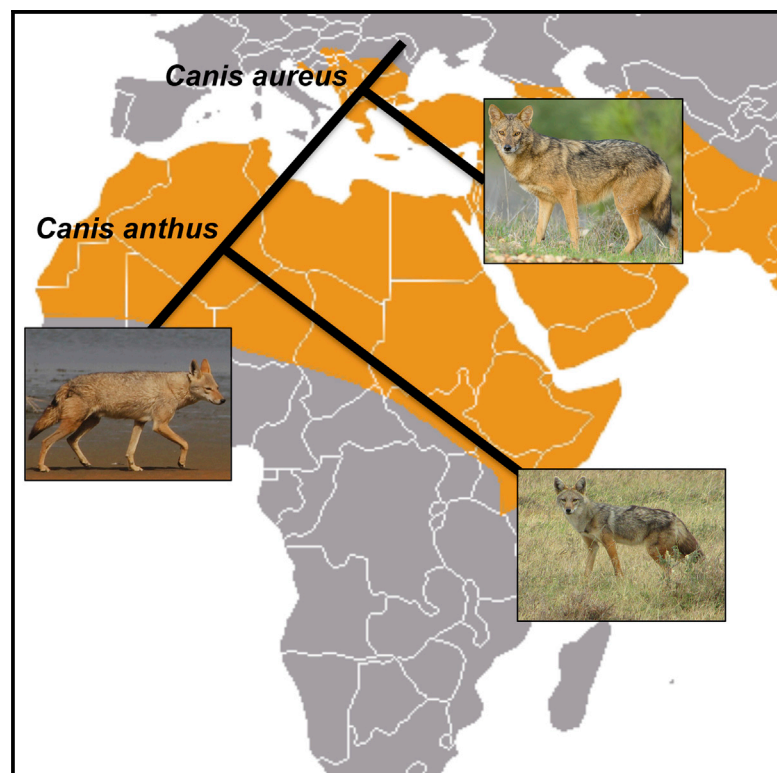


Current Biology

Genome-wide Evidence Reveals that African and Eurasian Golden Jackals Are Distinct Species

Graphical Abstract



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In Brief

Koepfli et al. assess divergence between golden jackals (*Canis aureus*) from Africa and Eurasia using data from the mitochondrial and nuclear genomes. They show that African and Eurasian golden jackals are genetically distinct and independent lineages, and that African golden jackals likely represent a separate species.

Highlights

- African and Eurasian golden jackals are genetically distinct lineages
- Divergence between lineages is concordant across multiple molecular markers
- Morphologic convergence is observed between African and Eurasian golden jackals
- African golden jackals merit recognition as a distinct species

Genome-wide Evidence Reveals that African and Eurasian Golden Jackals Are Distinct Species

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SUMMARY

The golden jackal of Africa (*Canis aureus*) has long been considered a conspecific of jackals distributed throughout Eurasia, with the nearest source populations in the Middle East. However, two recent reports found that mitochondrial haplotypes of some African golden jackals aligned more closely to gray wolves (*Canis lupus*) [1, 2], which is surprising given the absence of gray wolves in Africa and the phenotypic divergence between the two species. Moreover, these results imply the existence of a previously unrecognized phylogenetically distinct species despite a long history of taxonomic work on African canids. To test the distinct-species hypothesis and understand the evolutionary history that would account for this puzzling result, we analyzed extensive genomic data including mitochondrial genome sequences, sequences from 20 autosomal loci (17 introns and 3 exon segments), microsatellite loci, X- and Y-linked zinc-finger protein gene (*ZFX* and *ZFY*) sequences, and whole-genome nuclear

sequences in African and Eurasian golden jackals and gray wolves. Our results provide consistent and robust evidence that populations of golden jackals from Africa and Eurasia represent distinct monophyletic lineages separated for more than one million years, sufficient to merit formal recognition as different species: *C. anthus* (African golden wolf) and *C. aureus* (Eurasian golden jackal). Using morphologic data, we demonstrate a striking morphologic similarity between East African and Eurasian golden jackals, suggesting parallelism, which may have misled taxonomists and likely reflects uniquely intense interspecific competition in the East African carnivore guild. Our study shows how ecology can confound taxonomy if interspecific competition constrains size diversification.

RESULTS

Two recent studies based on mtDNA reported that the larger-sized golden jackals from Ethiopia and North and West Africa were more closely related to gray wolves than to other

populations of golden jackals, suggesting that some populations of African golden jackals represent a cryptic subspecies of gray wolf, designated *Canis lupus lupaster*, the African wolf [1, 2]. These results were consistent with earlier findings based on morphological and zoogeographic evidence that had suggested the large jackals of Egypt (*C. aureus lupaster*) were actually a small-sized subspecies of gray wolf [3]. However, this conclusion leaves the position of golden jackal populations in East Africa problematic, as they were never considered distinct from conspecifics in Eurasia. Consequently, either both golden jackal and African wolf occur in Africa, as has been suggested [2, 3], or these represent a single polytypic species. The former scenario suggests separate invasions of wolf- and jackal-like forms into North and East Africa, whereas the latter scenario suggests stable coexistence of distinct morphs within the same species that evolved in situ.

Evolutionary history is best verified through concordance among different molecular markers, which can provide a genome-wide history of divergence, and along with ecological and morphological data can be used to understand the context of evolutionary divergence [4–7]. Here, we present detailed analyses of the genome history of golden jackals employing a comprehensive set of molecular markers that include (1) mitochondrial genome sequences, (2) 20 autosomal DNA segments, (3) microsatellites, (4) sequences from the X- and Y-linked zinc-finger protein gene (*ZFX* and *ZFY*), and (5) ~7.6 million SNPs derived from whole-genome sequences. We compare the data generated from golden jackals to that from gray wolves and other wolf-like canids (see [Supplemental Experimental Procedures](#)).

Phylogenetic Analyses of Mitochondrial and Nuclear Sequences

Phylogenies estimated from sequences of the mitochondrial cytochrome *b* gene, 13 protein-coding and two rRNA genes from complete mitochondrial genomes (13,890 bp), and 17 intron plus 3 exon segments (13,727 bp) were all consistent in showing that golden jackals are separated into two well-supported clades. The cytochrome *b* phylogeny ([Figure 1A](#)) includes both published and novel sequences from golden jackals sampled in Africa and Eurasia ([Figure 1B](#)), which are assorted into two clades. Golden jackal haplotypes from Kenya, Mauritania, and Morocco are included in a clade containing haplotypes of canids from Algeria, Egypt, Mali, and Senegal referred to as *C. lupus lupaster* [1, 2]. This African golden jackal clade is closely related to Eurasian gray wolves with strong nodal support and up to 6.7% divergence from Eurasian golden jackals. The only exceptions to this geographic pattern are haplotypes of golden jackals from Israel, which are grouped into both Eurasian and African clades, and three canids originating from Egypt that are classified as African wolf, gray wolf, and golden jackal (the latter indicated by arrows in [Figure 1B](#)). The phylogeny of sequences derived from complete mitochondrial genomes also shows that African golden jackals from Kenya group strongly with gray wolves, and not with a Eurasian golden jackal from Israel ([Figure S1](#)).

Phylogenies estimated from nuclear data likewise suggest a close relationship between representative African golden jackals and gray wolves, but in these analyses, the gray wolf clade is sister to coyotes as found previously [8], suggesting that the divergence between golden jackals and gray wolves

preceded that of gray wolves and coyotes ([Figure 2](#)). Phylogenies estimated using both concatenation and multispecies coalescent approaches were identical, except for the relative placement of the Ethiopian wolf (*C. simensis*) ([Figure S2](#)). Divergence times estimated using the concatenated nuclear dataset show that gray wolf, coyote, Ethiopian wolf, and the two lineages of golden jackals diversified during the Pleistocene, beginning about 1.9 million years ago (mya) (95% highest posterior density [HPD] = 1.5–2.4 mya) with the divergence of the Eurasian lineage of golden jackals ([Figure 2](#)). The divergence between the African lineage of golden jackals and the gray wolf + coyote clade was estimated at 1.3 mya (95% HPD = 1.0–1.7 mya). These estimates are slightly earlier than the corresponding values from the mitochondrial genome analysis ([Figure S1](#)).

The mitochondrial gene trees and nuclear species trees differ significantly in topology, which may be due to differences in lineage sorting (see [Supplemental Experimental Procedures](#)). Nonetheless, topologies in which the two jackal lineages were constrained to be monophyletic were less significantly supported compared to their optimal topologies ([Table S1](#)).

Sex Chromosome Sequences

Genetic distinctness between African and Eurasian golden jackals is further supported by analyses of sequences from the final intron of the zinc-finger X-chromosomal (*ZFX*) and Y-chromosomal (*ZFY*) genes. Eurasian golden jackals, including most individuals from Israel, carry *ZFX* or *ZFY* haplotypes distinct from those seen in gray wolves, coyotes, and African golden jackals ([Figure 3A](#); [Table S2](#)). Notably, African golden jackals lack a 210 bp SINE II insertion, a 9 bp insertion, and a 2 bp insertion observed in Eurasian golden jackals ([Table S2](#)) [9]. A PCR assay of a larger panel of 31 male golden jackals from Eurasia and Africa confirmed that, with two exceptions (both from Israel), all male golden jackals from Eurasia had the *ZFY* SINE II element insertion, though this insert was absent in African golden jackals ([Table S3](#)).

Whole-Genome Sequences and Admixture

Whole-genome sequence analysis of three Eurasian wolves and one African (Kenya) and one Eurasian (Israel) golden jackal yielded 7,675,363 SNPs, and pairwise comparisons among these taxa confirm their distinctiveness across the genome ([Figure 3B](#)). We found relatively low diversity among gray wolf sequences (~42–45 ± 22 sites in 50,000 bp) despite the sampled wolves originating from geographically distant populations across Eurasia. The African golden jackal was equally divergent from all three gray wolves, differing at ~72 ± 30 sites in 50,000 bp. The Eurasian golden jackal showed a higher level of divergence from the gray wolves of ~87 ± 29 sites in 50,000 bp. Most strikingly, the African and Eurasian golden jackals were the most divergent, differing by ~94 ± 31 sites in 50,000 bp. Principal-component analysis (PCA) and historical trajectories of effective population size from the five canid genomes further reinforce the distinction between the two golden jackals relative to gray wolves ([Figure S3](#)).

We found evidence confirming historical gene flow among the canid lineages in *D* statistic analyses of the genome-wide SNP data ([Figure 3C](#); [Table S4](#)) [10]. Low *D* values (*D* = 0.0

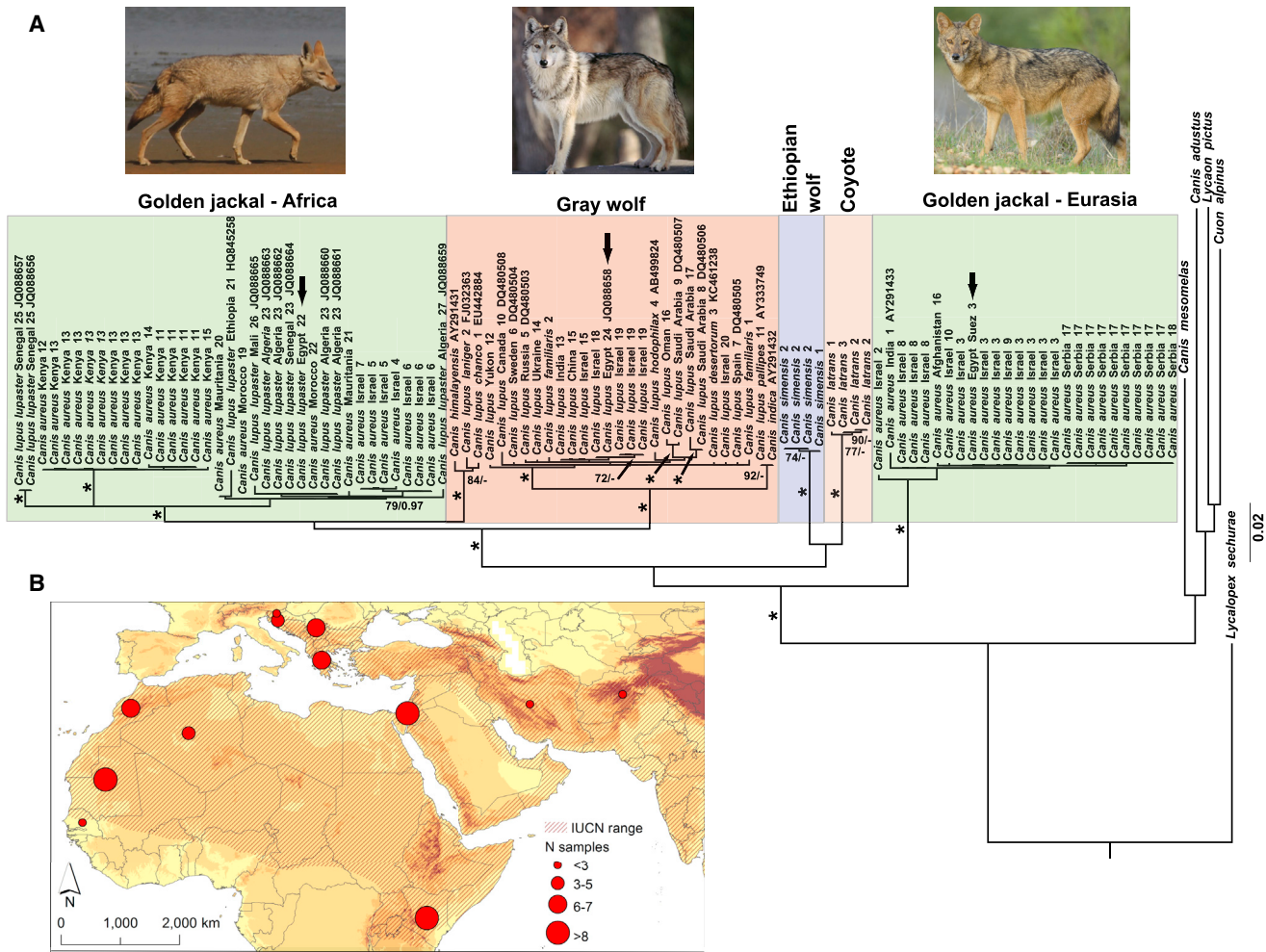


Figure 1. Phylogenetic Tree Based on Mitochondrial Cytochrome *b* Sequences and Sampling Localities of Golden Jackals Used in This Study
 (A) Maximum-likelihood phylogram of 104 cytochrome *b* sequences (1,140 bp). Haplotype number is shown next to taxon name and locality. Accession numbers indicate sequences downloaded from GenBank. Haplotypes without accession numbers are novel sequences generated for the present study. Asterisks at nodes indicate bootstrap support $\geq 80\%$ based on maximum-likelihood analyses (500 pseudoreplicates) and ≥ 0.95 posterior probability from Bayesian inference. *Canis* spp. from Egypt are indicated by thick arrows. Haplotypes labeled as *Canis lupus lupaster* refer to the African wolf. The tree was rooted using Sechuran fox (*Lyncalopex sechurae*) as outgroup. Scale bar indicates the number of substitutions per site. Photo credits: left, golden jackal from Senegal (© CIBIO/Monia Nakamura); center, Mexican gray wolf (© Tom and Pat Leeson); right, golden jackal from Israel (© Eyal Cohen).
 (B) Map of geographic localities showing where golden jackals were sampled. Relative number of animals sampled from each locality is shown. Hatched lines indicate geographic range of golden jackal based on IUCN distribution (<http://www.iucnredlist.org/details/3744/0>). See also Figure S1 and Table S1.

to -0.04) indicate only infrequent gene flow between the Kenyan golden jackal and gray wolf lineages, comparable to comparisons between anatomically modern humans and Neanderthals [10]. In contrast, higher *D* statistic values (0.16 to 0.18) suggest significant gene flow has occurred between Eurasian golden jackals and the gray wolf/dog group after the latter's divergence from the African golden jackal lineage (Figure 3C; Table S4).

Interestingly, the signal of gene flow is greatest between the Eurasian golden jackal and the basenji and dingo. The closeness of dog breeds and golden jackals may indicate recent admixture. Alternatively, some dog genome component may derive from admixture with gray wolves that have admixed with dogs in the past. However, previous genome analysis suggests

that the dog component in Middle Eastern gray wolves is $<9\%$ [11]. Additional evidence for genetic admixture in Israeli golden jackals comes from comparisons of our cytochrome *b*, nuclear DNA, microsatellite, and *ZFX/ZFY* sequence results (see above and Supplemental Experimental Procedures).

Microsatellite Analysis of Population Structure

Bayesian clustering analysis of 128 individuals genotyped at 38 microsatellite loci corroborates our findings above (Figure 3D). Our results showed that $K = 3$ had the highest likelihood (see Supplemental Experimental Procedures), with Eurasian golden jackals; golden jackals from Kenya; and a group containing North African golden jackals, gray wolves, and dogs resolved as distinct genetic clusters. Notably, at $K = 2$ all African golden

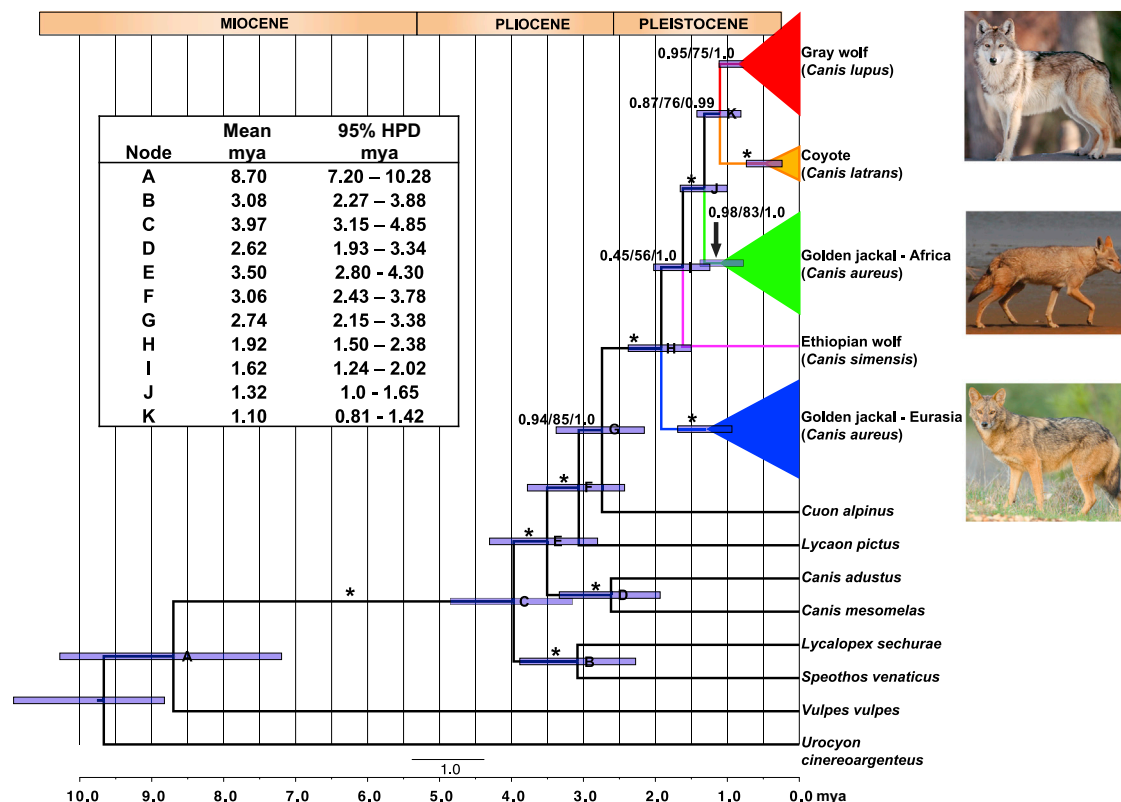


Figure 2. Chronogram Estimated from Concatenated Analysis of Twenty Nuclear Gene Segments Using a Relaxed Molecular Clock

Tree is based on analysis of 13,727 bp of sequence collected from 17 intron- and 3 exon-containing segments. Values shown at nodes are, respectively: Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT, PhyML), bootstrap support with 1,000 pseudoreplicates (BS, RAXML), and posterior probability from Bayesian inference (PP, BEAST). Asterisks indicate SH-aLRT = 100%, BS = 100%, and PP = 1.0. Node bars show 95% highest posterior density (HPD) for divergence times. Four individuals were used each for gray wolf, golden jackal (Africa), and golden jackal (Eurasia), and two individuals were used for coyote. Letters correspond to list of estimated divergence times and 95% HPD for internodes (inset). The tree was rooted using red fox (*Vulpes vulpes*) and gray fox (*Urocyon cinereoargenteus*) as outgroups. Scale bar indicates the number of substitutions per site. Timescale at bottom is in million years ago (mya), and geological timescale (epochs) are shown at top. Photo credits: top, Mexican gray wolf (© Tom and Pat Leeson); middle, golden jackal from Senegal (© CIBIO/Monia Nakamura); bottom, golden jackal from Israel (© Eyal Cohen). See also Table S1 and Figure S2.

jackals are grouped together with gray wolves and dogs in a single cluster, while at K = 4 North African golden jackals are resolved as a cluster distinct from gray wolves and dogs (Figure 3D). Critically, our results suggest that the presence of two mtDNA clades in golden jackals from Israel (see cytochrome *b* results above) does not reflect the occurrence of two reproductively distinct entities in this region, as the microsatellite results suggest that haplogroups do not form distinct genetic clusters (Figure 3D).

Size and Morphological Parallelism

We tested whether the patterns revealed by the genetic and genomic data were also manifested in morphology. PCA of 45 cranial and dental measurements taken from 140 golden jackals sampled from throughout the range of the species [12] revealed that golden jackals from North Africa are distinct from golden jackals from Eurasia and East Africa on PC1 (58.3% variation explained), which reflects the larger body size of North African golden jackals and is consistent with the equal loading across measurements on this PC (Figure 4A). PC2 (7.0% variation explained) does not segregate these three populations further,

but PC3 (4.4% variation explained) suggests that there are some differences in relative tooth size and skull shape between Eurasian and Middle Eastern golden jackals and all other African golden jackals (North, East, West, and Central) (Figure S4). To explore this further, we conducted PCA on the arcsine-transformed values of nine shape ratios for three groups: North African, East African, and Middle Eastern golden jackals (see Supplemental Experimental Procedures). The first PC accounted for 33% of the variance and separated East African from Middle Eastern golden jackals (Figure 4B). Compared with East African golden jackals, Eurasian golden jackals had high values on this axis, reflecting their broader muzzles, shorter molars, and the rounder cross-sections of their premolars and upper canines (see Supplemental Experimental Procedures). North African golden jackals overlap with the other two populations on the first PC, perhaps because this sample includes both larger “African wolf” individuals and others that are more closely related to Middle Eastern golden jackals. Notably, the North African golden jackals have more negative or near-zero values on the first PC and thus are more similar to East African than Middle Eastern golden jackals in shape. The North and East

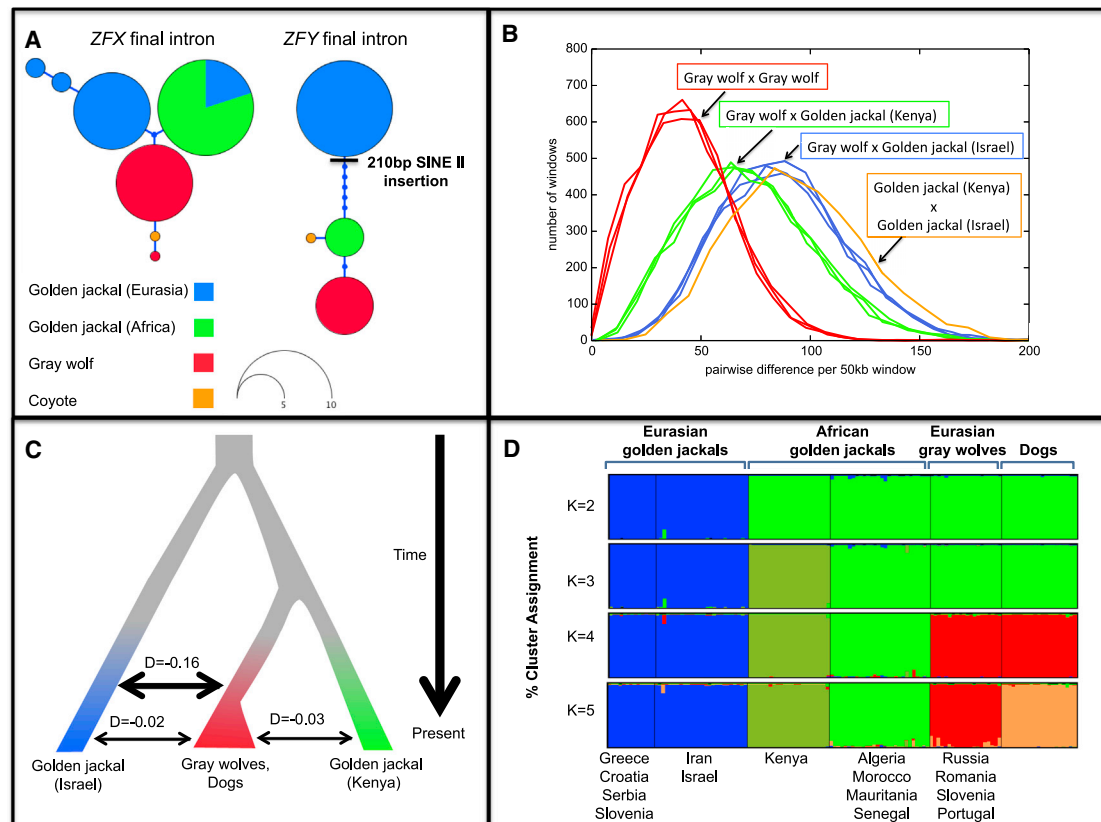


Figure 3. Patterns of Genetic Differentiation and Admixture of African and Eurasian Golden Jackals Based on Sex Chromosome Sequences, Genome-wide SNP Data, and Microsatellite Multilocus Genotypes

(A) Haplotype networks showing relationships among ZFX and ZFY final intron sequences among golden jackals from Africa and Eurasia, gray wolves, and coyotes. Circle size is proportional to haplotype frequency (see scale). Small dots on internodes indicate number of indels and nucleotide substitutions between haplotypes. Internodes without dots indicate single substitutions between haplotypes. The 210 bp SINE II insertion in the ZFY sequences separating Eurasian golden jackals from African golden jackals, gray wolves, and coyotes is indicated. See Table S2 for specific sequence features of each haplotype. See also Table S3.

(B) Comparison of genome-wide divergence between golden jackals and gray wolves. Histograms of genome-wide pairwise distance estimates were calculated from 50 kb non-overlapping windows (41,999 windows total) for all ten possible pairwise comparisons between the three gray wolf genomes and two golden jackal genomes. Gray wolves are from China, Croatia, and Israel. Pairwise differences are the number of differences per 50 kb. See also Figure S3.

(C) Diagram showing the phylogenetic relationships among dogs, gray wolves, African golden jackals (Kenya), and Eurasian golden jackals (Israel) used in the *D* statistic comparisons. The phylogeny was rooted using the Channel Island fox (not shown). *D* statistic values above double-headed arrows indicate detectable admixture (gene flow) between lineages. Gray wolves are from China, Croatia, and Israel, and domestic dogs represent the dingo and basenji breeds. See also Table S4.

(D) Estimated population structure of 128 individuals genotyped for 38 microsatellite loci. Analysis and posterior probability assignments to each cluster assuming two ($K = 2$) to five ($K = 5$) genetic clusters were estimated using STRUCTURE (see Supplemental Experimental Procedures). ΔK likelihood was highest for $K = 3$ (see Supplemental Information). The origin of individuals in each cluster is indicated at the bottom of the figure.

African golden jackals are similar in having narrower, more blade-like upper canines, as well as more slender premolars and muzzles, all of which are gray wolf-like features. These results suggest that parallelism in size and body conformation between Eurasian and East African jackals is accompanied by more subtle differences that support common ancestry of the latter with North African jackals.

DISCUSSION

Our results from mtDNA, nuclear loci, and whole genomes provide consistent, compelling evidence that golden jackals from Africa and Eurasia constitute largely distinct gene pools with in-

dependent evolutionary histories. We estimate that the African lineage has been on an independent trajectory for at least one million years. Our results extend and contrast with the findings of previous genetic studies, based exclusively on mitochondrial DNA, which suggested that some golden jackal populations in Africa constitute a subspecies of gray wolf [1, 2]. Specifically, we show that, given our current sampling, there are no golden jackals of Eurasian affinity in Africa. Instead, African golden jackals define a distinct lineage, which includes those from East Africa showing phenotypic similarity to Eurasian golden jackals. African golden jackals are distinct by all genetic measures in this study, showing diagnostic differences across a range of markers and with levels of genome divergence similar

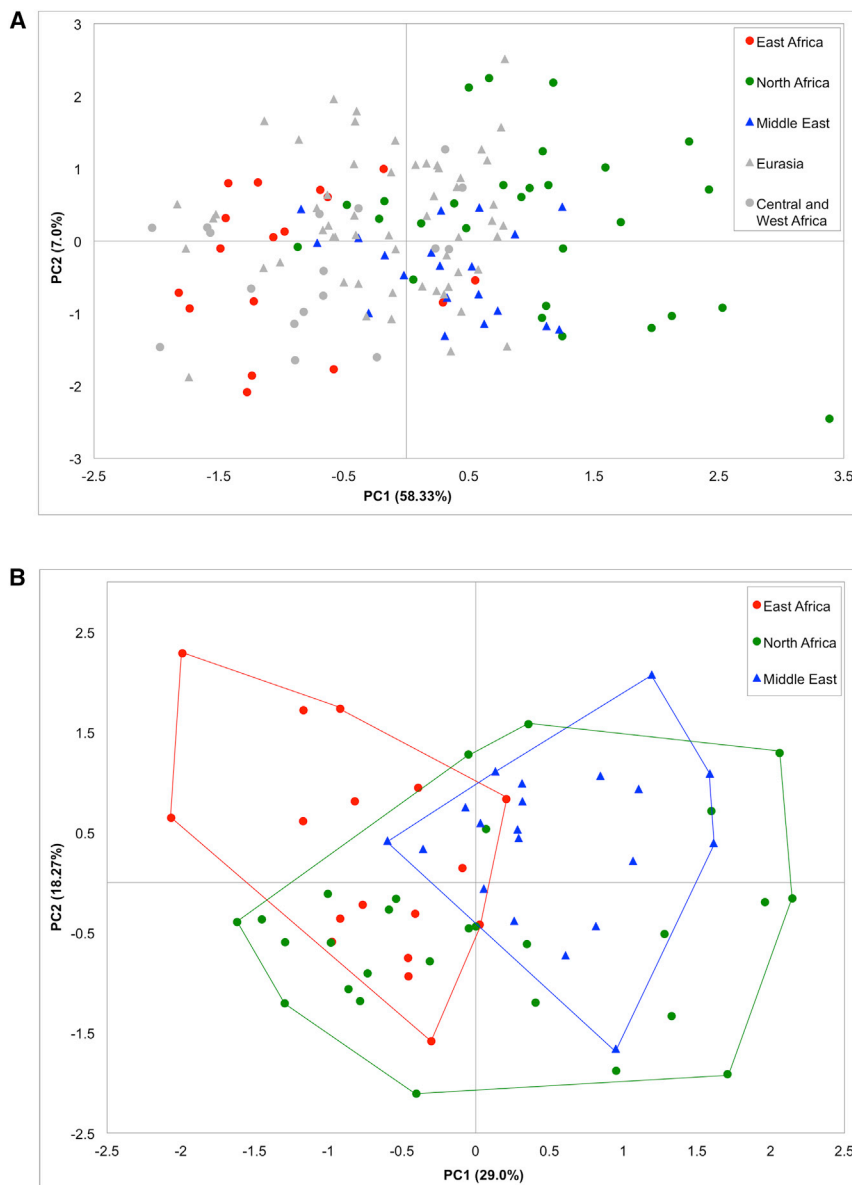


Figure 4. Principal Component Analyses of the Morphometric Data for African and Eurasian Golden Jackals

(A) Plot of principal component 2 (PC2) against PC1 based on 45 linear measurements of teeth and skulls of 140 African and Eurasian golden jackals from five different geographic regions. See [12] for details of geographic sampling of golden jackals. (B) Plot of PC2 against PC1 based on nine ratio variables that describe dental and cranial shape for three populations: North Africa (Egypt, Libya, Tunisia, Algeria, Morocco, Senegal, Western Sahara), East Africa (Kenya, Ethiopia) and the Middle East (Iran, Turkey, Jordan, Israel, Greece). Numbers in parentheses indicate percent variance explained on each axis. See also Figure S4.

in most taxonomic treatments since *C. aureus* was first formally described by Linnaeus [15]. *C. anthus* merits conservation concern and assessment independent of *Canis aureus*, as it represents a unique legacy of adaptation and divergence within the extant Canidae.

Our nuclear DNA analyses indicate that the African golden wolf lineage split from the gray wolf + coyote clade about 1.0–1.7 mya during the Pleistocene. More broadly, our phylogenetic analyses suggest that extant wolf-like canids have colonized Africa from Eurasia at least five times throughout the Pliocene and Pleistocene, which is consistent with fossil evidence suggesting that much of African canid fauna diversity resulted from the immigration of Eurasian ancestors [16, 17], likely coincident with Plio-Pleistocene climatic oscillations between arid and humid conditions [18, 19].

Our analyses of genome-wide SNP data revealed evidence of admixture in the histories of Eurasian golden jackals

in magnitude to those found between other recognized species. Thus, our results suggest that African golden jackals merit recognition as a full species, as they meet the primary defining criterion of a separate and independently evolving metapopulation lineage under the unified species concept [13]. Accordingly, we propose that African golden jackals be designated as *Canis anthus* (Cuvier, 1820) based on the earliest description of golden jackals from Senegal [14] (see [Supplemental Experimental Procedures](#)). Furthermore, we suggest that the common names “African golden wolf” (*C. anthus*) and “Eurasian golden jackal” (*C. aureus*) be applied to distinguish these taxa, and to distinguish the former from the Ethiopian wolf (*C. simensis*). We propose that the African golden wolf is distributed across Africa and includes individuals that have been referred to as *C. lupus lupaster* [1–3] or *C. aureus*, sensu lato. Morphologic parallelism of African golden wolves and Eurasian golden jackals may have resulted in their mistaken attribution to a single species

and African golden wolves. Eurasian golden jackals from Israel show signals of hybridization with gray wolves, dogs, and the African golden wolf based on *D* statistic analyses and comparisons of cytochrome *b*, microsatellite, and *ZFX/ZFY* sequence results. The close geographic proximity and connectivity between the Levant and Northeastern Africa (e.g., Egypt) may have facilitated admixture and mitochondrial capture of African golden wolf haplotypes by Eurasian golden jackals. Furthermore, Eurasian golden jackals have only recently recolonized parts of Israel following a large-scale eradication program begun in the 1960s to control rabies [20], and the greater amount of hybridization detected in Eurasian golden jackals from Israel may be related to colonization of migrants from elsewhere. Interestingly, microsatellites revealed no evidence of admixture, suggesting that the admixture we detected in the genome-wide SNP data was relatively ancient. Previous analysis of complete genome sequences of gray wolves and Israeli golden jackals

also supported ancient hybridization between the two species, suggesting that as much as 15% of the current Israeli wolf genome is derived from ancient admixture with golden jackals [11]. Our results suggest a dynamic genetic history among these canids in the Middle East and North Africa, similar to that observed in North American wolf-like canids and other carnivorous taxa such as brown and polar bears [21–23]. Increased sampling of gray wolves, African golden wolves, and Eurasian golden jackals from throughout the Middle East and North Africa will be required to fully resolve the details of this history.

Despite their distinct genetic ancestries, African golden wolves and Eurasian golden jackals are phenotypically similar in craniodental anatomy, and African golden wolves from East Africa and Eurasian golden jackals are similar in body size. This striking example of parallel evolution highlights the importance of natural selection in constraining morphologic divergence in sympatric carnivores [24–26]. However, there are subtle shape similarities in craniodental form that unite African golden wolves and distinguish them from Eurasian golden jackals. The phylogenetic affinities of the African golden wolves to gray wolves or gray wolves + coyotes, the canine fossil record, and macroevolutionary dynamics of canine body-size evolution suggest that they were derived from ancestors of larger body size [16, 27]. The convergent evolution of a smaller, more omnivorous jackal-like form, especially in East Africa, from larger, more carnivorous wolf-like forms is uncommon in canids [28, 29] and may have been facilitated by intense competition from a uniquely diverse carnivorous community including species larger and smaller than jackals, thus inhibiting size divergence [12].

ACCESSION NUMBERS

GenBank accession numbers for the sequences reported here will be available in the issue edition of the article, available on August 17, 2015. Additional files associated with the Supplemental Information have been deposited at the Dryad Digital Repository at <http://dx.doi.org/10.5061/dryad.3b77f>.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.06.060>.

AUTHOR CONTRIBUTIONS

K.-P.K. designed the study, performed experiments, analyzed data, and drafted the manuscript. J.P. designed the study, performed experiments, and analyzed the sex chromosome and SNP data. R.G. collected and analyzed the microsatellite data. J.R., A.L., S.H., and R.M.S. performed experiments and collected data. O.T. performed experiments and collected the whole-mitochondrial-genome data. P.S., Z.F., J.A.C., and B.S. analyzed the whole-genome SNP data. P.D. and A.M. analyzed the mitochondrial genome and nuclear DNA data. A.A.Y. and B.V.V. analyzed the morphological data. F.A., J.C.B., E.G., J.A.L., and K.M.H. provided materials and reagents to the study. W.E.J. and S.J.O. contributed to the scientific strategy and assisted with the manuscript. R.K.W. co-designed and supervised the study and co-drafted the manuscript. All authors contributed to and approved the final manuscript.

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