## SPECIAL ISSUE ARTICLE

Revised: 3 December 2020



# Pleistocene climate fluctuations drove demographic history of African golden wolves (*Canis lupaster*)

Carlos Sarabia<sup>1</sup> | Bridgett vonHoldt<sup>2</sup> | Juan C. Larrasoaña<sup>3</sup> | Vicente Uríos<sup>4</sup> | Jennifer A. Leonard<sup>1</sup>

<sup>1</sup>Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana (EBD-CSIC, Seville, Spain

<sup>2</sup>Faculty of Ecology and Evolutionary Biology, University of Princeton, Princeton, NJ, USA

<sup>3</sup>Instituto Geológico y Minero de España (IGME, Madrid, Spain

<sup>4</sup>Vertebrate Zoology Research Group, University of Alicante, Alicante, Spain

### Correspondence

Carlos Sarabia and Jennifer A. Leonard, Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana (EBD-CSIC), Seville, Spain Emails: cdomsar@gmail.com; jleonard@ ebd.csic.es

### **Funding information**

Ministerio de Ciencia e Innovación, Grant/ Award Number: BES-2015-074331 and SEV-2012-0262; Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía, Grant/Award Number: P18-FR-5099

### Abstract

Pleistocene climate change impacted entire ecosystems throughout the world. In the northern hemisphere, the distribution of Arctic species expanded during glacial periods, while more temperate and mesic species contracted into climatic refugia, where isolation drove genetic divergence. Cycles of local cooling and warming in the Sahara region of northern Africa caused repeated contractions and expansions of savannah-like environments which connected mesic species isolated in refugia during interglacial times, possibly driving population expansions and contractions; divergence and geneflow in the associated fauna. Here, we use whole genome sequences of African golden wolves (*Canis lupaster*), a generalist mesopredator with a wide distribution in northern Africa to estimate their demographic history and past episodes of geneflow. We detect a correlation between divergence times and cycles of increased aridity-associated Pleistocene glacial cycles. A complex demographic history with responses to local climate change in different lineages was found, including a relict lineage north of the High Atlas Mountains of Morocco that has been isolated for more than 18,000 years, possibly a distinct ecotype.

### KEYWORDS

Canis anthus, carnivore, genomics, MiSTI, PSMC

# 1 | INTRODUCTION

Pleistocene climatic fluctuations shaped phylogeographic and demographic histories of many species in the northern hemisphere (Bolfíková et al., 2017; Feliner, 2011; Gómez & Lunt, 2007; Hewitt, 1999, 2000; Tison et al., 2014), which was periodically glaciated (Clark et al., 2009). The patterns left from repeated range shifts into glacial refugia and subsequent expansion across the higher latitudes has been best characterized in Europe (Hewitt, 1999, 2000). Species adapted to temperate climates saw a reduction in range and numbers of individuals during glacial periods in European and North American ecosystems, and benefited from milder climates during interglacials (Bolfíková et al., 2017; Dufresnes et al., 2020; Gómez & Lunt, 2007; Sommer & Nadachowski, 2006; Stöck et al., 2012). In tropical regions a combination of changes in incoming solar radiation and glacial-interglacial cycles influenced the extension and activity of the monsoon systems, and resulted in shifts between humid and arid conditions (Drake et al., 2011; Ehrmann et al., 2017; Emeis et al., 2000; Heinrich, 1988; Hoffmann et al., 2016; Larrasoaña et al., 2013; Lézine et al., 2011; Rohling et al., 2003; Smith, 2012). A pattern of expansion of savannah associated north African species was observed during humid periods (Bertola et al., 2016; Cosson et al., 2005; Dinis et al., 2019; Husemann et al., 2014; Iyengar et al., 2007; Leite et al., 2015; Lerp et al., 2011). Desert adapted species show the opposite pattern of expansion during dry periods and reductions in distribution and population size in humid periods (Moutinho et al., 2020; Tamar et al., 2018).

The northern coast of north Africa is currently dominated by a temperate Mediterranean climate, which brings westerly rains that hardly penetrate 200 km away from coastal areas (Larrasoaña et al.,

<sup>2</sup> WILEY-MOLECULAR ECOLOGY

SARABIA ET AL.

2013). Further south, the climate of tropical north Africa is driven by two monsoonal systems - the west and east African monsoons - that result in higher rainfall in the equatorial zone that decreases progressively towards the north. Although westerly- and monsoon-driven precipitation extend into the continental interior during extreme events, the overall scarcity of rainfall dictates the presence of the Sahara Desert, a hyperarid zone between 28-30°N and 18°N where semi-arid or savannah fauna live isolated in refugia around the periphery and in oases (Dinis et al., 2019; Husemann et al., 2014; Nicolas et al., 2009; Rato et al., 2007) (Figure 1). Cyclic variations in the Earth's orbit have driven changes in the amount of solar radiation received in the tropics, which is the engine that powers the monsoon system and has resulted in periodical expansion of savannah landscapes throughout much of the Sahara Desert since its inception about 14 million years ago (Ma) (Castañeda et al., 2009; DeMenocal, 2004; Drake et al., 2008, 2011, 2013; Ehrmann et al., 2017; Geyh & Thiedig, 2008; Larrasoaña et al., 2013; Smith, 2012; Tjallingii et al., 2008; Weldeab et al., 2007). Such expansions, known as Green Sahara Periods (GSP), typically lasted for 3 to 6 thousand years (kyr) and occurred during bundles of high-amplitude boreal summer insolation maxima driven by maxima in the eccentricity of the Earth's orbit. During periods of eccentricity minima, when boreal summer insolation peaks were subdued, the monsoon system was weakened and influenced by glacial-boundary conditions via changes in north Atlantic sea-surface temperatures (DeMenocal, 2004; Ehrmann et al., 2017; Tjallingii et al., 2008; Weldeab et al., 2007). Intensification of glacial cycles in the northern hemisphere after 2.7 Ma amplified the effect of glacial-boundary conditions and led to an overall shift to drier conditions over the Sahara (DeMenocal, 2004). Glacial periods occurred with a 100-kyr cyclicity after ca. 1.2 Ma (McClymont et al., 2013) and, at least in the case of the last glacial period, were punctuated by short, colder periods (Heinrich stadials) that that lasted up to 1500 years (Heinrich, 1988; Hemming, 2004) and enhanced the dry conditions

over the desert (Ehrmann et al., 2017). If animals are tightly associated with their habitat, their populations should expand and contract with their preferred habitat, so during GSPs semi-arid species should expand in population size and gene flow should increase between regions isolated during drier, glacial times (Dinis et al., 2019; Karssene et al., 2019; Leite et al., 2015; Lerp et al., 2011; Rato et al., 2007). Short-lived epochs of enhanced rainfall also occurred during the last glacial period along the northernmost (Drake et al., 2013; Hoffmann et al., 2016; Smith, 2012) and southernmost (Castañeda et al., 2009; Weldeab et al., 2007) fringes of the Sahara, and might have also driven changes in population size and gene flow in north African species.

The African golden wolf (Canis lupaster) is a recently rediscovered (Koepfli et al., 2015) wild canid species of north and east Africa. Its current distribution encompasses approximately 11 million square kilometers from Morocco to Egypt in the north and from Senegal to Kenya in the south (Kebede, 2017; Kingdon, 2013) (Figure 1). This large distribution makes them useful for answering questions about how past climate periods could have affected mesic mammal communities in north Africa. Until recently, African golden wolves were considered the same species as the Eurasian golden jackal (Canis aureus), and most ecological data have been collected in Eurasia, making African golden wolves one of the least studied canid species in the world (Admasu et al., 2004; Amroun, Giraudoux, et al., 2006; Amroun, Oubellil, et al., 2014; McShane & Grettenberger, 1984). Their wide distribution and generalist predatory style has allowed them to adapt to a wide variety of habitats including arid or semi-arid landscapes, grasslands, savannahs, forests and high elevation areas in Morocco and Ethiopia as well as anthropized zones (Amroun et al., 2006; Amroun, Oubellil, et al., 2014; Cuzin, 2003; Kebede, 2017; McShane & Grettenberger, 1984).

Despite their large home ranges and ecological plasticity (Admasu et al., 2004; Amroun et al., 2006; Amroun, Oubellil, et al., 2014; Fuller et al., 1989; Karssene et al., 2018; McShane & Grettenberger, 1984; Moehlman, 1986), African golden wolves



FIGURE 1 Map of Africa showing isohyets, elevations, main water sources, African golden wolf distribution (in green) according to IUCN and location of the seven samples included in this study (in red).

- MOLECULAR ECOLOGY - WILEY-

have not been found in hyperarid areas (Kingdon, 2013) although they have been reported in archaeological sites in current-day desert areas that were greener in the past (Sereno et al., 2008). This suggests that African golden wolves had a wider distribution during GSP. In addition to climate, competition between African golden wolves and bigger carnivores with overlapping distributions, such as hyenas (Kebede, 2017; Kingdon, 2013) and black-backed jackals (Van Valkenburgh & Wayne, 1994), could have affected their distribution. Recent genetic evidence has suggested that African golden wolves may have benefited from increases in human populations since the Neolithic, either through the introduction of caprid livestock that could have served as a food source, and/or through predator control against competing species (Eddine et al., 2020). African golden wolves have been shown to scavenge around anthropized zones (Admasu et al., 2004; Amroun et al., 2006; Amroun, Oubellil, et al., 2014; McShane & Grettenberger, 1984), but have disappeared from very intensively exploited agricultural areas (Aulagnier, 1992; Cuzin, 2003). Finally, recent evidence from whole genomes of African golden wolves (Chavez et al., 2019; Gopalakrishnan et al., 2018; Liu et al., 2018) have shown a complex genetic history with two separate populations at the extremes of their distribution and possibly events of introgression from other related canids in the past.

Here, we use seven whole genome sequences from across the distribution of African golden wolves and recently developed analytical methods to evaluate population structure, deep demographic history, and past episodes of gene flow. We integrated the genomic results with past environmental variability in north Africa (Castañeda et al., 2009; Drake et al., 2008, 2011, 2013; Geyh & Thiedig, 2008; Larrasoaña et al., 2013; Tjallingii et al., 2008; Weldeab et al., 2007) in order to evaluate the role of monsoon variability and its teleconnection with glacial-interglacial climates in shaping the demography of the African golden wolf. We also compared the genomic diversity, structure, and inbreeding coefficients across the species with similar geographic distributions in two closely related canids (grey wolves, Canis lupus; and coyotes, C. latrans) with very different social systems and ecology. We found a complex demographic history with different, well defined populations that diverged thousands of generations ago coinciding with late Pleistocene climatic fluctuations, and an isolated mountain lineage with a high degree of inbreeding whose genome-wide heterozygosity has been drastically reduced. Although the morphology and size of the African golden wolves is more similar to North American coyotes, the observed genetic population structure is more similar to that found in grey wolves, possibly suggesting a social system and ecology more similar to the latter.

### 2 | MATERIALS AND METHODS

### 2.1 | Study sample

A sample from a previous study (Urios et al., 2015) from which the mitochondrial genome has already been published (KT378605) was used to construct a shotgun library as in Camacho-Sanchez et al. (2018) and sequenced. The individual was a roadkill found at N32°33.364' W5°50.848', in Beni Mellal province, Morocco. The area is a hill slope at around 2000 m asl elevation in the north of the High Atlas Mountains. We refer to this sample as "west Morocco" and a previously published genome (Gopalakrishnan et al., 2018) from another Moroccan individual as "east Morocco". An additional 26 genomes were obtained from the literature: six African golden wolves, seven domestic dogs, six grey wolves, two Eurasian golden jackals one Ethiopian wolf, one African hunting dog, and three coyotes (Table S1).

### 2.2 | Preprocessing pipeline

Adapters were trimmed with cutadapt (Martin, 2011) and quality of the reads was evaluated with FastQC (Andrews, 2010). Reads were mapped using BWA MEM v1.3 (Li & Durbin, 2010) to the reference genome CanFam3.1 (*Canis familiaris*, domestic dog) (Lindblad-Toh et al., 2005), with the Y chromosome (Oetjens et al., 2018). Reads were also mapped to an assembled reference genome of an African hunting dog (*Lycaon pictus*; Campana et al., 2016) to avoid bias from mapping to the dog genome, which is in the ingroup for some analyses. We sorted and filtered low quality mapped reads with SAMTOOLS v1.9 (Li et al., 2009) and removed duplicates with GATK v3.7 (McKenna et al., 2010). All reads had a sequencing quality higher than 20 and had a complementary read in the same chromosome (Table S1).

### 2.3 | Variant calling and quality filters

We filtered reads mapping to sex chromosomes and mitochondrial DNA and retained only autosomes. We filtered low quality mapped reads and called genotype likelihoods using ANGSD. Sites with depth <5x or more than twice the mean coverage depth were filtered out (Freedman et al., 2014). Genotype likelihood frequency files (.glf. gz) were used to call SNPs using the -doplink option in ANGSD and an exclusion zone of 10 kilobases (kb) upstream and downstream the genic regions was defined using a.refseq annotation file of CanFam3 from the UCSC Genome browser (Kent et al., 2002) and custom scripts (Supporting Information Code File 02.b). Genotype likelihoods were computed and SNPs called for autosomes mapped against both reference genomes (CanFam3.1 and African hunting dog; Li, 2011).

### 2.4 | Genetic structure

A principal component analysis (PCA) was performed using genotype posterior probabilities of genotypes of the five Old World species with the NGSCOVAR package in NGSTOOLS (Fumagalli et al., 2014) and RSCRIPT v3.4.4 (R Core Development Team, 2017). We estimated admixture proportions from genotype likelihoods using NGSadmix (Skotte & Albrechtsen, 2013), setting number of clusters (K) between

5 and 14. To ensure reproducibility of results, we ran the NGSadmix test five times and visually compared the plots.

We used.glf.gz files to call SNPs with ANGSD (-doPlink option, settings: -pvalue 1e-5, -doMaf 1, -doPost 1). Genic regions, deviations from Hardy-Weinberg (HW) equilibrium, and SNPs in linkage disequilibrium were filtered from the data set in PLINK v1.9 (Purcell et al., 2007) (settings: --hwe 0.001, --maf 0.05, --indep-pairwise 50 5 0.5). We performed a PCA using flashPCA (Abraham & Inouye, 2014) and estimated admixture composition using ADMIXTURE (Alexander et al., 2009) with a number of clusters (K) between 5 and 14. Visual comparisons were made of plots with genotype likelihoods and with discovered SNPs in genomes mapped against CanFam3.1 and the African hunting dog genome.

### 2.5 **Demographic history**

To avoid misinterpretations of heterozygous sites as homozygous in low coverage samples (e.g., 7×-15×) (Nadachowska-Brzyska et al., 2016), we repeated the process with the actual coverage and downsampled.bam files of the Kenyan African golden wolf (7×, 9×, 11.12×, 15× and 24×, using samtools view -bs, Li et al., 2009) to visually estimate the best false negative rate (FNR) following (Hawkins et al., 2018; Kim et al., 2014; Nadachowska-Brzyska et al., 2016). The different coverages of the Kenyan genome were plotted with and without FNR correction (Figures S1A,B). After determining the FNR, consensus sequences with genic regions were called with bcftools mpileup (settings: -C 50, d 5, -D 100). PSMC was called using 64 atomic time intervals (settings: -p "1 × 6 + 58 × 1") as in previous studies with AGW (Freedman et al., 2014), and initial theta per individual and coverage was corrected (Li & Durbin, 2011). A round of 50 iterations of bootstrapping per genome was applied to draw a multisample PSMC plot. A mutation rate of  $4.5 \times 10^{-9}$  (Koch et al., 2019) and generation time of three years (Chavez et al., 2019; Freedman et al., 2014; Gopalakrishnan et al., 2018; Koepfli et al., 2015; Liu et al., 2018) were used. To explore extreme mutation rates  $(2.7-7.1 \times 10^{-9}$  as estimated by Koch et al., 2019), we also generated PSMC plots for all AGWs except Egypt without bootstrapping with these values.

More recent demographic population history was studied with ngsPSMC (Shchur et al., 2017). We generated files for ngsPSMC in ANGSD (option: -dopsmc) filtering for a minimum depth of 5× per genome. We ran ngsPSMC for 50 iterations with the same atomic time intervals as in PSMC, using initial population sizes 105 years ago as observed in the PSMC plot. Calculated  $\Theta \pi$  was used as theta ( $\theta$ ) (see Supporting Information Code File 03) and we estimated genome wide rho from a recombination map for dogs (Auton et al., 2013). Mutation rate and generation time were the same as in PSMC. NgsPSMC is still under development, and bootstrapped plots could not be generated.

We considered both low- and high-latitude climate mechanisms influencing past environmental variability in the Sahara back to 1.5 Ma (Castañeda et al., 2009; Drake et al., 2013; Ehrmann

et al., 2017; Larrasoaña et al., 2013; McClymont et al., 2013; Rohling et al., 2003; Smith, 2012; Tjallingii et al., 2008; Weldeab et al., 2007) that could have affected the demographic history of AGW. We compared the timing of these events with the PSMC and ngsPSMC maxima and minima and observed possible correlations between climatic events and increases or decreases of population. We also added the speciation event that led to African golden wolves (Chavez et al., 2019; Koepfli et al., 2015) with a confidence interval of 400 kyr.

#### 2.6 Summary statistics

We inferred changes in Ne with thetas and neutrality tests based on likelihood-based estimation of site frequency spectrum (SFS) in ANGSD (Nielsen et al., 2012). The reference genome of the African hunting dog (Campana et al., 2016) was used as ancestral to call unfolded SFS, assuming HW equilibrium, multisample GL estimation (-dosaf 1), and an upper depth filter of 2.5×, mean read depth per sample and per population as in Table S1. We calculated the genome wide heterozygosity per individual as in Gopalakrishnan et al. (2018). Different coverages of the Kenyan AGW (7×, 9×, 11.2×, 15×, 24×, as above) were used to repeat SFS calling and correct mean heterozygosity for all samples.

We used a joint SFS between pairs of populations (2DSFS) to estimate average genome wide  $F_{ST}$  and 95% confidence intervals from a 50 kb sliding window scan. Finally, we computed a series of nucleotide diversity indexes (Tajima's D (Tajima, 1989), Fu and Li's F and D (Fu & Li, 1993), Fay's H (Zeng et al., 2006), Zeng's E (Zeng et al., 2006)) and thetas ( $\Theta_W$ ,  $\Theta_\pi$ ,  $\Theta_{FL}$ ,  $\Theta_H$ ,  $\Theta_L$ ) (Durrett, 2008; Fay & Wu, 2000; Fu & Li, 1993; Tajima, 1989; Watterson, 1975; Zeng et al., 2006) using the -doThetas 1 option in ANGSD with population-based SFS as prior information (-pest), divided in 50-kb windows across the genome and excluding genic regions.

### 2.7 | Heterozygosity

We defined four populations: afr north (AGW from Algeria, Egypt, east Morocco, west Morocco, Senegal), afr\_east (AGW from Ethiopia, Kenya), coyote (coyotes from California, Midwest and Mexico), and gwolf\_me (grey wolves from S. Arabia, Iran and Syria). We calculated the inbreeding coefficient or F<sub>i</sub> per individual based on individual genotype likelihoods using ngsF (Vieira et al., 2013), computed across 20 iterations in ngsF. Genotype-based calculation of F<sub>i</sub> per individual was performed in PLINK with -het on a data set of SNPs discovered using ANGSD (option -doPlink).

We used two approaches to calculate ROHs across the whole genome. The first method uses PLINK and the SNP data set from the F<sub>i</sub> calculation. In each population we removed SNPs in close linkage disequilibrium in 200 base pair (bp) windows with a step size of 100 bp and a  $R^2$  of 0.9 using option –indep-pairwise 200

MOLECULAR ECOLOGY - WILFY-

100 0.90 in PLINK and generated ROHs as in Sams and Boyko (2019). The second method uses the software ROHAN (Renaud et al., 2019) on mapped.bam files. We ran ROHan in windows of 500 kb in .bam files of all AGW, coyotes, and grey wolves of the Middle East. Expected theta in ROHs was set to  $2 \times 10^{-5}$  and default options for Illumina error rate. Plots of local heterozygosity were computed across the whole genome and a summary of ROHs was calculated. Finally, we calculated inbreeding coefficients from ROHs (F<sub>ROH</sub>) as in (McQuillan et al., 2008; Sams & Boyko, 2019):

$$F_{\text{ROH}_{j}} = \frac{\sum_{k} \text{length} \left( \text{ROH}_{k} \right)}{L}$$

where  $\text{ROH}_k$  is the *k*th ROH in individual *j*'s genome and L is the total length of the genome.

### 2.8 | Divergence dating

We used MiSTI (Shchur, 2019) to estimate times of divergence between local lineages represented by our seven AGW individuals. We used a list of green Sahara periods (Ehrmann et al., 2017; Larrasoaña et al., 2013) and of cold stadials (Heinrich, 1988; Hemming, 2004; Rohling et al., 2003) associated with increased aridity of the Sahara region (Ehrmann et al., 2017) to define humid (with potentially more connectivity among lineages) and dry (potential times for divergence) time segments. We used pairwise timescales generated using PSMC and 2DSFS files from previous sections. GNU Parallel (Tange, 2018) was used to model simultaneously different scenarios of divergence among lineages with different migration rates in dry periods and GSPs, permitting MiSTI to automatically optimize calculations of migration rate per time segment. We extracted a table of splitting times from MiSTI and plotted log likelihoods per proposed splitting time against time. Finally, a polynomial curve was fitted per group of data where  $R^2 \ge 0.99$  to estimate the "aximum point of the curve using" the Newton-Raphson approach and a confidence interval of the upper 5%, 1% and 0.1% of log likelihood points. Since this is new software, we evaluated the replicability of our results by testing divergence time estimations with different coverages of the same genomes and different combinations of predefined time segments. We also compared the results with an estimation of divergence time using the Cavalli-Sforza (1969) equation (Supporting Information Methods).

## 3 | RESULTS

Although coverage was different between samples, for each sample coverage across the genome was similar when reads were mapped against the two reference genomes, CanFam3.1 and the African hunting dog. Coverage for samples mapped against CanFam3.1 ranged from 5.16× to 34.54× and from 4.58× to 32.1× when mapped to the African hunting dog reference genome (Campana et al., 2016) (Tables S2 and S3). Mapping against CanFam3.1 resulted in a slightly higher mappability, higher coverage and less duplicates than against the African hunting dog genome (Table S4). The new sample, west Morocco, had a total of 284,332,735 raw reads. Average mappability for this sample against CanFam3.1 and African hunting dog reference genomes was 93.99% and 91.55%, respectively. Genomewide coverage was 11.26× mapped against CanFam3.1 and 10.58× mapped against African hunting dog (Tables S2 and S3).

# 3.1 | Population structure driven by a northsouth boundary

Genotype probabilities were calculated across 23 genomes of five species (seven African golden wolves [AGW], seven dogs, six grey wolves, one Ethiopian wolf, two Eurasian golden jackals) with the -doGeno 32 option in ANGSD. Nearly three million (2.98 M) sites were called when mapping against the CanFam3.1 genome, and 2.54 M sites when mapping against the African hunting dog. Genotype likelihood-based SNP calling with ANGSD produced 689,000 (698 K) sites for those genomes mapped against CanFam3.1, and 625 K sites for genomes mapped against the African hunting dog. All SNPs were more than 10 kb away from any genic zone and were in HW equilibrium. We identified population structure, with all wolves northwest (Senegal, east Morocco, west Morocco, Algeria) and southeast of the Sahara (Kenyan and Ethiopian) in different clusters (Figure S2). The Egyptian AGW appeared at an intermediate genetic distance between the northwestern cluster and a cluster that encompassed all grey wolves and dogs, which is consistent with previously published studies that suggested it has introgressed ancestry (Gopalakrishnan et al., 2018; Liu et al., 2018). While different PCAs showed Ethiopian wolves at different positions with respect to the African golden wolves depending on the reference genome and the source data (glf.gz or SNPs), the structure of African golden wolves, dogs, and grey wolves remained consistent regardless of the reference genome used to map reads against (CanFam3.1 or African hunting dog) or what method was used to generate the PCA plots (genotype likelihoods or SNPs) (Figure S3).

After filtering for genic sites plus 10 kb windows, NGSadmix retained 10.5 M genotype likelihood sites for admixture analyses in genomes mapped to CanFam3.1 and 9.89 M sites for genomes mapped against the African hunting dog. Using the full data set of 23 genomes, NGSadmix failed to identify a most probable value of K based on the maximum likelihood estimation implemented in the program (Skotte & Albrechtsen, 2013), with high numbers of K receiving the highest likelihood. We extracted and plotted in R the best likelihood values for every K and attempted to find the best-fit K, but the best likelihood was found at very large K values, even beyond the number of individuals included in the analysis (Figure S4). A relative maximum was found at K = 21. This issue may indicate

subclustering within the species studied (Pilot et al., 2019), and does not necessarily mean lack of population structure. Nonetheless, a series of general trends could be identified. Starting from K = 6 onwards, the Ethiopian and Kenyan African golden wolves clustered together in a different population from the other AGWs. From K = 5 to K = 10, the Egyptian AGW appears to have mixed ancestry between African golden wolves and possibly Middle Eastern grey wolves or dogs from Africa (Figure 2a). Notably, at K = 9 the Ethiopian AGW seems to share some proportion of alleles from a postulated ancestral population with Ethiopian wolves, but this trend is not shown at any other K or level of clustering.

When considering African golden wolves alone (Figure 2b), some structure is detected between the southeast population (Ethiopia,



Kenya) and the northern population (Algeria, Egypt, Morocco, Senegal). This pattern is consistent with a general trend in big African carnivores and ungulates to cluster in northern and southeast populations divided by the Rift Valley (Bertola et al., 2016; Brown et al., 2007; Charruau et al., 2011; Flagstad et al., 2001; Lorenzen et al., 2012; Moodley & Bruford, 2007; Muwanika et al., 2003; Smitz et al., 2013), and is also consistent with previous studies on African golden wolves (Gopalakrishnan et al., 2018). No mixed composition was detected in the Egyptian individual from other different African golden wolves when no dogs or grey wolves were present at the NGSadmix study. SNP-based Admixture plots showed very similar results, and only small local proportions of admixture between dog and grey wolf were detected when comparing NGSadmix and Admixture plots



**FIGURE 2** NGSadmix plots of Old World canids showing admixture proportions, including the 23 individuals used at this study (a) mapped against African hunting dog; and (b) only African golden wolves. Eastern (Kenya, Ethiopia) African golden wolves cluster in a different group from those from the north (Egypt, Algeria, east Morocco, west Morocco, Senegal). The Egyptian individual also seems to have ancestry from grey wolves or domestic dogs. EW, Ethiopian wolf; EGJ, Eurasian golden jackal. Plots are based in 9.885 M sites (see text for details).

LAK LCOLOGI

generated for mapped genomes against CanFam3.1 and African hunting dog (Figure S5). Since we did not detect major reference biases in African golden wolf genomes, all subsequent analyses were performed using autosomes mapped against CanFam3.1.

# 3.2 | Complex and heterogeneous demographic history

With PSMC, three possible groups of lineages with similar demographic histories could be seen: one for eastern AGW from Kenya and Ethiopia, another for the Senegalese and Algerian individuals, and another one for the two Moroccan individuals (Figure 3). The Egyptian AGW showed a very steep increase in effective population size ( $N_e$ ) towards the end of the graph that could be interpreted as introgression and could not be placed with the other samples at the plot.

While the Moroccan lineages could have benefited from a series of GSP identified at 124 kyr (Eemian), ca. 100 kyr and 80 kyr ago (Ehrmann et al., 2017; Larrasoaña et al., 2013), the eastern group showed a relatively constant decrease in population since 300 kyr ago, reaching a minimum Ne of ca. 40,000 at around 45 kyr ago, after which animals from northwest and east of the Sahara follow different demographic trajectories (Figure 3). Both Moroccan lineages seem to have followed a similar trajectory until about 28 kyr ago, after which the west Moroccan lineage has a steep reduction in population effective size, while the east Moroccan lineage remains constant. The Senegalese and Algerian individuals shared a demographic history until about 100 kyr ago, when they diverge, with a constant decrease in population for the Algerian individual and a very steep decrease for the Senegalese lineage after 24 kyr ago. Some proposed events of enhanced dry conditions in north Africa driven by Heinrich stadials could have caused decreases in these populations. Heinrich Event 6 (58.25–58.85 kyr ago, Rohling et al., 2003) appears to coincide with a reduction of population size in the Moroccan lineages, while all populations experienced a reduction after Heinrich Event 2 (ca. 23.6–25.9 kyr ago; Rohling et al., 2003) (Figure 3). However, the resolution for this time is not sufficient to confidently associate climate events with population contractions. Pleistocene climate changes in north Africa did not impact the African golden wolves equally or synchronously across their range.

After 70 kyr ago all lineages, including the Egyptian individual, show an increase in population size according to ngsPSMC (Figure 4a), although at different times. The first lineages to experience this increase are found in the western part of the range, and later in the east when the Kenyan lineage reached an effective population size of more than 16,000 by 28–30 kyr ago (Figure 4a). Strikingly, around 53 kyr ago we observe an increase in population size in the Algerian and Egyptian AGW that can be connected to a local wetter event in the northernmost fringe of the Sahara (Hoffmann et al., 2016). Western populations reached minimum effective population sizes around 30–40 kyr ago (Moroccan individuals), and 35–25 kyr (Algeria, Ethiopia), followed by local recoveries and relative maximums around 15–25 kyr ago. The Kenyan, Egyptian and Senegalese lineages (from the extremes of the distribution) show no recovery after their initial minima.

A general decrease in population sizes was observed from the last part of the last Glacial Maximum (LGM) around 18 kyr ago through the Younger Dryas (YD) with no recoveries during the wetter phase of the Holocene GSP ca. 10–5.5 kyr ago (Figure 4b). Consistent with a differentiation in paleodistribution of these animals, there are



FIGURE 3 PSMC plot of six African golden wolf individuals: Algeria, west Morocco, east Morocco, Senegal, Ethiopia, Kenya. Consensus sequences were extracted from .bam files and  $\Theta_0$  values were corrected according to the false negative rate (FNR) calculated for each actual coverage by downsampling the Kenyan genome (24x). Individual PSMC plots were bootstrapped 50 times each. Proposed Green Sahara Periods (GSPs) were included from Larrasoaña et al. (2013) and Ehrmann et al. (2017). Heinrich events (H) of local cooling were also included according to Rohling et al. (2003) and Ehrmann et al. (2017). The black line marks the speciation event (ca. 1.3 million years ago [Ma]) according to Koepfli et al. (2015) and Chavez et al. (2019) and a confidence interval of 1.10–1.5 Ma (Chavez et al., 2019). Numbers after each individual are mean FNR calculated by visually adjusting using the psmc\_plot.py program from the PSMC package.

7



FIGURE 4 ngsPSMC plots of the seven African golden wolf individuals of this study. (a) ngsPSMC plot of all individuals using maximum Ne =17,000. (b) ngsPSMC plot using maximum Ne =2000. Cooling events (H) are the same as in Figure 3. Local events of wetter conditions are marked up (north Sahara: 65–61 ka, 52.5– 50.5 ka and 37.5–33 ka (Hoffmann et al., 2016)) and down (Sahel: 55–60 ka; (Tjallingii et al., 2008; Weldeab et al., 2007)) of Figure 4a. HO =Holocene Optimum as in Larrasoaña et al. (2013). YD =Younger Dryas.

general increases of effective population size that start at different times: while the easternmost (Ethiopia, Kenya) lineages reach a local minimum in population size around 3–3.5 kyr ago, the northwestern Moroccan lineages experience their minimum more recently at ca. 2.7–3 kyr ago and Senegal and Algeria reach theirs around 2–2.2 kyr ago. The western populations all reach their local maximum around 1000 years ago, to decrease again afterwards (Figure 4b). A final steep increase in population size is seen only in the Egyptian and Kenyan lineages ca. 150 and 90 years ago, respectively. While the first one could be attributed to local hybridization with another species, the Kenyan increase could be the result of two converging lineages of the same species. Our analysis failed to identify any more changes in population size after 70 years ago (ca. 23 generations), and attempts to do so with IBD-based methods did not yield any results. TABLE 1 Mean values and standard deviations of genome wide thetas per site and neutrality tests of four populations: northwest African Golden Wolves (AGW) (Algeria, east Morocco, west Morocco, Senegal), east AGW (Ethiopia, Kenya), grey wolves of the Middle East (ME) (S. Arabia, Iran, Syria), and coyote (California, Midwest, Mexico)

|                   | Thetas                       |                           |                            |                           |                           | Neutrality tests            |                              |                              |                  |                           |
|-------------------|------------------------------|---------------------------|----------------------------|---------------------------|---------------------------|-----------------------------|------------------------------|------------------------------|------------------|---------------------------|
| Populations       | θ                            | θπ                        | $\boldsymbol{\theta}_{FL}$ | θ                         | θ <sup>Γ</sup>            | Tajima's D                  | Fu's F                       | Fu's D                       | Fay's H          | Zeng's E                  |
| Northwest<br>AGW  | 8.52 ± 3.96*10 <sup>-4</sup> | $8.55 \pm 4.15^* 10^{-4}$ | $4.62 \pm 2.35^* 10^{-4}$  | $1.7 \pm 0.77^* 10^{-3}$  | $1.28 \pm 0.59^* 10^{-3}$ | $-0.34 \pm 3.98^{*}10^{-1}$ | $7.92 \pm 4.32^* 10^{-1}$    | 9.06 ± 3.63*10 <sup>−1</sup> | $-1.54 \pm 0.41$ | $9.13 \pm 2.08*10^{-1}$   |
| East AGW          | $7.77 \pm 4.42^* 10^{-4}$    | $7.93 \pm 4.53^*10^{-4}$  | $4.61 \pm 2.99^* 10^{-4}$  | $1.17 \pm 0.64^* 10^{-3}$ | $9.55 \pm 5.43^* 10^{-4}$ | $2.09 \pm 6.12^* 10^{-1}$   | $1.04 \pm 0.65$              | $1.04 \pm 0.55$              | $-1.22 \pm 0.58$ | $7.79 \pm 2.69^* 10^{-1}$ |
| ME grey<br>wolves | 8.47 ± 4.06*10 <sup>-4</sup> | $8.49 \pm 4.19^*10^{-4}$  | $4.87 \pm 2.53^*10^{-4}$   | $1.49 \pm 0.71^* 10^{-3}$ | $1.17 \pm 0.56^* 10^{-3}$ | $-0.19 \pm 4.02^*10^{-1}$   | 8.03 ± 4.45*10 <sup>-1</sup> | 8.87 ± 3.77*10 <sup>-1</sup> | -1.46 ± 0.44     | $8.81 \pm 2.2^* 10^{-1}$  |
| Coyote            | $7.61 \pm 3.88^* 10^{-4}$    | $7.71 \pm 4.02^*10^{-4}$  | $4.24\pm2.48^*10^{-4}$     | $1.33 \pm 0.68^* 10^{-3}$ | $1.05 \pm 0.54^* 10^{-3}$ | $-0.19 \pm 4.02^{*}10^{-1}$ | $8.03 \pm 4.45^* 10^{-1}$    | $8.87 \pm 3.77*10^{-1}$      | -1.46 ± 0.44     | $8.81 \pm 2.2^* 10^{-1}$  |
| We considered     | 50-kb nonoverlapp            | aing windows acros        | s the whole genome         | e and filtered out tl     | hose windows with         | a number of sites of        | utside the 99.7% of          | the distribution (me         | ean ± 3 standarc | l deviations). Theta      |

Li's theta (Fu & Li, 1993).  $\Theta_{H}$ : Fay and Wu's theta (Fay & Wu, 2000).  $\Theta_{L}$ : Fu and Li's L (Fu & Li, 1993). Neutrality tests: Tajima's D (Tajima, 1989); Fu's F and D (Fu & Li, 1993); Fay's H (Zeng et al., 2006); Zeng's statistics were calculated dividing by the total number of sites. Neutrality tests were averaged per window.  $\Theta_{w'}$ : Watterson's theta (Watterson, 1975).  $\Theta \pi$ : Nucleotide diversity (Tajima, 1989).  $\Theta_{e_1}$ : Fu and E (Zeng et al., 2006).

| (A) African golden wolves            |                   |                       |                         |                                  |                       |                       |                       |              |
|--------------------------------------|-------------------|-----------------------|-------------------------|----------------------------------|-----------------------|-----------------------|-----------------------|--------------|
|                                      | Algeria           | Egypt                 | East Morocco            | West Morocco                     | Senegal               | Ethiopia              | Kenya                 |              |
| Distances (km)                       |                   | $2.01 \times 10^{-1}$ | $6.09 \times 10^{-2}$   | $2.05 \times 10^{-1}$            | $9.5 \times 10^{-2}$  | $2.38 \times 10^{-1}$ | $4.05 \times 10^{-1}$ | Algeria      |
|                                      | 2590              |                       | $1.67 \times 10^{-1}$   | $3.04 \times 10^{-1}$            | $2.09 \times 10^{-1}$ | $2.92 \times 10^{-1}$ | $4.4 \times 10^{-1}$  | Egypt        |
|                                      | 920               | 3400                  |                         | $1.7 \times 10^{-1}$             | $8.11 \times 10^{-2}$ | $2.15 \times 10^{-1}$ | $3.62 \times 10^{-1}$ | East Morocco |
|                                      | 1300              | 3780                  | 390                     |                                  | $2.19 \times 10^{-1}$ | $3.46 \times 10^{-1}$ | $5.04 \times 10^{-1}$ | West Morocco |
|                                      | 3250              | 5270                  | 2420                    | 2060                             |                       | $2.35 \times 10^{-1}$ | $4.04 \times 10^{-1}$ | Senegal      |
|                                      | 4610              | 2530                  | 5170                    | 5420                             | 6110                  |                       | $2.89 \times 10^{-1}$ | Ethiopia     |
|                                      | 5170              | 3290                  | 5630                    | 5840                             | 6220                  | 850                   |                       | Kenya        |
| (B) Grey wolves                      |                   |                       |                         |                                  |                       |                       |                       |              |
|                                      |                   | S. Arabia             | -                       | ran                              | Syria                 |                       |                       |              |
| Distances (km)                       |                   |                       | H                       | $1.73 \times 10^{-1}$            | 2.54 × 1              | 0 <sup>-1</sup>       | Ś                     | Arabia       |
|                                      |                   | 1270                  |                         |                                  | 1.66 × 1              | 0 <sup>-1</sup>       | <u> </u>              | an           |
|                                      |                   | 1390                  | -                       | 1360                             |                       |                       | Ś                     | ria          |
| (C) Coyotes                          |                   |                       |                         |                                  |                       |                       |                       |              |
|                                      |                   | California            | 2                       | Mexico                           | Midwest               |                       |                       |              |
| Distances (km)                       |                   |                       | F                       | $1.62 \times 10^{-1}$            | $1.15 \times 10^{-1}$ | 0 <sup>-1</sup>       | U                     | alifornia    |
|                                      |                   | 1060                  |                         |                                  | 1.34 × 1              | 0 <sup>-1</sup>       | 2                     | lexico       |
|                                      |                   | 2770                  |                         | 2590                             |                       |                       | Σ                     | lidwest      |
| $F_{ m cr}$ values were calculated w | vith the NGSTOOLS | package. Distances in | ı km between coordinato | es were calculated in Marble (Li | nux).                 |                       |                       |              |

TABLE 2 Pairwise genome wide  $F_{ST}$  values of African golden wolves (A), grey wolves (B) and coyotes (C)

We observed similar behavior for all four populations in Watterson's theta ( $\Theta_W$ ) (Watterson, 1975), nucleotide diversity ( $\Theta \pi$ ) (Tajima, 1989), and Fu & Li's theta ( $\Theta_{FL}$ ) (Fu & Li, 1993) (Table 1, Figure S6A). Although means showed very similar trends, Fu and Li's ( $\Theta_L$ ) (Fu & Li, 1993) and Fay and Wu's ( $\Theta_H$ ) (Fay & Wu, 2000) thetas were very different between the northwest and eastern African golden wolf populations.

Neutrality tests show very similar results in all four populations considered (Table 1, Figure S6B). While Tajima's D (Tajima, 1989) is negative and very close to zero in northwestern AGW, North American coyotes and grey wolves from the Middle East; D is positive for eastern AGW, which could be indicative of an ongoing population contraction (Durrett, 2008; Zeng et al., 2006). However, trends were very similar among all populations. These values are not significantly different from zero for a population of four individuals according to Tajima (1989) (-0.876, 2.336, *p*-value: .001) (Tajima, 1989), so the neutral mutation hypothesis could explain the DNA polymorphism of these neutral sites. Fu and Li's D and F are also not significantly different from zero (Fu and Li's D and F intervals: [-1.87, 2.38] and [-1.96, 2.78],  $\alpha$  = .01) (Fu & Li, 1993), so our values are within the normal variation expected under the neutral mutation hypothesis. This is consistent with nongenic data and does not provide evidence for recent population expansions or contractions.

An exception occurs with Fay's H (Fay & Wu, 2000) and Zeng's E (Zeng et al., 2006) neutrality tests. Fay's H was uniformly negative and Zeng's E was uniformly positive in all four populations studied (Table 1, Figure S6B). A slightly higher value for northwestern than eastern African golden wolves was detected in Zeng's E values, which could be representative of a recent population growth (Zeng et al., 2006). However, values for all four populations were very similar and it was difficult to detect major differences. Fay's

H trend was slightly higher in eastern than in northwestern African golden wolves, which could be representative of a recent population decline (Fay & Wu, 2000; Zeng et al., 2006). However, all standard deviations overlapped with each other, so they were not significantly different (Table 1, Figure S6B).

We tested whether the three population model of the PSMC plot was consistent for genome wide  $F_{ST}$  values across lineages without accounting for genic regions. Surprisingly, the Algerian, east Moroccan and Senegal individuals showed a very low  $F_{ST}$  between lineages (0.06–0.09) (Table 2A). These  $F_{st}$  values were lower than those found within North American coyote lineages at similar or even shorter geographic distances (Table 2C), although we found a greater standard deviation in genome wide values of  $F_{sT}$  (Table S5). These results were striking considering that coyotes are known for being highly mobile animals, with high rates of heterozygosity and forming populations much closer to panmixia and less affected by isolation by distance than grey wolves (DeCandia et al., 2019; Heppenheimer, Brzeski, et al., 2018; Heppenheimer, Cosio, et al., 2018; Koblmüller et al., 2012; Pilot et al., 2006). Although the west Moroccan individual was found less than 1000 km away from the east Moroccan individual, it had large genome wide pairwise  $F_{st}$ values with the east Morocco, Senegal and Algerian individuals (Table 2A). Algeria, east Morocco and Senegal individuals showed a fairly similar genetic distance to both east African lineages (Ethiopia and Kenya).

### 3.3 | Different histories of inbreeding

We used an SFS-based method to analyze the proportion of singletons with respect to the rest of sites and infer genome wide

TABLE 3 Values of inbreeding coefficients (Fi) using genomewide genotype likelihood and SNP-based approaches (ngsF, PLINK and ROHan, respectively) and using the ROH-based method of McQuillan et al. (2008) with ROHs calculated with genotype likelihood and SNP-based approaches (ROHan and PLINK, respectively)

|         |              | F <sub>i (ngsF)</sub>   | F <sub>i (PLINK)</sub>  | F <sub>ROH (PLINK)</sub><br>500 kb | F <sub>ROH (ROHan) min</sub><br>500 kb | F <sub>ROH (ROHan) med</sub><br>500 kb | <b>F<sub>ROH (ROHan)</sub></b><br>max 500 kb |
|---------|--------------|-------------------------|-------------------------|------------------------------------|--|--|--|
| AGW     | Algeria      | 1.17 × 10 <sup>-2</sup> | 4.66 × 10 <sup>-2</sup> | 2.34 × 10 <sup>-7</sup>            | 0                                      | 0                                      | $2.27 \times 10^{-4}$                        |
|         | Egypt        | 1.35 × 10 <sup>-1</sup> | 6.49 × 10 <sup>-2</sup> | 8.46 × 10 <sup>-6</sup>            | 0                                      | 0                                      | 0  |
|         | East Morocco | $5.88 \times 10^{-4}$   | 0                       | 0                                  | 0                                      | 0                                      | $2.27 \times 10^{-4}$                        |
|         | West Morocco | $2.51 \times 10^{-1}$   | 2.93 × 10 <sup>−1</sup> | $3.34 \times 10^{-6}$              | 0                                      | 0                                      | 0  |
|         | Senegal      | $2.65 \times 10^{-2}$   | $8.49 \times 10^{-2}$   | 7.17 × 10 <sup>−7</sup>            | 0                                      | 0                                      | $6.81 \times 10^{-4}$                        |
|         | Ethiopia     | $2.20 \times 10^{-5}$   | 0                       | 9.70 × 10 <sup>-4</sup>            | 0                                      | 0                                      | $2.27 \times 10^{-4}$                        |
|         | Kenya        | 6.04 × 10 <sup>-2</sup> | 1.47 × 10 <sup>-1</sup> | 9.53 × 10 <sup>-4</sup>            | 1.16 x 10 <sup>-2</sup>                | 2.68 x 10 <sup>-2</sup>                | $2.68 \times 10^{-2}$                        |
| GW      | S. Arabia    | 8.47 × 10 <sup>-2</sup> | $1.81 \times 10^{-1}$   | $1.47 \times 10^{-5}$              | 0                                      | 0                                      | $4.54 \times 10^{-4}$                        |
|         | Iran         | $1.90 \times 10^{-5}$   | 0                       | $1.05 \times 10^{-5}$              | 4.76 x 10 <sup>-3</sup>                | 1.18 x 10 <sup>-2</sup>                | 1.66 × 10 <sup>-2</sup>                      |
|         | Syria        | 7.21 × 10 <sup>-2</sup> | $1.57 \times 10^{-1}$   | 1.77 × 10 <sup>-5</sup>            | 0                                      | 0                                      | $4.54 \times 10^{-4}$                        |
| Coyotes | California   | 9.60 × 10 <sup>-5</sup> | 0                       | $4.27 \times 10^{-5}$              | 1.86 x 10 <sup>-2</sup>                | 4.13 x 10 <sup>-2</sup>                | $4.70 \times 10^{-2}$                        |
|         | Mexico       | 9.77 × 10 <sup>−3</sup> | 5.70 × 10 <sup>-2</sup> | $2.10 \times 10^{-5}$              | 0                                      | 0                                      | $8.17 \times 10^{-3}$                        |
|         | Midwest      | $3.89 \times 10^{-4}$   | $5.01 \times 10^{-3}$   | 8.58 × 10 <sup>-7</sup>            | 0                                      | 0                                      | $2.27 \times 10^{-4}$                        |

Values higher than 0.1 are marked in grey.

MOLECULAR ECOLOGY - WILFY

heterozygosity per individual corrected by genome wide depth (Gopalakrishnan et al., 2018). We found that African golden wolves had a mean genome wide heterozygosity of 7.83 ×  $10^{-4}$  (standard deviation (SD):  $1.82 \times 10^{-4}$ ); closer to North America coyotes (7.95 ×  $10^{-4}$ , SD:  $9.38 \times 10^{-5}$ ) than to grey wolves from the Middle East ( $6.33 \times 10^{-4}$ , SD:  $1.89 \times 10^{-4}$ ) and higher than domestic dogs ( $5.17 \times 10^{-4}$ , SD:  $9.71 \times 10^{-5}$ ) (Figure S7). However, the west Moroccan individual had a low genome wide heterozygosity ( $5.01 \times 10^{-4}$ ).

Although inbreeding coefficients varied among individuals, the Egyptian, Kenyan and west Moroccan African golden wolves were more inbred than the rest of the individuals of the species (Table 3), partially consistent with being at the extremes of the distribution. PLINK and ngsFs estimation of inbreeding coefficient yielded some differences that could be explained by differences in the calculation methods of each software. Our efforts to determine the influence or bias across genomes of varying depths of coverage (7×, 9×, 11.2× and 15–24×) found that ngsF is less sensitive to changes in coverage than PLINK, and tends to overestimate the homozygosity of the Kenyan over the Egyptian individual (Table S6). In any case, PLINK also identified the Kenyan, Egyptian, and west Moroccan individuals as the most inbred.

This approach could not be replicated, however, when using ROH-based methods to estimate inbreeding. Except for samples with a high mean genome wide coverage (Kenyan AGW – 26×, Iranian grey wolf – 26×, Californian coyote – 23×), we detected almost no ROHs in AGWs (Table 3). ROH-based calculations mostly underestimated Fi ratios detected by both ngsF and PLINK, especially in lower coverage genomes. This failure could be due to the dependency of these methods to detect long stretches with homozygous sites when using low coverage samples, or on the wide variety in methods and thresholds to infer ROHs (Sams & Boyko, 2019).



FIGURE 5 Times of divergence between pairs of lineages of African golden wolves.  $\delta^{18}$ O data from the North Greenland Ice Core Project (NGICP members, 2004) are shown, along with main glacial and interglacials periods, for the last 125,000 years. Intervals for divergence of pairs of lineages were considering the top 5% (light grey) and 1% (dark grey) of values for polynomial equations of adjusted graphs to the data points after  $R^2$  = 0.99. For further explanation, see Table S8. H bars, Heinrich events; YD, Younger Dryas.

Using a 50-kb windows-based approach across the west Moroccan AGW and the east Moroccan AGW genomes (Figure S8), we observed several regions where heterozygosity approaches zero in the west Moroccan individual. This trend is especially remarkable when compared with the east Moroccan individual, who only has a single possible ROH in chromosome 11. Furthermore, the Senegal sample (7× coverage) did not show any increased homozygosity as compared to other samples of higher coverage.

The west Moroccan genome is the African golden wolf with the highest inbreeding coefficient observed in this data set and the east Moroccan is the one with the lowest. This difference between geographically close lineages is remarkable when considering that other lineages more distant from each other share more alleles, have higher rates of heterozygosity, and lower pairwise Fst values (Table 2A).

### **Divergence during glacial periods** 3.4

We observed highly consistent results in divergence time estimation regardless of genome coverage (see Supporting Information File: "Replicability tests: results", Table S7). MiSTI also gave coherent results regardless of the time segment definition used (Table S8), so we used time segments defining humid/dry periods of Sahara according to Larrasoaña et al. (2013). Every divergence time estimation was run covering all time steps until 150 kyr ago. We also found good consistency between our MiSTI and Cavalli-Sforza divergence estimates except for those divergence times involving either the Ethiopian or the west Moroccan genomes (Table S9), which had the lowest genome wide heterozygosity (see Table 3).

We found a recurrent pattern of isolation of local lineages at times corresponding to glacial maxima in the Northern Hemisphere. We failed to detect the splitting time of three lineages (Algeria, Senegal, east Morocco) (Figure 5, Table S10), which is consistent with very low genome wide pairwise  $F_{ST}$  values (Table 2). Since the last splitting time considered by MiSTI was ca. 3 kyr ago, the separation of east Morocco, Algeria and Senegal lineages (referred to as the EMAS cluster) may have happened very recently (less than 3 kya), or they may maintain geneflow. Splitting times of the EMAS cluster from Egyptian AGW happened around 9-1 kyr ago (Table 1), which overlaps or follows the Younger Dryas in north Africa ca. 10-13 kyr ago (Ehrmann et al., 2017).

Most lineage divergences happened between ca. 16 kyr and 30 kyr ago (Table S10), overlapping with cold Heinrich stadials (Ehrmann et al., 2017; Heinrich, 1988; Rohling et al., 2003). The west Moroccan lineage diverged from the EMAS cluster ca. 16-21 kyr ago (Table S10), overlapping with Heinrich stadial H2 (Ehrmann et al., 2017; Heinrich, 1988; Rohling et al., 2003), as well as the divergence time of Ethiopian and Kenyan lineages, which probably happened around 17.5 kyr ago. The Kenyan lineage shows a more widespread divergence with all other lineages in the north, with a likely splitting time of 22.3 kyr with the EMAS cluster, 24.1 kyr with the Egyptian lineage, and ca. 29.4 kyr with the west Moroccan lineage, which could have followed cooling periods known as Heinrich stadials

H2 and H3. Divergence times between the Ethiopian lineage and EMAS cluster and the Egyptian lineage probably happened between 26.9-30.3 kyr ago, coinciding with Heinrich stadial H3 (Figure S9B, Table S10). We have not found any signatures of divergence after the Holocene GSP ca. 10-5.5 kyr ago (Drake et al., 2013; Larrasoaña et al., 2013; Smith, 2012).

After calculating migration rates during time segments, we observe a general trend with increased migration rates at around 80 kyr, 100 kyr and 120 kyr for some lineages, therefore increasing gene flow (Table S11). This is consistent with previous paleoclimatological data that point to three GSPs around those periods (Drake et al., 2013; Ehrmann et al., 2017; Larrasoaña et al., 2013; Smith, 2012). Furthermore, we have detected certain periods of increased migration that could coincide with minor wet phases reported at ca 52.5-50.5 kyr and 37.5-33 kyr ago in the northern Sahara (Hoffmann et al., 2016), such as west Morocco  $\rightarrow$ Egypt (35.2-51 kyr) and Senegal →east Morocco (29.9-36.3 kyr). However, time segments as defined by PSMC are wide and more resolution is needed to properly infer when these migration rates increased in the past.

### DISCUSSION 4

# 4.1 | Climate change-driven differentiation of African golden wolf lineages

Our results indicate that the divergence times of all lineages of African golden wolves occurred during the latest Pleistocene, between 50 and 10.5 kyr ago, with most divergence times clustered between 16 and 30 kyr ago, broadly coinciding with the Late Glacial Maximum (LGM) at ca. 33-16 kyr ago (Clark et al., 2009) (Figure 5). Strikingly, all divergence events are associated to either Heinrich stadials H1 to H4 or to the Younger Dryas, clearly linking divergence times with periods of enhanced dry conditions in the Sahara. We hypothesize that these 1500-year-long drier periods reinforced the isolation of mesic and water-dependent species in refugia (Brito et al., 2014), where isolation during hundreds of generations caused genetic divergence. Although some relative warming and cooling periods have been proposed since the middle Holocene that could be linked to relative changes in population size, the worldwide impact of these local events is heavily debated (Neukom et al., 2019) and we could not find a direct correlation.

It has been proposed that the onset of wetter conditions during later GSP permitted mesic species to expand and reconnect, a pattern seen in a wide number of species in north Africa (Ben Faleh et al., 2012; Cosson et al., 2005; Lerp et al., 2011; Nicolas et al., 2009; Rato et al., 2007). The last GSP (ca. 10-5.5 kyr ago) reconnected animals isolated in several refugia (Kuper & Kröpelin, 2006; Linstädter & Kröpelin, 2004; Yeakel et al., 2014). However, we have not detected strong signatures of gene flow between African golden wolf lineages during this period (except for the EMAS cluster), suggesting that this period was not wet enough to erase patterns of

genetic differentiation. This is consistent with the drier nature of the Holocene GSP in comparison with previous GSP (Ehrmann et al., 2017).

An exception to this is the east Moroccan AGW. While the west Moroccan population seems to have been isolated for the last 18,000 years and have undergone a high degree of inbreeding, the east Moroccan individual presents one of the highest levels of genome wide heterozygosity and is genetically close to lineages from Senegal and Algeria, forming the EMAS cluster. It is characterized by a wide distribution (3200 km wide), a relatively recent divergence (less than 3000 years ago), and the exclusion of the west Moroccan lineage, all of which are intriguing features. African golden wolves are known to have large home ranges (up to  $22 \text{ km}^2$  in Kenya, Fuller et al., 1989, and 64.8 km<sup>2</sup> in Ethiopia, Admasu et al., 2004) and large dispersal capabilities (in Tunisia, an individual was detected to have walked 230 km in 98 days, Karssene et al., 2018). A recent study (Eddine et al., 2020) detected almost no genetic structuring in a wide variety of samples from Algeria and Tunisia that encompassed roughly 1200 km. The east and west Moroccan individuals are less than 1000 km apart from each other. Why then would the west Moroccan individual present such high levels of inbreeding and be isolated from other African golden wolves for nearly 18,000 years?

### 4.2 | Atlas: Refugium during glacial times

Previous studies (Cosson et al., 2005; Husemann et al., 2014; Leite et al., 2015; Rato et al., 2007) have identified the Atlas Mountains of Maghreb as a glacial refugium during drier times for a variety of species. The highly heterogeneous landscape is formed by several mountain ranges (Anti-Atlas, High Atlas, Middle Atlas, Rif) with some of the highest peaks in north Africa (Jbel Toubkal, 4165 m), deep humid valleys, and relict high mountain cedar forests (Abel-Schaad et al., 2018) with isolated and endangered populations of *Macaca sylvanus* (Ciani et al., 2005) and rivers that act as barriers to aridadapted species (Rato et al., 2007). These features are key for the establishment of "refugia within refugia", with high endemism, barriers that subdivide habitats and the potential for genetic divergence and speciation (Dufresnes et al., 2020; Gómez & Lunt, 2007).

The isolation of the west Moroccan lineage could have arisen in a variety of ways. The "rear edge hypothesis" of Hampe and Petit (2005) proposes a scenario where previously isolated demes in interglacial refugia converge in an admixture zone, leaving behind groups that have undergone local adaptations in response to abiotic stresses. This could explain why the east Moroccan individual appears to have merged with the Algerian and Senegalese lineages in recent times, even though the demographic histories of the west and east Moroccan individuals appear overlapped until 60,000 years ago (Figure 3). In this scenario, the west Moroccan lineage could be one of the groups from the rear edge that failed to reconnect with others. Hampe and Petit (2005) explain how relict populations are not necessarily the source of postglacial expansions and this could be the case of the west Moroccan lineage. - MOLECULAR ECOLOGY - WILEY

Another possibility is that the west Moroccan lineage belongs to a population that has not contributed to the EMAS cluster for thousands of years. In a previous study on red fox (Vulpes vulpes), Leite et al. (2015) detected two main lineages in northwestern Africa: animals from Atlantic Sahara to Tunisia (Maghreb 1) and a more isolated lineage restricted to the valley of Oukaimeden, north of the High Atlas Mountains (Maghreb 2). The west Moroccan individual was found north of the High Atlas, which is the tallest mountain range of the Atlas Mountains (over 4000 m high) and could have restricted movements of African golden wolves. However, African golden wolves have been observed living at medium to high elevations (2000-3000 m) in Algeria (Amroun, Oubellil, et al., 2014), Ethiopia (Admasu et al., 2004; Gaubert et al., 2012; Rueness et al., 2011; Simeneh, 2010), Tanzania (Temu et al., 2018), and Morocco (Cuzin, 2003; Urios et al., 2015; Waters et al., 2015). There is a possibility, therefore, that the west Moroccan lineage represents a different, adapted ecotype of the same species of African golden wolf, parallel to what has been seen with North American grey wolves, where ecology drives differentiation in local populations (Leonard, 2014). Further studies and more sampling across the High Atlas Mountains will reveal whether African golden wolves follow a localized pattern of specialized demes associated with different ecosystems.

### 4.3 | Recent admixture in the EMAS cluster

In a previous study based on 13 autosomal microsatellites and mitochondrial control region sequences, Eddine et al. (2020) failed to identify structure between Algerian and Tunisian African golden wolves, and estimated an increase in population size between 3840 and 6720 years ago. However, across this time period we found an almost constant decrease of population size with ngsPSMC (Figure 4b). Higher values of  $\Theta_{\rm H}$  indicate a greater abundance of high-frequency variants than in northwest AGW, which could suggest a high number of shared variants among the northwestern individuals and possible recent gene flow between different lineages of wolves. In contrast, eastern AGW show a lower abundance of high frequency variants, which is possibly indicative of a process of local divergence of the two lineages in the region (Ethiopia, Kenya).

The high genome wide heterozygosity observed in the EMAS cluster could come from a recent admixture of distant lineages (as we failed to detect a divergence time using MiSTI), increasing genetic variability. Previous work in red fox also detected a wide-spread lineage that extended from Atlantic Sahara to Tunisia (Leite et al., 2015). Allele-based estimations of Ne have been found to be affected by recent admixture processes in human populations (Lohmueller et al., 2010) and recent studies have focused on independently ascertaining demographic histories of genomic portions of diverse origin in admixed individuals (Browning & Browning, 2015; Browning et al., 2018; Skov et al., 2020). Future IBD-based studies of whole genomes of individuals from the EMAS cluster could help disentangle the hypotheses of a recent Neolithic expansion vs. a recent admixture that enriched the diversity of local demes.

### 4.4 | Dynamic demographic histories

With PSMC we have found a complex demographic history, where lineages do not follow the same trends of expansion or contraction through time (Figure 3). This could be due to a variety of causes. First, current lineage locations might not represent those of the past. Consistent with the detected pattern in the west Moroccan sample, other lineages might have remained isolated for thousands of generations in a refugium and not have contributed for several GSP to the postglacial admixed populations, therefore increasing genome wide inbreeding and decreasing  $N_e$ .

Second, GSP did not lead to homogeneous savannah landscapes throughout the desert, a circumstance that has been shown for the two GSP for which more paleoenvironmental evidence is available (Eemian, ca. 122-128. kyr ago; and Holocene, 5.5-10 kyr ago). Complex topography modulated the S-N and W-E gradient of decreasing monsoonal rainfall, thereby creating areas of more or less aridity with different environments (Larrasoaña et al., 2013). With such a heterogeneous landscape, mild GSP would not have had much impact on local populations closer to arid zones while other populations would have benefitted from more humid environments. Different refugia within the Sahara could have hosted distinct populations of African golden wolves with diverse reactions to climate change. Further, competitive pressure from black-backed jackals (Van Valkenburgh & Wayne, 1994) and/or hyaenas (Kebede, 2017; Kingdon, 2013) may not have been equal throughout the entire Sahara region if their past distributions were not entirely overlapping with the distribution of African golden wolves.

A third possibility is that we are detecting different ecotypes of the same species. In grey wolves, the onset of postglacial conditions benefited some populations, while others, possibly adapted to colder conditions and/ or bigger prey, declined or went extinct (Ersmark et al., 2016; Leonard et al., 2007). Although current African golden

wolves are generalist predators and maintain a varied diet composed of small mammals, birds, plants, insects and waste (Amroun, Oubellil, et al., 2014; McShane & Grettenberger, 1984), predation of gazelles has been detected in Niger, Kenya and Tanzania (Fuller et al., 1989; McShane & Grettenberger, 1984; Moehlman, 1986; Temu et al., 2016, 2018). It is unknown if the decline of gazelles after ca. 25 kyr ago (Lerp et al., 2011) impacted local populations of AGW, or if some of them were more specialized predators of ungulates. Another factor is habitat preference. While African golden wolves have been detected in all sorts of environments in Algeria (Amroun et al., 2006; Amroun, Bensidhoum, et al., 2014; Amroun, Oubellil, et al., 2014), Tunisia (Karssene et al., 2017, 2018), Morocco (Cuzin, 2003) and Niger (McShane & Grettenberger, 1984), some prefer farmlands and covered woodlands over open environments in Ethiopia (Admasu et al., 2004; Simeneh, 2010). In places where AGW are sympatric with competitors such as black-backed jackals, they are found mostly in open grasslands and rarely in woodlands (Fuller et al., 1989; Moehlman, 1986). Human pressure is another important factor to take into consideration. African golden wolves are found close to highly anthropized zones in Algeria and Ethiopia (Admasu et al., 2004; Amroun, Bensidhoum, et al., 2014; Simeneh, 2010), while they tend to avoid humans in Morocco, where higher human pressures exist (Brito et al., 2014; Cuzin, 2003). While it has been proposed that increasing anthropization could have driven a recent population expansion in northeast Africa, either through the arrival of caprid livestock or through predator control (Eddine et al., 2020), almost negligible amounts of livestock remains were found in faecal diet studies in Niger, Tanzania and Ethiopia (Admasu et al., 2004; Fuller et al., 1989; McShane & Grettenberger, 1984; Simeneh, 2010). It remains plausible to consider that different abiotic and biotic factors could have driven different populations of African golden wolf to specialize into ecotypes. It is unknown whether current African golden wolves arose from bottlenecked specialized ecotypes from the past, but it is a possibility that cannot be excluded.



FIGURE 6 Dust flux record of Ocean Drilling Program (ODP) Leg 160, site 967 (Larrasoaña et al., 2003) of eastern Mediterranean Sea from the last three million years (Myr). We have incorporated an estimation of the onset of the mid-Pleistocene climate Transition (MPT) (McClymont et al., 2013), the shift from 41-kyr- to 100-kyr-long aridity cycles and a represents a breakpoint in north African aridity (Trauth et al., 2009). Estimation of the speciation event of African golden wolves circa 1.32 million years ago (Ma) is also included with confidence intervals (blue: 1.0–1.65, Koepfli et al., 2015; red: 1.1–1.5, Chavez et al., 2019).

tribution that received support from the Spanish Ministry of Economy and the exception Competitiveness under the 'Centro de Excelencia Severo Ochoa er, "jackal-like" 2013-2017' program, SEV-2012-0262.

### AUTHOR CONTRIBUTIONS

J.A.L. developed the initial concept which was further developed with C.S., and B.V.H. V.U. provided the sample of the west Moroccan African golden wolf. C.S. conducted data analyses. B.V.H. and J.A.L. assisted with interpretation of genomic results. J.C.L. assisted with interpretation of paleoclimatological results. All authors discussed the results and provided edits and approval of the manuscript.

### DATA AVAILABILITY STATEMENT

All code generated in this study have been made available in Github: https://github.com/cdomsar/DivgLupaster. Newly sequenced African golden wolf has been deposited into EMBL-EBI server under accession number ERP123054.

### ORCID

Carlos Sarabia b https://orcid.org/0000-0002-6980-3480 Bridgett vonHoldt b https://orcid.org/0000-0001-6908-1687 Juan C. Larrasoaña b https://orcid.org/0000-0003-4568-631X Vicente Uríos https://orcid.org/0000-0001-6444-379X Jennifer A. Leonard b https://orcid.org/0000-0003-0291-7819

### REFERENCES

- Abel-Schaad, D., Iriarte, E., López-Sáez, J. A., Pérez-Díaz, S., Sabariego Ruiz, S., Cheddadi, R., & Alba-Sánchez, F. (2018). Are Cedrus atlantica forests in the Rif Mountains of Morocco heading towards local extinction? *Holocene*, 28(6), 1023–1037. https://doi. org/10.1177/0959683617752842
- Abraham, G., & Inouye, M. (2014). Fast principal component analysis of large-scale genome-wide data. *PLoS One*, 9(4), 1–5. https://doi. org/10.1371/journal.pone.0093766
- Admasu, E., Thirgood, S. J., Bekele, A., & Laurenson, M. K. (2004). Spatial ecology of golden jackal in farmland in the Ethiopian Highlands. African Journal of Ecology, 42(2), 144–152. https://doi. org/10.1111/j.1365-2028.2004.00497.x
- Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19(9), 1655–1664. https://doi.org/10.1101/gr.094052.109
- Amroun, M., Bensidhoum, M., Delattre, P., & Gaubert, P. (2014). Feeding habits of the common genet (genetta genetta) in the area of Djurdjura, north of Algeria. *Mammalia*, 78(1), 35–43. https://doi. org/10.1515/mammalia-2012-0111
- Amroun, M., Giraudoux, P., & Delattre, P. (2006). A comparative study of the diets of two sympatric carnivores - The golden jackal (Canis aureus) and the common genet (Genetta genetta) - In Kabylia, Algeria. Mammalia, 70(3-4), 247-254. https://doi.org/10.1515/ MAMM.2006.040
- Amroun, M., Oubellil, D., & Gaubert, P. (2014). Trophic ecology of the Golden Jackal in Djurdjura National Park (Kabylie, Algeria). Revue D'ecologie (La Terre Et La Vie), 69(3-4), 304-317.
- Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/proje cts/fastqc
- Aulagnier, S. (1992). Zoogéographie des mammifères du Maroc: de l'analyse spécifique à la typologie de peuplement à l'échelle régionale. (PhD thesis). Université des sciences et techniques de Montpellier 2.

In this study we sampled the extremes of the distribution that were most probably not directly (re)connected (with the exception of the EMAS cluster) after the Younger Dryas; however, "jackal-like" forms have been discovered in several archaeological sites with dates closer to Holocene GSP conditions (Guagnin, 2015; di Lernia, 1998; Sereno et al., 2008) that could indicate higher connectivity among locations that we have not detected with our data set. A higher number of samples from more diverse locations -and possibly, historic or ancient DNA – could ascertain whether extinct ecotypes of African golden wolf roamed the once green Sahara landscape.

## 4.5 | Speciation and the mid-Pleistocene transition

The mid-Pleistocene transition was characterized by the shift of 41 kyr long glacial-interglacial cycles to much longer and more intense 100 kyr long glacial-interglacial cycles (McClymont et al., 2013) and a general trend of increased aridity in northern Africa (DeMenocal, 2004; Trauth et al., 2009). Based on genetic evidence, two studies suggested a date of 1.32 Ma for the speciation event that gave rise to AGW, with a confidence interval of either 1.0-1.65 Ma (Koepfli et al., 2015) or 1.1-1.5 Ma (Chavez et al., 2019). This may be consistent with the oldest "jackal-like" fossils referred to as golden jackals (Canis aureus) dating back to the Middle Pleistocene in Morocco (Geraads, 2011). Overall, these data suggest that a shift to enhanced aridity in the Sahara at  $1.44 \pm 0.2$  Ma (Trauth et al., 2009) drove the speciation of African golden wolves (Figure 6) (Chavez et al., 2019; Koepfli et al., 2015) and reinforces the view that the mid-Pleistocene transition was a prime driver of speciation events in north Africa (DeMenocal, 2004). The increase of aridity ca. 1.2-1.4 Ma also coincides with the expansion and formation of new haplogroups of scimitar-horned onyx (lyengar et al., 2007), the appearance of several clades of rodents (Praomys rostratus; Nicolas et al., 2008; genus Acomys; Nicolas et al., 2009; desert-adapted Gerbillus tarabuli, Ndiaye et al., 2011) and the divergence of red and Rüppell's foxes, the latter being a species more adapted to arid conditions (Leite et al., 2015). In east Africa, the mid-Pleistocene transition is suggested to be connected to the appearance of modern carnivores, especially those of the genus Canis (Werdelin & Lewis, 2005).

### ACKNOWLEDGEMENTS

We are grateful to I. Salado, S. Ravagni, A. Cornellas and M. Camacho-Sánchez for their assistance in laboratory work and in the analyses. Logistical support was provided by Laboratorio de Ecología Molecular (LEM-EBD) and by the infrastructures offered by Doñana's Singular Scientific-Technical Infrastructure (ICTS-EBD). Informatic technical support was provided by the High-Performance Computing (HPC) Research Center in Princeton University. CS was supported by a PhD fellowship from Programa Internacional de Becas "La Caixa-Severo Ochoa" of the Spanish Ministerio de Economía y Competitividad and La Caixa bank (BES-2015-074331). This project was funded by the Frontera grant P18-FR-5099 from the Junta de Andalucia. EBD-CSIC

- Auton, A., Rui Li, Y., Kidd, J., Oliveira, K., Nadel, J., Holloway, J. K., Hayward, J. J., Cohen, P. E., Greally, J. M., Wang, J., Bustamante, C. D., & Boyko, A. R. (2013). Genetic recombination is targeted towards gene promoter regions in dogs. *PLoS Genetics*, 9(12), e1003984. https://doi.org/10.1371/journal.pgen.1003984
- Ben Faleh, A., Granjon, L., Tatard, C., Boratyński, Z., Cosson, J. F., & Said, K. (2012). Phylogeography of two cryptic species of African desert jerboas (Dipodidae: Jaculus). *Biological Journal of the Linnean Society*, 107(1), 27–38. https://doi.org/10.1111/j.1095-8312.2012.01920.x
- Bertola, L. D., Jongbloed, H., van der Gaag, K. J., de Knijff, P., Yamaguchi, N., Hooghiemstra, H., Bauer, H., Henschel, P., White, P. A., Driscoll, C. A., Tende, T., Ottosson, U., Saidu, Y., Vrieling, K., & de longh, H. H. (2016). Phylogeographic Patterns in Africa and High Resolution Delineation of Genetic Clades in the Lion (Panthera leo). *Scientific Reports*, 6, 1–11. https://doi.org/10.1038/srep30807
- Bolfíková, B. Č., Eliášová, K., Loudová, M., Kryštufek, B., Lymberakis, P., Sándor, A. D., & Hulva, P. (2017). Glacial allopatry vs. postglacial parapatry and peripatry: The case of hedgehogs. *PeerJ*, 2017(4), 1–21. https://doi.org/10.7717/peerj.3163
- Brito, J. C., Godinho, R., Martínez-Freiría, F., Pleguezuelos, J. M., Rebelo, H., Santos, X., Vale, C. G., Velo-Antón, G., Boratyński, Z., Carvalho, S. B., Ferreira, S., Gonçalves, D. V., Silva, T. L., Tarroso, P., Campos, J. C., Leite, J. V., Nogueira, J., Álvares, F., Sillero, N., ... Carranza, S. (2014). Unravelling biodiversity, evolution and threats to conservation in the sahara-sahel. *Biological Reviews*, 89(1), 215–231. https:// doi.org/10.1111/brv.12049
- Brown, D. M., Brenneman, R. A., Koepfli, K.-P., Pollinger, J. P., Milá, B., Georgiadis, N. J., Louis, E. E., Grether, G. F., Jacobs, D. K., & Wayne, R. K. (2007). Extensive population genetic structure in the giraffe. *BMC Biology*, 5(57), https://doi.org/10.1186/1741-7007-5-57
- Browning, S. R., & Browning, B. L. (2015). Accurate non-parametric estimation of recent effective population size from segments of identity by descent. American Journal of Human Genetics, 97(3), 404– 418. https://doi.org/10.1016/j.ajhg.2015.07.012
- Browning, S. R., Browning, B. L., Daviglus, M. L., Durazo-Arvizu, R. A., Schneiderman, N., Kaplan, R. C., & Laurie, C. C. (2018). Ancestryspecific recent effective population size in the Americas. *PLoS Genetics*, 14(5), 1–22. https://doi.org/10.1371/journ al.pgen.1007385
- Camacho-Sanchez, M., Quintanilla, I., Hawkins, M. T. R., Tuh, F. Y. Y., Wells, K., Maldonado, J. E., & Leonard, J. A. (2018). Interglacial refugia on tropical mountains: Novel insights from the summit rat (Rattus baluensis), a Borneo mountain endemic. *Diversity and Distributions*, 24, 1252–1266. https://doi.org/10.1111/ddi.12761
- Campana, M. G., Parker, L. D., Hawkins, M. T. R., Young, H. S., Helgen, K. M., Szykman Gunther, M., Woodroffe, R., Maldonado, J. E., & Fleischer, R. C. (2016). Genome sequence, population history, and pelage genetics of the endangered African wild dog (*Lycaon pictus*). BMC Genomics, 17(1), 1–10. https://doi.org/10.1186/s1286 4-016-3368-9
- Castañeda, I. S., Mulitza, S., Schefuß, E., Lopes, R. A., Damste, J. S. S., & Schouten, S. (2009). Wet phases in the Sahara / Sahel region and human migration patterns in North Africa. Proceedings of the National Academy of Sciences of the United States of America, 106(48), 20159–20163.
- Cavalli-Sforza, L. L. (1969). Human diversity. Proceedings of 12th International Congress Genetics, 3, 405–416.
- Charruau, P., Fernandes, C., OROZCO-terWENGEL, P., Peters, J., Hunter, L., Ziaie, H., Jourabchian, A., Jowkar, H., Schaller, G., Ostrowski, S., Vercammen, P., Grange, T., Schlötterer, C., Kotze, A., Geigl, E.-M., Walzer, C., & Burger, P. A. (2011). Phylogeography, genetic structure and population divergence time of cheetahs in Africa and Asia: Evidence for long-term geographic isolates. *Molecular Ecology*, 20(4), 706–724. https://doi. org/10.1111/j.1365-294X.2010.04986.x

- Chavez, D. E., Gronau, I., Hains, T., Kliver, S., Koepfli, K.-P., & Wayne, R. K. (2019). Comparative genomics provides new insights into the remarkable adaptations of the African wild dog (*Lycaon pictus*). *Scientific Reports*, 9(1), 8329. https://doi.org/10.1038/s41598-019-44772-5
- Ciani, A. C., Palentini, L., Arahou, M., Martinoli, L., Capiluppi, C., & Mouna, M. (2005). Population decline of *Macaca sylvanus* in the middle atlas of Morocco. *Biological Conservation*, 121(4), 635–641. https://doi.org/10.1016/j.biocon.2004.06.009
- Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B., Mitrovica, J. X., Hostetler, S. W., & McCabe, A. M. (2009). The last glacial maximum. *Science*, 325(5941), 710–714. https://doi. org/10.1126/science.1172873
- Cosson, J. F., Hutterer, R., Libois, R., Sarà, M., Taberlet, P., & Vogel, P. (2005). Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic fluctuations in the western Mediterranean: A case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). *Molecular Ecology*, 14(4), 1151–1162. https:// doi.org/10.1111/j.1365-294X.2005.02476.x
- Cuzin, F. (2003). Les grands mammiferes du maroc meridional. (PhD thesis) Université des sciences et techniques de Montpellier 2.
- DeCandia, A. L., Henger, C. S., Krause, A., Gormezano, L. J., Weckel, M., Nagy, C., & vonHoldt, B. M. (2019). Genetics of urban colonization: Neutral and adaptive variation in coyotes (Canis latrans) inhabiting the New York metropolitan area. *Journal of Urban Ecology*, 5(1), 1–12. https://doi.org/10.1093/jue/juz002.
- DeMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters*, 220(1–2), 3–24. https://doi.org/10.1016/S0012-821X(04)00003-2
- di Lernia, S. (1998).Cultural control over wild animals during the early Holocene: The case of Barbary sheep in central Sahara. In S. di Lernia, & G. Manzi (Eds.), *Before food production in North Africa* (Vol. 1, pp. 113–126).
- Dinis, M., Merabet, K., Martínez-Freiría, F., Steinfartz, S., Vences, M., Burgon, J. D., Elmer, K. R., Donaire, D., Hinckley, A., Fahd, S., Joger, U., Fawzi, A., Slimani, T., & Velo-Antón, G. (2019). Allopatric diversification and evolutionary melting pot in a North African Palearctic relict: The biogeographic history of Salamandra algira. *Molecular Phylogenetics and Evolution*, 130, 81–91. https://doi.org/10.1016/j. ympev.2018.10.018
- Drake, N. A., Blench, R. M., Armitage, S. J., Bristow, C. S., & White, K. H. (2011). Ancient watercourses and biogeography of the Sahara explain the peopling of the desert. *Proceedings of the National Academy* of Sciences of the United States of America, 108(2), 458–462. https:// doi.org/10.1073/pnas.1012231108
- Drake, N. A., Breeze, P., & Parker, A. (2013). Palaeoclimate in the Saharan and Arabian Deserts during the Middle Palaeolithic and the potential for hominin dispersals. *Quaternary International*, 300, 48–61. https://doi.org/10.1016/j.quaint.2012.12.018
- Drake, N. A., El-Hawat, A. S., Turner, P., Armitage, S. J., Salem, M. J., White, K. H., & McLaren, S. (2008). Palaeohydrology of the Fazzan Basin and surrounding regions: The last 7 million years. *Palaeogeography*, *Palaeoclimatology*, *Palaeoecology*, 263(3–4), 131–145. https://doi. org/10.1016/j.palaeo.2008.02.005
- Dufresnes, C., Nicieza, A. G., Litvinchuk, S. N., Rodrigues, N., Jeffries, D. L., Vences, M., Perrin, N., & Martínez-Solano, Í. (2020). Are glacial refugia hotspots of speciation and cytonuclear discordances? Answers from the genomic phylogeography of Spanish common frogs. *Molecular Ecology*, 29(5), 986–1000. https://doi.org/10.1111/mec.15368
- Durrett, R. (2008). Probability models for DNA sequence evolution, second edition by Richard Durrett. *International Statistical Review*, 77(2), 304. https://doi.org/10.1111/j.1751-5823.2009.00085\_5.x
- Eddine, A., Rocha, R. G., Mostefai, N., Karssene, Y., De Smet, K., Brito, J. C., Klees, D., Nowak, C., Cocchiararo, B., Lopes, S., van der Leer, P., & Godinho, R. (2020). Demographic expansion of an African

MOLECULAR ECOLOGY – WI

opportunistic carnivore during the Neolithic revolution. *Biology Letters*, 16(1), 1–7.

- Ehrmann, W., Schmiedl, G., Beuscher, S., & Krüger, S. (2017). Intensity of african humid periods estimated from saharan dust fluxes. *PLoS One*, 12(1), 1–18. https://doi.org/10.1371/journal.pone.0170989
- Emeis, K. C., Sakamoto, T., Wehausen, R., & Brumsack, H. J. (2000). The sapropel record of the eastern Mediterranean Sea - Results of Ocean Drilling Program Leg 160. Palaeogeography, Palaeoclimatology, Palaeoecology, 158(3–4), 371–395. https://doi.org/10.1016/S0031 -0182(00)00059-6
- Ersmark, E., Klütsch, C. F. C., Chan, Y. L., Sinding, M. H. S., Fain, S. R., Illarionova, N. A., & Savolainen, P. (2016). From the past to the present: Wolf phylogeography and demographic history based on the mitochondrial control region. *Frontiers in Ecology and Evolution*, 4(DEC), 1–12. https://doi.org/10.3389/fevo.2016.00134
- Fay, J. C., & Wu, C. I. (2000). Hitchhiking under positive Darwinian selection. Genetics, 155(3), 1405–1413.
- Feliner, G. N. (2011). Southern European glacial refugia: A tale of tales. Taxon, 60(2), 365–372. https://doi.org/10.1002/tax.602007
- Flagstad, S. P. O., Stenseth, N. C., & Jakobsen, K. S. (2001). Environmental change and rates of evolution: The phylogeographic pattern within the hartebeest complex as related to climatic variation. *Proceedings* of the Royal Society B: Biological Sciences, 268(1468), 667–677. https://doi.org/10.1098/rspb.2000.1416
- Freedman, A. H., Gronau, I., Schweizer, R. M., Ortega-Del Vecchyo, D., Han, E., Silva, P. M., & Novembre, J. (2014). Genome sequencing highlights the dynamic early history of dogs. *PLoS Genetics*, 10(1), https://doi.org/10.1371/journal.pgen.1004016
- Fu, Y. X., & Li, W.-H.-H. (1993). Statistical tests of neutrality of mutations. Genetics, 133(3), 693–709.
- Fuller, T. K., Biknevicius, A. R., Kat, P. W., Van Valkenburgh, B., & Wayne, R. K. (1989). The ecology of 3 sympatric jackal species in the Rift-Valley of Kenya. *African Journal of Ecology*, 27(4), 313–323. https:// doi.org/10.1111/j.1365-2028.1989.tb01025.x
- Fumagalli, M., Vieira, F. G., Linderoth, T., & Nielsen, R. (2014). NgsTools: Methods for population genetics analyses from next-generation sequencing data. *Bioinformatics*, 30(10), 1486–1487. https://doi. org/10.1093/bioinformatics/btu041
- Gaubert, P., Bloch, C., Benyacoub, S., Abdelhamid, A., Pagani, P., Djagoun, C. A. M. S., Couloux, A., & Dufour, S. (2012). Reviving the African Wolf Canis lupus lupaster in north and west africa: A mitochondrial lineage ranging more than 6,000 km wide. *PLoS One*, 7(8), https:// doi.org/10.1371/journal.pone.0042740
- Geraads, D. (2011). A revision of the fossil Canidae (Mammalia) of north-western Africa. *Palaeontology*, 54(2), 429-446. https://doi. org/10.1111/j.1475-4983.2011.01039.x
- Geyh, M. A., & Thiedig, F. (2008). The Middle Pleistocene Al Mahrúqah Formation in the Murzuq Basin, northern Sahara, Libya evidence for orbitally-forced humid episodes during the last 500,000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology, 257*(1–2), 1–21. https://doi.org/10.1016/j.palaeo.2007.07.001
- Gómez, A., & Lunt, D. H. (2007). Refugia within Refugia: Patterns of Phylogeographic Concordance in the Iberian Peninsula. In Phylogeography of Southern European Refugia: Evolutionary Perspectives on the Origins and Conservation of European Biodiversity (pp. 155-188). Springer. https://doi. org/10.1007/1-4020-4904-8\_5
- Gopalakrishnan, S., Sinding, M.-H., Ramos-Madrigal, J., Niemann, J., Samaniego Castruita, J. A., Vieira, F. G., Carøe, C., Montero, M. D. M., Kuderna, L., Serres, A., González-Basallote, V. M., Liu, Y.-H., Wang, G.-D., Marques-Bonet, T., Mirarab, S., Fernandes, C., Gaubert, P., Koepfli, K.-P., Budd, J., ... Gilbert, M. T. P. (2018). Interspecific gene flow shaped the evolution of the genus *Canis. Current Biology*, *28*(21), 3441–3449.e5. https://doi.org/10.1016/j. cub.2018.08.041

- Guagnin, M. (2015). Animal engravings in the central Sahara: A proxy of a proxy. Environmental Archaeology, 20(1), 52-65. https://doi. org/10.1179/1749631414Y.0000000026
- Hampe, A., & Petit, R. J. (2005). Conserving biodiversity under climate change: The rear edge matters. *Ecology Letters*, 8(5), 461–467. https://doi.org/10.1111/j.1461-0248.2005.00739.x
- Hawkins, M. T. R., Culligan, R. R., Frasier, C. L., Dikow, R. B., Hagenson, R., Lei, R., & Louis, E. E. (2018). Genome sequence and population declines in the critically endangered greater bamboo lemur (*Prolemur simus*) and implications for conservation. *BMC Genomics*, 19(1), 1–15. https://doi.org/10.1186/s12864-018-4841-4
- Heinrich, H. (1988). Origin and consequences of cyclic ice rafting in the Northeast Atlantic Ocean during the past 130,000 years. *Quaternary Research*, *29*(2), 142–152.
- Hemming, S. R. (2004). Heinrich events: Massive late Pleistocene detritus layers of the North Atlantic and their global climate imprint. *Reviews* of *Geophysics*, 42(1), https://doi.org/10.1029/2003RG000128
- Heppenheimer, E., Brzeski, K. E., Hinton, J. W., Patterson, B. R., Rutledge, L. Y., DeCandia, A. L., Wheeldon, T., Fain, S. R., Hohenlohe, P. A., Kays, R., White, B. N. & vonHoldt, B. M. (2018). High genomic diversity and candidate genes under selection associated with range expansion in eastern coyote (Canis latrans) populations. *Ecology and Evolution*, 8(24), 12641–12655. https://doi.org/10.1002/ ece3.4688
- Heppenheimer, E., Cosio, D. S., Brzeski, K. E., Caudill, D., Van Why, K., Chamberlain, M. J., Hinton, J. W., & vonHoldt, B. (2018). Demographic history influences spatial patterns of genetic diversityin recently expanded coyote (Canis latrans) populations. *Heredity*, 120(3), 183–195. https://doi.org/10.1038/s41437-017-0014-5
- Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society, 68(1-2), 87-112. https:// doi.org/10.1006/bijl.1999.0332
- Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405(6789), 907–913. https://doi.org/10.1038/35016000
- Hoffmann, D. L., Rogerson, M., Spötl, C., Luetscher, M., Vance, D., Osborne, A. H., Fello, N. M., & Moseley, G. E. (2016). Timing and causes of North African wet phases during the last glacial period and implications for modern human migration. *Scientific Reports*, 6(September), 1–7. https://doi.org/10.1038/srep36367
- Husemann, M., Schmitt, T., Zachos, F. E., Ulrich, W., & Habel, J. C. (2014). Palaearctic biogeography revisited: Evidence for the existence of a North African refugium for Western Palaearctic biota. *Journal of Biogeography*, 41(1), 81–94. https://doi.org/10.1111/jbi.12180
- Iyengar, A., Gilbert, T., Woodfine, T., Knowles, J. M., Diniz, F. M., Brenneman, R. A., Louis, E. E., & Maclean, N. (2007). Remnants of ancient genetic diversity preserved within captive groups of scimitar-horned oryx (Oryx dammah). *Molecular Ecology*, 16(12), 2436– 2449. https://doi.org/10.1111/j.1365-294X.2007.03291.x
- Karssene, Y., Chammem, M., Khorchani, T., Nouira, S., & Li, F. (2017). Global warming drives changes in carnivore communities in the North Sahara desert. *Climate Research*, 72, 153–162.
- Karssene, Y., Chammem, M., Nowak, C., de Smet, K., Castro, D., Eddine, A., Lopes, S., Muñoz-Fuentes, V., Cocchiararo, B., Klees, D., Van Der Leer, P., Nouira, S., & Godinho, R. (2018). Noninvasive genetic assessment provides evidence of extensive gene flow and possible high movement ability in the African golden wolf. *Mammalian Biology*, 92, 94–101. https://doi.org/10.1016/j.mambio.2018.05.002
- Karssene, Y., Nowak, C., Chammem, M., Cocchiararo, B., & Nouira, S. (2019). Genetic diversity of the genus Vulpes (Red fox and Fennec fox) in Tunisia based on mitochondrial DNA and noninvasive DNA sampling. *Mammalian Biology*, 96, 118–123. https://doi. org/10.1016/j.mambio.2018.09.008
- Kebede, Y. (2017). A Review on: Distribution, Ecology and Status of Golden Jackal (Canis aureus), Africa. Journal of Natural Sciences Research 7(1), 32–43.

- Kent, J., Charles, S., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., & Haussler, D. (2002). The human genome browser at UCSC. *Genome Research*, 12(6), 996–1006. https://doi.org/10.1101/ gr.229102
- Kim, H. L., Ratan, A., Perry, G. H., Montenegro, A., Miller, W., & Schuster, S. C. (2014). Khoisan hunter-gatherers have been the largest population throughout most of modern-human demographic history. *Nature Communications*, 5, https://doi.org/10.1038/ncomms6692
- Kingdon, J. (2013). Mammals of Africa. In *Bloomsbury natural history* (Vol. 50). https://doi.org/10.5860/choice.50-4188
- Koblmüller, S., Wayne, R. K., & Leonard, J. A. (2012). Impact of Quaternary climatic changes and interspecific competition on the demographic history of a highly mobile generalist carnivore, the coyote. *Biology Letters*, 8(4), 644–647. https://doi.org/10.1098/rsbl.2012.0162
- Koch, E. M., Schweizer, R. M., Schweizer, T. M., Stahler, D. R., Smith, D. W., Wayne, R. K., & Novembre, J. (2019). De novo mutation rate estimation in wolves of known pedigree. *Molecular Biology and Evolution*, 36(11), 2536–2547. https://doi.org/10.1093/molbev/ msz159
- Koepfli, K.-P., Pollinger, J., Godinho, R., Robinson, J., Lea, A., Hendricks, S., Schweizer, R. M., Thalmann, O., Silva, P., Fan, Z., Yurchenko, A. A., Dobrynin, P., Makunin, A., Cahill, J. A., Shapiro, B., Álvares, F., Brito, J. C., Geffen, E., Leonard, J. A., ... Wayne, R. K. (2015). Genome-wide evidence reveals that African and Eurasian golden jackals are distinct species. *Current Biology*, 25(16), 2158–2165. https://doi.org/10.1016/j.cub.2015.06.060
- Kuper, R., & Kröpalin, S. (2006). Climate-controlled Holocene occupation in the Sahara: Motor of Africa's evolution. *Science*, 313(5788), 803–807. https://doi.org/10.1126/science.1130989
- Larrasoaña, J. C., Roberts, A. P., & Rohling, E. J. (2013). Dynamics of green sahara periods and their role in hominin evolution. *PLoS One*, 8(10), https://doi.org/10.1371/journal.pone.0076514
- Larrasoaña, J. C., Roberts, A. P., Rohling, E. J., Winklhofer, M., & Wehausen, R. (2003). Thre million years of monsoon variability over the northern Sahara. *Climate Dynamics*, 21, 689–698. https:// doi.org/10.1007/s00382-033-0355-z
- Leite, J. V., Álvares, F., Velo-Antón, G., Brito, J. C., & Godinho, R. (2015). Differentiation of North African foxes and population genetic dynamics in the desert. Insights into the evolutionary history of two sister taxa, Vulpes rueppellii and Vulpes vulpes. Organisms Diversity and Evolution, 15(4), 731–745. https://doi.org/10.1007/s1312 7-015-0232-8
- Leonard, J. A. (2014). Ecology drives evolution in grey wolves. Evolutionary Ecology Research, 16(6), 461–473.
- Leonard, J. A., Vila, C., Fox-Dobbs, K., Koch, P. L., Wayne, R. K., & Van Valkenburgh, B. (2007). Megafaunal extinctions and the disappearance of a specialized wolf ecomorph. *Current Biology*, 17(13), 1146– 1150. https://doi.org/10.1016/j.cub.2007.05.072
- Lerp, H., Wronski, T., Pfenninger, M., & Plath, M. (2011). A phylogeographic framework for the conservation of Saharan and Arabian Dorcas gazelles (Artiodactyla: Bovidae). Organisms Diversity and Evolution, 11(4), 317–329. https://doi.org/10.1007/s1312 7-011-0057-z
- Lézine, A. M., Hély, C., Grenier, C., Braconnot, P., & Krinner, G. (2011). Sahara and Sahel vulnerability to climate changes, lessons from Holocene hydrological data. *Quaternary Science Reviews*, 30(21–22), 3001–3012. https://doi.org/10.1016/j.quasc irev.2011.07.006
- Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, 27(21), 2987–2993. https://doi.org/10.1093/bioinformatics/btr509
- Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, 26(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

- Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. *Nature*, 475(7357), 493–496. https://doi.org/10.1038/nature10231
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. https:// doi.org/10.1093/bioinformatics/btp352
- Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., Kamal, M., Clamp, M., Chang, J. L., Kulbokas, E. J., Zody, M. C., Mauceli, E., Xie, X., Breen, M., Wayne, R. K., Ostrander, E. A., Ponting, C. P., Galibert, F., Smith, D. R., deJong, P. J., ... Lander, E. S. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, 438(7069), 803–819. https://doi.org/10.1038/nature04338
- Linstädter, J., & Kröpelin, S. (2004). Wadi Bakht revisited: Holocene climate change and prehistoric occupation in the Gilf Kebir Region of the eastern Sahara, SW Egypt. *Geoarchaeology*, 19(8), 753–778. https://doi.org/10.1002/gea.20023
- Liu, Y.-H., Wang, L. U., Xu, T., Guo, X., Li, Y., Yin, T.-T., Yang, H.-C., Hu, Y., Adeola, A. C., Sanke, O. J., Otecko, N. O., Wang, M., Ma, Y., Charles, O. S., Sinding, M.-H., Gopalakrishnan, S., Alfredo Samaniego, J., Hansen, A. J., Fernandes, C., ... Zhang, Y.-P. (2018). Whole-Genome sequencing of African dogs provides insights into adaptations against tropical parasites. *Molecular Biology and Evolution*, 35(2), 287–298. https://doi.org/10.1093/molbev/ msx258
- Lohmueller, K. E., Bustamante, C. D., & Clark, A. G. (2010). The effect of recent admixture on inference of ancient human population history. *Genetics*, 185(2), 611–622. https://doi.org/10.1534/genet ics.109.113761
- Lorenzen, E. D., Heller, R., & Siegismund, H. R. (2012). Comparative phylogeography of African savannah ungulates. *Molecular Ecology*, 21(15), 3656–3670. https://doi.org/10.1111/j.1365-294X.2012.05650.x
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*, 10–12.
- McClymont, E. L., Sosdian, S. M., Rosell-Melé, A., & Rosenthal, Y. (2013). Pleistocene sea-surface temperature evolution: Early cooling, delayed glacial intensification, and implications for the mid-Pleistocene climate transition. *Earth-Science Reviews*, 123, 173–193. https://doi.org/10.1016/j.earscirev.2013.04.006
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., & DePristo, M. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Proceedings of the International Conference on Intellectual Capital, Knowledge Management & Organizational Learning, 20, 254–260. https://doi.org/10.1101/gr.107524.110.20
- McQuillan, R., Leutenegger, A.-L., Abdel-Rahman, R., Franklin, C. S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., MacLeod, A. K., Farrington, S. M., Rudan, P., Hayward, C., Vitart, V., Rudan, I., Wild, S. H., Dunlop, M. G., Wright, A. F., ... Wilson, J. F. (2008). Runs of homozygosity in European populations. *American Journal of Human Genetics*, *83*(3), 359–372. https://doi.org/10.1016/j.ajhg.2008.08.007
- McShane, T. O., & Grettenberger, J. F. (1984). Food of the golden jackal (Canis aureus) in central Niger. *African Journal of Ecology*, 22(1), 49– 53. https://doi.org/10.1111/j.1365-2028.1984.tb00673.x
- Moehlman, P. D. (1986). Ecological aspects of social evolution. *Ecological* aspects of social evolution. Princeton University Press. https://doi. org/10.2307/j.ctt7zvwgq
- Moodley, Y., & Bruford, M. W. (2007). Molecular biogeography: Towards an integrated framework for conserving Pan-African biodiversity. *PLoS One*, *2*(5), https://doi.org/10.1371/journal.pone.0000454
- Moutinho, A. F., Serén, N., Paupério, J., Silva, T. L., Martínez-Freiría, F., Sotelo, G., Faria, R., Mappes, T., Alves, P. C., Brito, J. C., & Boratyński, Z. (2020). Evolutionary history of two cryptic species of northern

MOLECULAR ECOLOGY – WI

African jerboas. BMC Evolutionary Biology, 20(1), 1–16. https://doi. org/10.1186/s12862-020-1592-z

- Muwanika, V. B., Nyakaana, S., Siegismund, H. R., & Arctander, P. (2003). Phylogeography and population structure of the common warthog (Phacochoerus africanus) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity*, *91*(4), 361–372. https://doi.org/10.1038/sj.hdy.6800341
- Nadachowska-Brzyska, K., Burri, R., Smeds, L., & Ellegren, H. (2016). PSMC analysis of effective population sizes in molecular ecology and its application to black-and-white Ficedula flycatchers. *Molecular Ecology*, 25(5), 1058–1072. https://doi.org/10.1111/mec.13540
- Ndiaye, A., Bâ, K., Aniskin, V., Benazzou, T., Chevret, P., Konečný, A., Sembène, M., Tatard, C., Kergoat, G. J., & Granjon, L. (2011). Evolutionary systematics and biogeography of endemic gerbils (Rodentia, Muridae) from Morocco: an integrative approach. *Zoologica Scripta*, 41, 11–28.
- Neukom, R., Steiger, N., Gómez-Navarro, J. J., Wang, J., & Werner, J. P. (2019). No evidence for globally coherent warm and cold periods over the preindustrial Common Era. *Nature*, 571, https://doi. org/10.1038/s41586-019-1401-2
- Nicolas, V., Bryja, J., Akpatou, B., Konecny, A., Lecompte, E., Colyn, M., Lalis, A., Couloux, C., Denys, C., & Granjon, L. (2008). Comparative phylogeography of two sibling species of forest-dwelling rodent (*Praomys rostratus* and *P. tullbergi*) in West Africa: different reactions to past forest fragmentation. *Molecular Ecology*, 17, 5118-5134. https://doi. org/10.1111/j.1365-294X.2008.03974.x
- Nicolas, V., Granjon, L., Duplantier, J. M., Cruaud, C., & Dobigny, G. (2009). Phylogeography of spiny mice (genus Acomys, Rodentia: Muridae) from the south-western margin of the sahara with taxonomic implications. *Biological Journal of the Linnean Society*, 98(1), 29–46. https://doi.org/10.1111/j.1095-8312.2009.01273.x
- Nielsen, R., Korneliussen, T., Albrechtsen, A., Li, Y., & Wang, J. (2012). SNP calling, genotype calling, and sample allele frequency estimation from new-generation sequencing data. *PLoS One*, 7(7), https:// doi.org/10.1371/journal.pone.0037558
- North Greenland Ice Core Project members (2004). High-resolution record of Northern Hemisphere climate extending into the last interglacial period. *Nature*, 431, 147–151.
- Oetjens, M. T., Martin, A., Veeramah, K., & Kidd, J. (2018). Analysis of the canid Y-chromosome phylogeny using short-read sequencing data reveals the presence of distinct haplogroups among Neolithic European dogs. BMC Genomics, 19, 350. https://doi.org/10.1186/ s12864-018-4749-z
- Pilot, M., Jedrzejewski, W., Branicki, W., Sidorovich, V. E., Jedrzejewska, B., Stachura, K., & Funk, S. M. (2006). Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology*, 15(14), 4533–4553. https://doi. org/10.1111/j.1365-294X.2006.03110.x
- Pilot, M., Moura, A. E., Okhlopkov, I. M., Mamaev, N. V., Alagaili, A. N., Mohammed, O. B., Yavruyan, E. G., Manaseryan, N. H., Hayrapetyan, V., Kopaliani, N., Tsingarska, E., Krofel, M., Skoglund, P., & Bogdanowicz, W. (2019). Global phylogeographic and admixture patterns in grey wolves and genetic legacy of an ancient Siberian lineage. *Scientific Reports*, 9(1), 1–13. https://doi. org/10.1038/s41598-019-53492-9
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81(3), 559–575. https://doi. org/10.1086/519795
- R Core Team (2017). R: A language and environment for Statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

- Rato, C., Brito, J. C., Carretero, M. A., Larbes, S., Shacham, B., & Harris, D. J. (2007). Phylogeography and genetic diversity of Psammophis schokari (Serpentes) in North Africa based on mitochondrial DNA sequences. *African Zoology*, 42(1), 112-117. https://doi. org/10.1080/15627020.2007.11407383
- Renaud, G., Hanghøj, K., Korneliussen, T. S., Willerslev, E., & Orlando, L. (2019). Joint estimates of heterozygosity and runs of homozygosity for modern and ancient samples. *Genetics*, 212(July), 587–614. https://doi.org/10.1534/genetics.119.302057
- Rohling, E. J., Mayewski, P. A., & Challenor, P. (2003). On the timing and mechanism of millennial-scale climate variability during the last glacial cycle. *Climate Dynamics*, 20(2-3), 257-267. https://doi. org/10.1007/s00382-002-0266-4
- Rueness, E. K., Asmyhr, M. G., Sillero-Zubiri, C., Macdonald, D. W., Bekele, A., Atickem, A., & Stenseth, N. C. (2011). The cryptic African wolf: Canis aureus lupaster is not a golden jackal and is not endemic to Egypt. *PLoS One, 6*(1), https://doi.org/10.1371/journ al.pone.0016385
- Sams, A. J., & Boyko, A. R. (2019). Fine-scale resolution of runs of homozygosity reveal patterns of inbreeding and substantial overlap with recessive disease genotypes in domestic dogs. G3: Genes, Genomes, Genetics, 9(1), 117–123. https://doi.org/10.1534/g3.118.200836
- Sereno, P. C., Garcea, E. A. A., Jousse, H., Stojanowski, C. M., Saliège, J.-F., Maga, A., Ide, O. A., Knudson, K. J., Mercuri, A. M., Stafford, T. W., Kaye, T. G., Giraudi, C., N'siala, I. M., Cocca, E., Moots, H. M., Dutheil, D. B., & Stivers, J. P. (2008). Lakeside cemeteries in the Sahara: 5000 years of holocene population and environmental change. *PLoS One*, 3(8), https://doi.org/10.1371/journ al.pone.0002995
- Shchur, V. (2019). MiSTI: PSMC-based Migration and Split Time Inference from two genomes. https://github.com/vlshchur/MiSTI
- Shchur, V., Korneliussen, T. S., & Nielsen, R. (2017). ngsPSMC: genotype likelihood-based PSMC for analysis of low coverage NGS. https:// github.com/ANGSD/ngsPSMC
- Simeneh, G. (2010). Habitat use and Diet of Golden Jackal (Canis aureus) and Human-Carnivore Conflict in Guassa Community Conservation Area, Menz. (MSc thesis). Department of Biology, Addis Ababa University.
- Skotte, L., & Albrechtsen, A. (2013). Estimating Individual Admixture Proportions from. *Genetics*, 195(November), 693–702. https://doi. org/10.1534/genetics.113.154138
- Skov, L., Coll Macià, M., Sveinbjörnsson, G., Mafessoni, F., Lucotte, E. A., Einarsdóttir, M. S., Jonsson, H., Halldorsson, B., Gudbjartsson, D. F., Helgason, A., Schierup, M. H., & Stefansson, K. (2020). The nature of Neanderthal introgression revealed by 27,566 Icelandic genomes. *Nature*, *582*(7810), 78–83. https://doi.org/10.1038/s4158 6-020-2225-9
- Smith, J. R. (2012). Spatial and temporal variations in the nature of Pleistocene pluvial phase environments across Africa. In Modern origins a north african perspective (pp. 35–77). Springer.
- Smitz, N., Berthouly, C., Cornélis, D., Heller, R., Van Hooft, P., Chardonnet, P., Caron, A., Prins, H., van Vuuren, B. J., De longh, H., & Michaux, J. (2013). Pan-African genetic structure in the African Buffalo (Syncerus caffer): Investigating intraspecific divergence. *PLoS One*, 8(2), e56235.
- Sommer, R. S., & Nadachowski, A. (2006). Glacial refugia of mammals in Europe: Evidence from fossil records. *Mammal Review*, *36*(4), 251– 265. https://doi.org/10.1111/j.1365-2907.2006.00093.x
- Stöck, M., Dufresnes, C., Litvinchuk, S. N., Lymberakis, P., Biollay, S., Berroneau, M., Borzée, A., Ghali, K., Ogielska, M., & Perrin, N. (2012). Cryptic diversity among Western Palearctic tree frogs: Postglacial range expansion, range limits, and secondary contacts of three European tree frog lineages (Hyla arborea group). *Molecular Phylogenetics and Evolution*, 65(1), 1–9. https://doi.org/10.1016/j. ympev.2012.05.014

- Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123(3), 585-595.
- Tamar, K., Metallinou, M., Wilms, T., Schmitz, A., Crochet, P. A., Geniez, P., & Carranza, S. (2018). Evolutionary history of spiny-tailed lizards (Agamidae: Uromastyx) from the Saharo-Arabian region. *Zoologica Scripta*, 47(2), 159–173. https://doi.org/10.1111/zsc.12266
- Tange, O. (2018). GNU Parallel 2018. https://doi.org/10.5281/ zenodo.1146014
- Temu, S. E., Nahonyo, C. L., & Moehlman, P. D. (2016). Comparative foraging efficiency of two sympatric jackals, silver-backed jackals (Canis mesomelas) and Golden Jackals (Canis aureus), in the Ngorongoro Crate, Tanzania, 2016. https://doi.org/10.1155/2016/6178940
- Temu, S. E., Nahonyo, C. L., & Moehlman, P. D. (2018). Diet composition of the golden jackal (Canis aureus) in the Ngorongoro Crater, Tanzania. *Tanzania Journal of Science*, 44(1), 52–61.
- Tison, J.-L., Edmark, V. N., Sandoval-Castellanos, E., Van Dyck, H., Tammaru, T., Välimäki, P., Dalén, L., & Gotthard, K. (2014). Signature of post-glacial expansion and genetic structure at the northern range limit of the speckled wood butterfly. *Biological Journal of the Linnean Society*, 113(1), 136–148. https://doi.org/10.1111/bij.12327
- Tjallingii, R., Claussen, M., Stuut, J.-B., Fohlmeister, J., Jahn, A., Bickert, T., Lamy, F., & Röhl, U. (2008). Coherent high- and low-latitude control of the northwest African hydrological balance. *Nature Geoscience*, 1, 670–675. https://doi.org/10.1038/ngeo289
- Trauth, M. H., Larrasoaña, J. C., & Mudelsee, M. (2009). Trends, rhythms and events in Plio-Pleistocene African climate. *Quaternary Science Reviews*, 28(5–6), 399–411. https://doi.org/10.1016/j.quasc irev.2008.11.003
- Urios, V., Donat-Torres, M. P., Monroy-Vilchis, C. R. O., & Idrissi, H. R. (2015). El análisis del genoma mitocondrial del cánido estudiado en Marruecos manifiesta que no es ni lobo (Canis lupus) ni chacal euroasiático (Canis aureus). *Altotero*, 3.
- Van Valkenburgh, B., & Wayne, R. K. (1994). Shape divergence associated with size convergence in sympatric East African Jackals. *Ecology*, 75(6), 1567–1581.
- Vieira, F. G., Fumagalli, M., Albrechtsen, A., & Nielsen, R. (2013). Estimating inbreeding coefficients from NGS data: Impact on genotype calling and allele frequency estimation. *Genome Research*, 23(11), 1852–1861. https://doi.org/10.1101/gr.157388.113

- Waters, S., Harrad, A. E., Amhaouch, Z., Taiqui, L., & Senn, H. (2015). Distribution update DNA analysis confirms African wolf in Morocco. *Canid Biology & Conservation*, 18(5), 15–17. https://doi. org/10.1371/journal.pone.0016385
- Watterson, G. (1975). On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology*, 7, 256–276.
- Weldeab, S., Lea, D. W., Schneider, R. R., & Andersen, N. (2007). 155,000 years of West African monsoon and ocean thermal evolution. *Science*, 316, 1303–1307. https://doi.org/10.1126/science.1140461
- Werdelin, L., & Lewis, M. E. (2005). Plio-Pleistocene Carnivora of eastern Africa: species richness and turnover patterns. *Zoological Journal of the Linnean Society*, 144(2), 121–144. https://doi. org/10.1111/j.1096-3642.2005.00165.x
- Yeakel, J. D., Pires, M. M., Rudolf, L., Dominy, N. J., Koch, P. L., Guimarães, P. R., & Gross, T. (2014). Collapse of an ecological network in Ancient Egypt. Proceedings of the National Academy of Sciences of the United States of America, 111(40), 14472–14477. https://doi.org/10.1073/ pnas.1408471111
- Zeng, K., Fu, Y. X., Shi, S., & Wu, C. I. (2006). Statistical tests for detecting positive selection by utilizing high-frequency variants. *Genetics*, 174(3), 1431–1439. https://doi.org/10.1534/genetics.106.061432

### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Sarabia C, vonHoldt B, Larrasoaña JC, Uríos V, Leonard JA. Pleistocene climate fluctuations drove demographic history of African golden wolves (*Canis lupaster*). *Mol Ecol.* 2021;00:1–20. <u>https://doi.org/10.1111/</u>mec.15784