Electronic supplementary material

Demographic expansion of an African opportunistic carnivore during the Neolithic revolution

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Appendix 1
Materials and Methods: details on sample collection, laboratory procedures, data analysis

(a) Sampling and DNA extraction
Sampling was carried out in Algeria across different ecosystems (forest, steppe and desert) between April 2014 and July 2016. It comprised a total of 22 tissue and hair samples from road-kills and poached individuals and three scats (electronic supplementary material, table S1). All samples were preserved in 96% ethanol immediately after collection and then at -20ºC until DNA extraction. The geographical location of each sample was GPS recorded.

DNA extraction of tissue and hair samples was performed using the Genomic DNA Minipreps Tissue Kit (EASY SPIN) following manufacturer’s instructions. DNA isolation from scat samples was performed using the GuSCN/silica method of [1]. Handling of non-invasive samples was performed in dedicated laboratory. Negative controls were included throughout the procedures to monitor possible contamination.

(b) Mitochondrial DNA amplification and sequencing
Mitochondrial (mtDNA) control region was amplified using primers DLH and ThrH [2]. Polymerase chain reactions (PCR) were prepared using 5 μl of the QIAGEN Taq PCR Master Mix, 0.4 μM of each primer, 1 μl of DNA extract and water up to a final
volume of 10 μl. Reactions were performed in a BioRad T100 Thermal Cycler (for
thermoprofile see electronic supplementary material, table S2). A negative control was
included in each PCR to monitor possible contaminations. PCR products were purified
using ExoSap IT® (Affymetrix) following manufacturer instructions, and then
sequenced using DLH primer using the Big-Dye Terminator v3.1 Cycle Sequencing
protocol (Applied Biosystems). Electropherograms were checked and aligned using
GENEIOUS 7.1.5 (https://www.geneious.com). All sequences blasted to African wolf
in NCBI GenBank database.

(c) Microsatellites genotyping and individual identification
A set of 47 microsatellite loci was amplified in five multiplex reactions for tissue
samples following the methodology proposed by [3] and [4] (for details on markers see
electronic supplementary material, table S3). For scat samples, we genotyped a subset
of 13 microsatellites previously optimized in three pools by [5] following the
methodology of these authors. Four PCR replicas of each marker were accomplished
per non-invasive sample. Negative controls were included in all PCR amplifications to
monitor possible DNA contamination. PCRs were performed in a BioRad T100
Thermal Cycler in final volume reactions of 10 μl including 5 μl of QIAGEN Multiplex
PCR Kit, 1 μl of primer multiplex, 3 μl of H2O and 1 μl of DNA (2.5 μl of DNA for
non-invasive samples). PCR profile was specific for each multiplex and according to
previously published information referred to above. Amplification products were
separated and detected on the ABI 3130xl Genetic Analyser (AB Applied Biosystems)
and alleles were scored by comparison to the GeneScan™ 500 LIZ size standard using
GENEMAPPER 4.1 (Applied Biosystems), and manually checked to control automatic
binning. Identical genotypes corresponding to the same individual were grouped using
GIMLET 1.3.3 [6] and excluded from subsequent analysis.

(d) Diversity and genetic structure
Mitochondrial diversity was assessed using sequences generated in this study (n=22),
and then together with 46 sequences from Algeria and Tunisia, respectively, retrieved
from previous works [5,7; supplementary material, table S5]. Diversity indices were
assessed using DnaSP 5 [8]. Intraspecific genetic distances were estimated in MEGA 7
[9] using p-distance model. Phylogeographic relationships among the different mtDNA
haplotypes were estimated using the Median-joining (MJ) network algorithm [10] implemented in PopArt [11].

The 47 microsatellite dataset was evaluated for deviations from Hardy–Weinberg equilibrium (HWE) using GENALEX 6.5 [12], and loci with significant departure from expectations after Bonferroni correction were excluded from the subsequent analysis. Genetic diversity was estimated separately for the dataset in Algeria (n=18), and for the subset of 13 microsatellites in Algerian samples including 2 additional genotypes obtained from non-invasive samples from Algeria, and 27 genotypes from Tunisia [5] generated previously in our lab. Diversity measures were calculated using GENALEX 6.5 [12]. Population structure was tested using the Bayesian clustering approach implemented in STRUCTURE 2.3.4 [13]. Analyses were performed independently 5 times for 10⁶ iterations after a burn-in period of 5x10⁵ iterations, using the admixture model with correlated allele frequencies among populations. We tested 1 to 10 clusters (K) without prior population information. Structure Harvester [14] was used to summarize the posterior probabilities of each K over all runs [15]. We carried out a Principal Components Analysis (PCA) using the Adegenet package in R [16].

Isolation by Distance was evaluated through Mantel tests for mitochondrial and microsatellite loci separately. Three matrices were built including: i) pairwise genetic distance between individuals for each molecular markers estimated in GENALEX 6.5 and, ii) pairwise geographic distance in kilometers from the latitude and longitude of the sampling sites calculated using Geographic Distance Matrix Generator [17]. The Mantel tests were performed in GENALEX 6.5, with significance determined via 999 permutation tests. The same software was used to test population structure between the two sampling areas (Algeria and Tunisia) through an Analysis of Molecular Variance (AMOVA).

(e) Demographic analysis

Demographic history of the African wolf was inferred using mitochondrial and microsatellite loci separately, compiling data from Algeria and Tunisia in a single dataset.

For mtDNA we estimated mismatch distributions and Harpending’s raggedness statistics [18], and tested deviation from neutrality through Tajima’s D [19] and Fu’s Fs [20] statistics, using DnaSP 5 [8]. Coalescence simulations with 1,000 replicates were applied to determine the p-value of each statistic. Smooth and unimodal mismatch
distributions, non-significant Harpending’s raggedness statistics [18], and significant negative values (p-value <0.05) of Tajima’s D and Fu’s Fs were taken as evidencing a scenario of demographic expansion. Past population dynamics was inferred using Extended Bayesian Coalescent Skyline (EBSP) implemented in BEAST 2.3.2 [21]. We used the strict clock, an evolutionary rate of 5.48% per million years estimated for canids [22] and previously used in this species [7], and HKI+G as best model of nucleotide substitution as selected in MrModelTest2.3 [23]. Two independent runs of 10^8 generations each and sampled every 10^4 generations were performed. Tracer 1.6 [24] was used to check convergence of the MCMC chains. The Extended Bayesian skyline plot (EBSP) was constructed in R platform (R Core Team, 2018).

For microsatellite loci we estimated the variation of effective population sizes (Ne) from present to ancestral time with a coalescent approach using the method VarEff [25] implemented in a R package. The method uses an approximate likelihood of the distribution of distance frequencies between alleles in a Monte Carlo Markov Chain framework [25]. After several trial runs, the final analyses were conducted using the two phase mutation model assuming a proportion of 0.22 for multi-step mutations [26], a mutation rate of 3.5x10^{-3} [27] and allowing three population size changes (JMAX = 3). Prior for current Ne was set according to estimation on trial runs (NBAR = 1,600). Prior for the number of generations since the origin of the population (GBAR) was set to 8000 generations (equivalent to 32 kya, based on a generation time of 4 years for wolves from [28]) to encompass timing of Neolithic expansion in North Africa. Final run was carried out using 10,000 batches with a length of 10, saved every 10 batches in the MCMC chain and with a burn-in period of 10,000 batches.

(f) Verification of demographic inference results

Demographic inference can be affected by population structure, non-random sampling, lack of information in molecular markers and natural selection [29–31]. In order to rule out obvious confounders and possible batch effect, we performed additional demographic analyses for microsatellites using subsets of our samples per country.

We implemented the same coalescent approach using the method VarEff with the same priors as described above, except for the current Ne which was set to 1,600 and 1,200 for Algeria and Tunisia, respectively, as estimated in trial runs. This coalescent approach confirmed a pronounced signature of population expansion for both Algerian and Tunisian datasets (electronic supplementary material, figure S4). The
expansion event estimated separately for each subset of samples is concordant with that estimated using the combination of Algerian and Tunisian samples. This event happened between 960 and 1,680 generations in the past, corresponding in time to the interval between 3,840 and 6,720 years BP. This supports that the observed signature of population expansion is not a result of Northwestern African wolf population substructuring.

Table S1. African wolf samples collected throughout Algeria, including type of sample, geographic location (longitude and latitude), indication of available microsatellite genotypes, mtDNA haplotype code and GenBank accession numbers for the new haplotypes of mtDNA control region.

| Sample | Type | Longitude | Latitude | Microsatellites | mtDNA | GenBank     |
|--------|------|-----------|----------|-----------------|-------|-------------|
| CH04   | Scat | 9.281255  | 24.843176| Yes             | -     | MK659615    |
| CH06   | Tissue | 3.159227 | 33.790477| Yes             | H1    | MK659615    |
| CH07   | Hair | 2.337539  | 33.797816| Yes             | H1    |             |
| CH08   | Hair | 0.016947  | 34.542942| Yes             | H2    |             |
| CH09   | Scat | -1.477441 | 34.778433| No              | H3    | MK659616    |
| CH10   | Tissue | -1.454867| 34.794498| Yes             | H4    | MK659617    |
| CH12   | Tissue | 0.450367  | 35.142239| Yes             | -     |             |
| CH13   | Tissue | -0.849889 | 34.45156 | Yes             | H5    | MK659618    |
| CH18   | Hair | 5.118635  | 35.65465 | Yes             | H6    |             |
| CH19   | Tissue | -1.906344 | 34.935076| Yes             | H7    | MK659619    |
| CH20   | Tissue | -1.454867 | 34.822239| Yes             | H1    |             |
| CH21   | Tissue | -0.858917 | 34.809296| Yes             | H4    |             |
| CH22   | Hair | -0.353265 | 35.493187| Yes             | H8    | MK659620    |
| CH24   | Scat | 9.353495  | 24.93196 | Yes             | -     |             |
| CH23   | Tissue | -1.472927 | 34.764904| Yes             | H1    |             |
| CH26   | Tissue | -1.725753 | 34.616928| Yes             | H9    | MK659621    |
| CH27   | Hair | -0.389381 | 35.837722| Yes             | H10   | MK659622    |
| CH28   | Hair | 3.881595  | 35.544562| Yes             | H1    |             |
| CH29   | Hair | 1.741591  | 35.86015 | Yes             | H6    |             |
| CH30   | Hair | 1.253995  | 35.280347| Yes             | H6    |             |
| CH31   | Hair | 3.403027  | 34.113405| Yes             | H6    |             |
| CH32   | Hair | 8.080331  | 36.430028| Yes             | H11   | MK659623    |
| CH34   | Tissue | -1.238159 | 35.238424| Yes             | H2    |             |
| CH35   | Hair | -1.283305 | 35.171836| Yes             | H12   | MK659624    |
| CH36   | Tissue | -1.021449 | 35.430792| Yes             | H12   |             |
**Table S2.** Polymerase Chain Reaction (PCR) thermoprofile for amplification of the mtDNA control region fragment.

| Temperature | Time | N cycles |
|-------------|------|----------|
| 95°C        | 15’  | 1        |
| 95°C        | 30’’ | 1        |
| 50°C        | 30’’ | 40       |
| 72°C        | 45’’ |          |
| 60°C        | 10’  | 1        |
Table S3. Microsatellite multiplex and PCR thermocycling conditions for the African wolf (multiplexes following [4]; PCR thermocycling adjusted for this study). *loci excluded from the analysis; #loci used to amplify non-invasive samples.

| Multiplex | Microsatellites | Dye   | Temperature | Time       | PCR profile |
|-----------|-----------------|-------|-------------|------------|-------------|
| MS1       | AHT132          | VIC   | 95ºC        | 15'        | 1           |
|           | C27.442         | PET   | 95ºC        | 30''       |             |
|           | FH2010          | FAM   | 58ºC        | 45''       | 20 (-0.1ºC/cycle) |
|           | FH2079          | NED   | 72ºC        | 45''       |             |
|           | PEZ1            | FAM   | 56ºC        | 45''       | 15          |
|           | PEZ3#           | NED   | 72ºC        | 45''       |             |
|           | PEZ5*           | VIC   | 95ºC        | 30''       |             |
|           | PEZ8*           | XXX   | 53ºC        | 45''       | 10          |
|           |                 |       | 72ºC        | 45''       |             |
|           |                 |       | 60ºC        | 30'        | 1           |
| MS2       | AHT103*         | NED   | 95ºC        | 15'        | 1           |
|           | AHT111#         | VIC   | 95ºC        | 30''       |             |
|           | C04.140         | PET   | 56ºC        | 45''       | 35          |
|           | C09.173         | NED   | 72ºC        | 45''       |             |
|           | C13.758         | FAM   | 95ºC        | 30''       |             |
|           | C14.866         | VIC   | 53ºC        | 45''       | 8           |
|           | C20.253#        | PET   | 72ºC        | 45''       |             |
|           | CPH14           | FAM   | 60ºC        | 30'        | 1           |
|           | FH2001          | FAM   | 60ºC        | 30'        | 1           |
|           | VWF#            | NED   | 60ºC        | 30'        | 1           |
| MS3       | C08.140#        | VIC   | 95ºC        | 15'        | 1           |
|           | C08.618         | VIC   | 95ºC        | 30''       |             |
|           | C09.474         | PET   | 60ºC        | 45''       | 7 (-0.5ºC/cycle) |
|           | C20.446         | NED   | 72ºC        | 45''       |             |
|           | C22.763*        | XXX   | 95ºC        | 30''       |             |
|           | CPH02#          | NED   | 57ºC        | 45''       | 22          |
|           | CPH05#          | FAM   | 72ºC        | 45''       |             |
|           | CPH09*          | NED   | 95ºC        | 30''       |             |
|           | CXX.459*        | VIC   | 53ºC        | 45''       | 8           |
|           | FH2161          | NED   | 72ºC        | 45''       |             |
|           | REN64E19#       | FAM   | 60ºC        | 30'        | 1           |
| ThermoFisherScientificGenotypesPanel2.1Kit | AHT121 | PET | 98ºC | 3' | 1 |
|           | AHT137          | VIC   | 98ºC        | 15''       |             |
|           | AHTh171*        | PET   | 98ºC        | 15''       |             |
|           | AHTh260*        | VIC   | 98ºC        | 15''       |             |
|           | AHTk211*        | FAM   | 98ºC        | 15''       |             |
|           | AHTk253         | VIC   | 98ºC        | 15''       |             |
|           | C22.279#        | FAM   | 60ºC        | 75''       | 40          |
|           | FH2054#         | PET   | 57ºC        | 45''       | 8           |
|           | FH2848          | NED   | 72ºC        | 45''       |             |
|           | INRA21#         | VIC   | 95ºC        | 30''       |             |
|           | INU005          | NED   | 95ºC        | 30''       |             |
|           | INU030*         | NED   | 95ºC        | 30''       |             |
|           | INU055          | FAM   | 95ºC        | 30''       |             |
|           | REN162C04#      | PET   | 95ºC        | 30''       |             |
|           | REN169D01       | VIC   | 95ºC        | 30''       |             |
|           | REN169O18       | FAM   | 95ºC        | 30''       |             |
|           | REN247M23       | PET   | 95ºC        | 30''       |             |
|           | REN54P11        | FAM   | 95ºC        | 30''       |             |
Table S4. Genetic diversity of the African wolf based on mitochondrial control region (mtDNA) and microsatellite data for samples collected in Algeria, Tunisia and a combined dataset from Algeria and Tunisia. Information for mtDNA includes number of samples (n), sequence length in base pairs (bp), number of haplotypes (h), segregation sites (S), haplotype (Hd) and nucleotide (π) diversities, standard deviations between parentheses, neutrality tests of Tajima’s D and Fu’s Fs, and raggedness index r. Information for microsatellites includes number of loci (loci), average number of alleles/locus (Na), observed (Ho) and expected (He) heterozygosities and fixation index (Fis), and standard errors between parentheses. ¹this study only. Statistical significance: * P < 0.05, ** P < 0.01, *** P < 0.001.

|                | Algeria+Tunisia | Algeria¹ | Tunisia |
|----------------|-----------------|----------|---------|
| **mtDNA**      |                 |          |         |
| N              | 68              | 22       | 41      |
| bp             | 223             | 369      | 223     |
| H              | 26              | 12       | 15      |
| S              | 21              | 9        | 22      |
| Hd             | 0.944 (0.013)   | 0.918 (0.040) | 0.918 (0.02) |
| Nl             | 0.016 (0.001)   | 0.011 (0.001) | 0.018 (0.005) |
| D              | -0.878          | -0.166   | -0.733  |
| Fs             | -15.634***      | -6.096** | -3.113  |
| r              | 0.022           | 0.052    | 0.020*  |
| **Microsatellites** |                 |          |         |
| N              | 47              | 18       | 27      |
| Loci           | 13              | 38       | 13      |
| Na             | 9.3 (0.3)       | 7.7 (0.3) | 9.3 (0.4) |
| Ho             | 0.770 (0.02)    | 0.715 (0.02) | 0.775 (0.02) |
| He             | 0.830 (0.01)    | 0.773 (0.01) | 0.821 (0.01) |
| Fis            | 0.071 (0.02)    | 0.078 (0.02) | 0.054 (0.03) |
Table S5. Correspondence between mtDNA haplotype code and GenBank accession numbers.

|     | This work  | GenBank accession numbers Karssene et al. 2018[5] | GenBank accession numbers Gaubert et al. 2012[7] |
|-----|------------|--------------------------------------------------|--------------------------------------------------|
|  H1 | MK659615   |                                                  |                                                  |
|  H2 |            |                                                  | JQ088680                                         |
|  H3 | MK659616   |                                                  |                                                  |
|  H4 | MK659617   |                                                  |                                                  |
|  H5 | MK659618   |                                                  |                                                  |
|  H6 |            |                                                  | JQ088678                                         |
|  H7 | MK659619   |                                                  |                                                  |
|  H8 | MK659620   |                                                  |                                                  |
|  H9 | MK659621   |                                                  |                                                  |
| H10 | MK659622   |                                                  |                                                  |
| H11 | MK659623   |                                                  |                                                  |
| H12 | MK659624   |                                                  |                                                  |
| H13 |            |                                                  | JQ088682                                         |
| H14 |            |                                                  | JQ088681                                         |
| H15 |            |                                                  | JQ088679                                         |
| H16 |            | MK392560                                         |                                                  |
| H17 |            | MK392566                                         |                                                  |
| H18 |            | MK392562                                         |                                                  |
| H19 |            | MK392568                                         |                                                  |
| H20 |            | MK392563                                         |                                                  |
| H21 |            | MK392572                                         |                                                  |
| H22 |            | MK392569                                         |                                                  |
| H23 |            | MK392564                                         |                                                  |
| H24 |            | MK392561                                         |                                                  |
| H25 |            | MK392565                                         |                                                  |
| H26 |            | MK392571                                         |                                                  |
Table S6. Analyses of Molecular Variance (AMOVA) results for both Algeria and Tunisia based on mitochondrial DNA and microsatellites, including degrees of freedom (df) and percentage of variance (%).

|                | df | % var. |
|----------------|----|--------|
| **mtDNA**      |    |        |
| Among populations | 1  | 9      |
| Within populations | 67 | 91     |
| **Total**      | 68 | 100    |

|                | df | % var. |
|----------------|----|--------|
| **Microsatellites** |    |        |
| Among populations | 1  | 1      |
| Within populations | 45 | 11     |
| Within individuals | 47 | 87     |
| **Total**      | 93 | 100    |

Table S7. Correlation coefficient (R) between genetic and geographic matrices estimated throughout Mantel test for both Algeria and Tunisia, and for each country separately, including p-value. Significant values are in bold.

|                | Algeria + Tunisia | Algeria | Tunisia |
|----------------|------------------|---------|---------|
| IBD            | mtDNA            | microsatellites | mtDNA | microsatellites | mtDNA | microsatellites |
| R              | 0.075            | 0.149    | 0.124   | 0.174   | 0.069   | 0.194  |
| p-value        | 0.09             | **0.004**| 0.09    | 0.14    | 0.09    | **0.01**|

Table S8. Posterior estimates of effective population size (Ne) for the African wolf at different time in the past (240 generations intervals), calculated with VarEff, for both Algeria and Tunisia (ALG+TUN). Time is given in generations. h. mean: harmonic mean; HPD: highest posterior density (intervals).

| Time          | Effective population size |
|---------------|---------------------------|
| Generations   | h. mean | Mode | median | HPD 5% | HPD 95% |
| 0             | 1122    | 1705 | 1717   | 519    | 2474    |
| 240           | 1901    | 1712 | 1826   | 1404   | 4447    |
| 480           | 1907    | 1702 | 1844   | 1402   | 4949    |
| 720           | 1751    | 1723 | 1823   | 1047   | 5001    |
| 960           | 1199    | 1706 | 1729   | 390    | 4204    |
| 1200          | 609     | 353  | 1442   | 219    | 2873    |
| 1440          | 314     | 295  | 390    | 122    | 2020    |
| 1680          | 202     | 246  | 295    | 73     | 1609    |
| 1920          | 176     | 231  | 269    | 63     | 1071    |
| 2160          | 174     | 231  | 270    | 62     | 1096    |
Figure S1. Mean log-likelihood distribution for each cluster (K) and standard deviation bars as summarized through Structure Harvester [14] for (a) Algerian dataset using 38 loci, and (b) Algerian and Tunisian datasets using 13 loci.
Figure S2. Results of the clustering analysis in STRUCTURE for Algerian and Tunisian datasets using 13 loci. (a) Barplot based on the K=2 and (b) barplot based on the K=3, both showing admixture between individuals. Each vertical bar represents an individual.
Figure S3. Demographic analysis of the African wolf population from Algeria and Tunisia using 223bp of the mitochondrial control region. (a) Mismatch distribution graph inferred in DNAsp where dashed curve indicates the observed frequency distribution of pairwise differences and solid curve indicates the distribution expected under a population growth–decline model. (b) Extended Bayesian skyline plot inferred in BEAST2, with dashed curve indicating changes in effective population size and shaded region representing 95% highest posterior density (HPD) interval. Insert represent the number of counts per number of population changes (mean=1.2, median=1, 95% HPD=0–2).
Figure S4. Demographic analysis of the African wolf population from (a and c) Algeria and (b and d) Tunisia inferred in VarEff. Upper panel: Kernel density of the posterior distribution of the effective population size (Ne) over time. Lower panel: Posterior density distribution of Ne at present and at 1,680 generations ago.
References

1. Frantz AC, Pope LC, Carpenter PJ, Roper TJ, Wilson GJ, Delahay RJ, Burke T. 2003 Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. *Mol. Ecol.* **12**, 1649–1661. (doi:10.1046/j.1365-294X.2003.01848.x)

2. Kocher TD, Thomas WK, Meyer A, Edwards S V, Pääbo S, Villablanca FX, Wilson AC. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 6196–200. (doi:10.1073/PNAS.86.16.6196)

3. Godinho R et al. 2011 Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. *Mol. Ecol.* **20**, 5154–5166. (doi:10.1111/j.1365-294X.2011.05345.x)

4. Silva P et al. 2018 Cryptic population structure reveals low dispersal in Iberian wolves. *Sci. Rep.*

5. Karssene Y et al. 2018 Noninvasive genetic assessment provides evidence of extensive gene flow and possible high movement ability in the African golden wolf. *Mamm. Biol.* **92**, 94–101. (doi:10.1016/j.mambio.2018.05.002)

6. Valière N. 2002 gimlet: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes* **2**, 377–379.

7. Gaubert P, Bloch C, Benyacoub S, Abdelhamid A, Pagani P, Djugouë CAMS, 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**. (doi:10.1371/journal.pone.0042740)

8. Librado P, Rozas J. 2009 DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

9. Kumar S, Stecher G, Tamura K. 2016 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. *Mol. Biol. Evol.* **33**, 1870–1874.

10. Bandelt HJ, Forster P, Röhl A. 1999 Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48.

11. Leigh JW, Bryant D. 2015 PopART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116.

12. Peakall R, Smouse PE. 2012 GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* **28**, 2537–2539.
13. Falush D, Stephens M, Pritchard JK. 2003 Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* **164**, 1567 LP – 1587.

14. Earl DA, VonHoldt BM. 2012 STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**, 359–361.

15. Evanno G, Regnaut S, Goudet J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)

16. Jombart T. 2008 adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405. (doi:10.1093/bioinformatics/btn129)

17. Ersts PJ. In press. Geographic Distance Matrix Generator(version 1.2.3). *Am. Museum Nat. Hist. Cent. Biodivers. Conserv.* See http://biodiversityinformatics.amnh.org/open_source/gdmg (accessed on 29 April 2019).

18. Harpending HC. 1994 Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum. Biol.* **66**, 591–600.

19. Tajima F. 1989 Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.

20. Fu YX. 1997 Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915–925.

21. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014 BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**, e1003537.

22. Li Q et al. 2008 Origin and phylogenetic analysis of Tibetan Mastiff based on the mitochondrial DNA sequence. *J. Genet. Genomics* **35**, 335–340. (doi:10.1016/S1673-8527(08)60049-1)

23. Nylander JAA. 2004 MrModeltest, version 2. Program distributed by the author.

24. Rambaut A, Drummond AJ. 2013 Tracer.

25. Nikolic N, Chevalet C. 2014 Detecting past changes of effective population size. *Evol. Appl.* **7**, 663–681. (doi:10.1111/eva.12170)

26. Peery MZ, Kirby R, Reid BN, Stoelting R, Jonathan N, Robinson S, Va C. 2012 Reliability of genetic bottleneck tests for detecting recent population declines. *Mol. Ecol.* **21**, 3403–3418. (doi:10.1111/j.1365-294X.2012.05635.x)
27. Parra D, García D, Mendez S, Cañon J, Dunner S. 2009 High Mutation Rates in Canine Tetranucleotide Microsatellites : Too Much Risk for Genetic Compatibility Purposes? *Open Forensic Sci. J.* 2, 1–5.

28. Mech LD, Barber-meyer SM, Erb J. 2016 Wolf (Canis lupus) Generation Time and Proportion of Current Breeding Females by Age. *PLoS One* 11, e0156682. (doi:10.1371/journal.pone.0156682)

29. Stiller M et al. 2010 Withering away-25,000 years of genetic decline preceded cave bear extinction. *Mol. Biol. Evol.* 27, 975–978. (doi:10.1093/molbev/msq083)

30. Heller R, Chikhi L, Siegismund HR. 2013 The Confounding Effect of Population Structure on Bayesian Skyline Plot Inferences of Demographic History. *PLoS One* 8, e62992. (doi:10.1371/journal.pone.0062992)

31. Chikhi L, Sousa VC, Luisi P, Goossens B, Beaumont MA. 2010 The confounding effects of population structure, genetic diversity and the sampling scheme on the detection and quantification of population size changes. *Genetics* 186, 983–995. (doi:10.1534/genetics.110.118661)