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GETTING TO KNOW ANCIENT DOGS THROUGH A GENOMIC APPROACH

10th ICAZ APGM - 10th-13th October 2023

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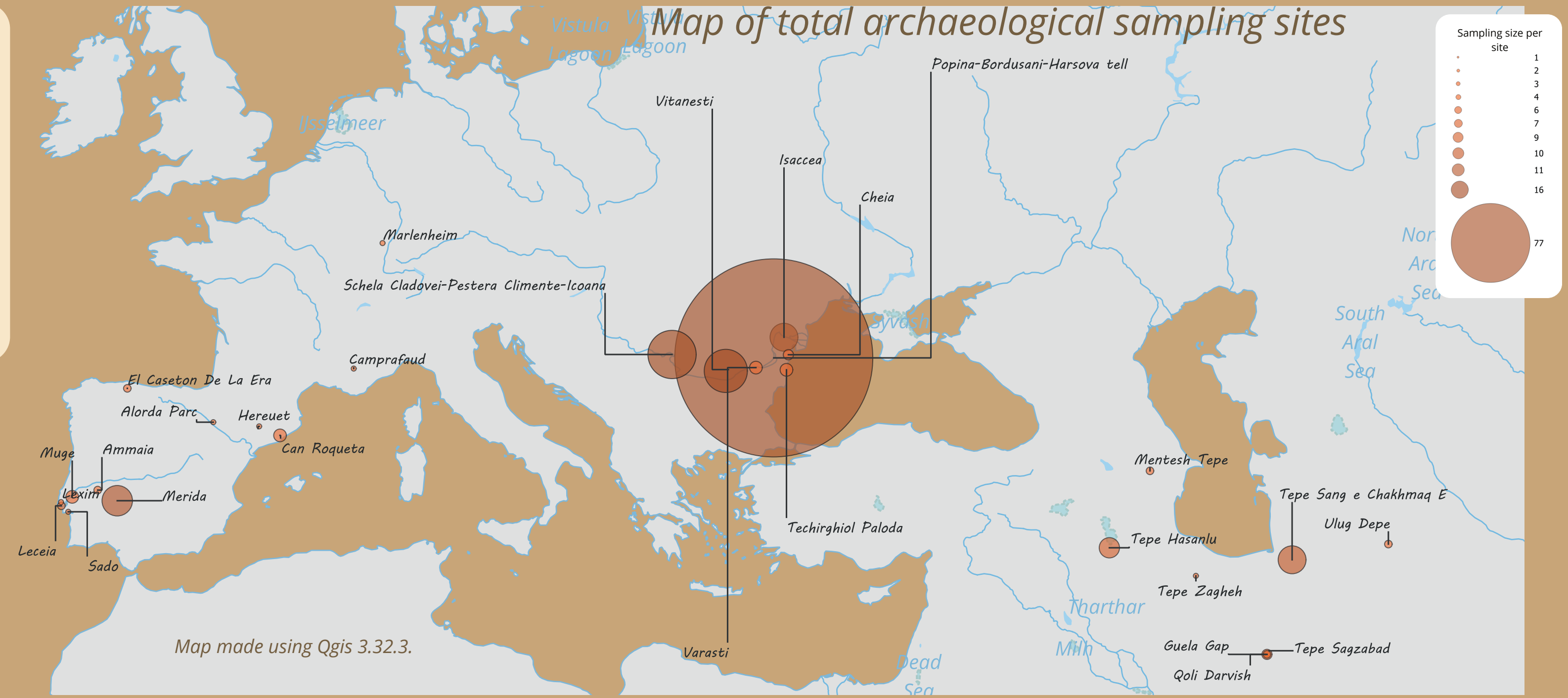
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Studying ancient dogs' genomes offers valuable information about past populations, genetic ancestries, origins, and evolution. As humans shifted from hunter-gatherer to agropastoral societies during Neolithic, *Canis lupus familiaris* is the only domestic species that accompanied humans throughout these changes, and is therefore an excellent model to study the impact of human cultural shifts on their immediate environment.

AIM: This research explores the origins and diversity of Western Eurasian dogs from SouthWestern Europe to the Near & Middle-East dated from Palaeolithic (>33,000yrs BP) to the Middle Ages (750 AD).

Sampling: In order to study dogs' genetic diversity and their spread throughout Europe, a total of 240 dogs and wolves' mitochondrial genomes were obtained through Whole Genome Sequencing and a target-enrichment assay and applying specific bioinformatic pipelines. Among those newly-generated mitogenomes, 151 have a coverage >90%, with a depth of >5X.

A nuclear DNA enrichment assay targeting 4,003 specific Single Nucleotide Polymorphism (SNPs) [5] was performed on 174 samples. In 74 samples, >2% of the total SNPs array with >3X coverage were present.



Mitochondrial DNA sequences are used to assess relationships intra & interspecies, mainly through the study of haplogroups, which are specific clusters of diversity. To our dataset, we added other genomes from present-day dogs [4][12][13][14][15][16][17] and other canids as well as from ancient dogs [1][2][16][18][19][20][22]. Below is presented a preliminary Maximum Likelihood phylogenetic tree (Iqtree).



Preliminary cladogram (ML tree) of 466 ancient and present-day mitochondrial dog genomes

The substitution model is TN+I+H+R4 (model Finder). All monophyletic clades were collapsed if they were from the same location (eg: Romania). Sample's nomenclature as: ID_Sites_Country_Hg/Sp_Date(direct or indirect) in BP. Purple = Wolves, Red=dog HgA, Blue=HgB, Yellow=HgC, Green=HgD

NEXT STEPS ?

- Recover radiocarbon dating for all crucial samples
- Refine the phylogenetic tree using Bayesian approach
- Test our hypothesis of different scenarios of phylogeographic diffusion using models and demo-genetic analysis
- Test for admixture between contemporaneous, ancient dogs and wolves populations in each studied regions to highlight possible links between phenotypes and their origin

SPECIFIC MARKERS UNDER EVOLUTION ?

We present preliminary results about 19 SNP markers associated with morphology and function. The study of such markers can ultimately help addressing relations between the evolution of morphological features of ancient specimens, evidence of adaptation under new environmental conditions and/or selection events. In dogs there are an increased prevalence of large-effect loci, nevertheless morphological features are often influenced by several loci and interpretation of molecular data should be cautious.

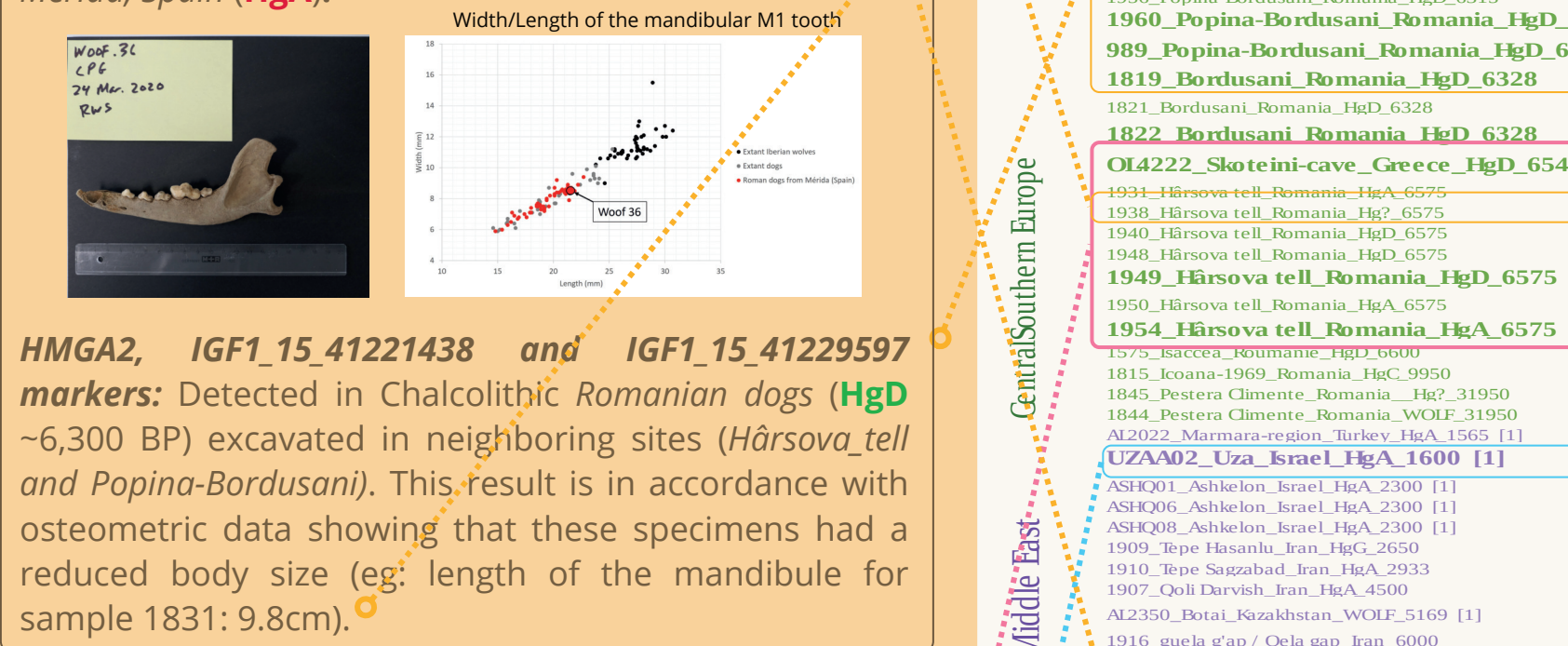
We were able to retrieve these markers for 62 ancient samples with a minimum coverage of 5X

BODY SIZE

IGSF1 gene: Presence of derived allele (homozygous form) associated with an increase in body size-by-weight [6]. We demonstrate the first appearance of this variant around 6,575 BP in a dog from Romania (with unknown Hg (no mitogenome data)).

Derived alleles in GHR-1[11], HMG2 [11], IGF1_15.41221438 [21] and IGF1_15.41229597 [21] genes are all associated with small body size in present-day dogs.

GHR-1 gene: earliest presence of the derived allele around 7,000 BP detected in a sample from Azerbaijan (Hg B). In contrast, the reference allele in homozygosity is associated with a large body size. Osteometric and odontometric data from a mandible (length: ~16cm) and a M1 tooth supports this finding for a Roman dog from Merida, Spain (HgA).



HMG2, IGF1_15.41221438 and IGF1_15.41229597 markers: Detected in Chalcolithic Romanian dogs (HgD ~6,300 BP) excavated in neighboring sites (Hârsova, tell and Popina-Bardusani). This result is in accordance with osteometric data showing that these specimens had a reduced body size (eg: length of the mandible for sample 1831: 9.8cm).

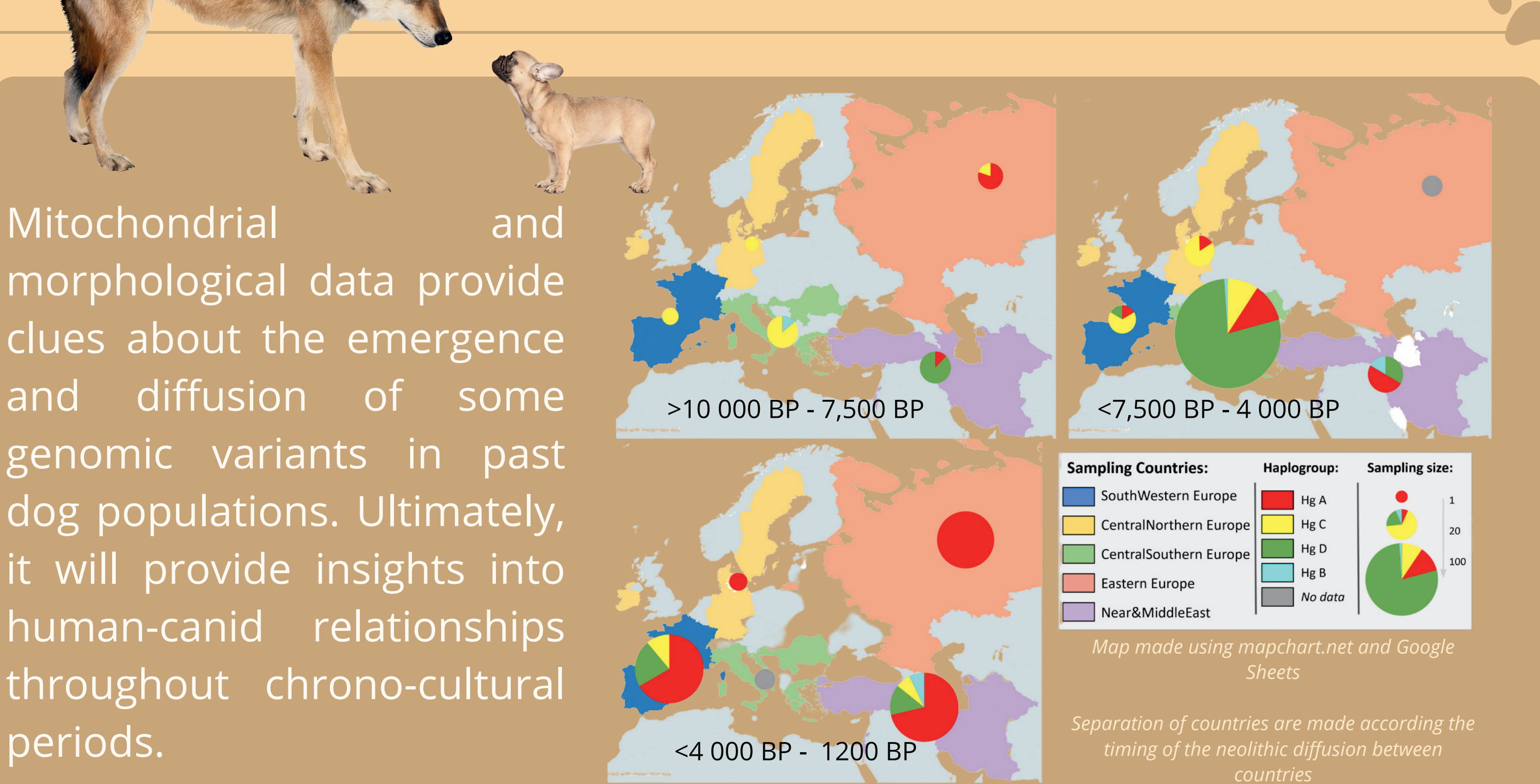
COAT COLOR
ASIP [25] - TYRP - MITF genes: Presence of derived alleles as early as Neolithic Romania (HgA ~6,575 BP) and Greece (HgD ~6544 BP) showing apparent diversity in those genes, that can imply coat color variations.

FUR
FGF5 gene [24]: we notice the first appearance of derived allele associated with long-hair trait in Israel around 1,600 BP as well as variant related with less hair shedding (MC5R) in the same dog (HgA).

Hypothesis: The ancient dogs bearing derived variants mostly belong to Hg A and B and could have been introduced in Europe following the Neolithic wave from the Near and Middle East, we hypothesize that these variants could have originated in those regions before being disseminated.

Figure made using Google Sheets (pink = REF/REF, light red = REF/ALT, dark red = ALT/ALT, white = missing data)

Dogs genotypes for 19 targeted nuclear variants associated with morphology and function



Map made using mapchart.net and Google Sheets

Separation of countries are made according the timing of the neolithic diffusion between countries

[1]: Bergström, A et al., 2020, Science (370) [2]: Frantz L, et al., 2016, Science (352) [3]: Gopalakrishnan et al., Current Biology (28), 2018 [4]: Lindblad-Toh K et al., 2005, Nature - Kim KS et al., 1998, Mol Phylogenet Evol [5]: Manin et al., 2023, submitted [6]: Plassais J, et al., 2019, Nature [7]: Skoglund et al., 2015 [8]: WOOF project [9]: Ollivier et al., 2018 [10]: Cadieu et al., 2009 [11]: Rimbault M, et al., 2013, Genome Res [12]: Björnfeldt et al., 2006 [13]: Webb Allard et al., 2009, J Forensic Sci [14]: Pang JF, et al., 2009, Mol Biol Evol [15]: Baranowska I, et al., 2009, Pgen [16]: Thalman O, et al., 2013, Science [17]: Strakova A, et al., 2016, Elife [18]: Armeen et al., 2019, Biological Science (286) [19]: Bergström et al., 2022, Nature 607 [20]: Botigué L, et al., 2017, Nat Commun 8 [21]: Sutter et al., 2007, Science et Gray et al., 2010, BMC Biol [22]: Leathlobair et al., 2018, Science 361 [23]: Mikkel-Holger S. Sinding et al., 2020, Science 368, [24]: Dierks et al., 2013, Epub [25]: Berryere TG, et al., 2005, Genome

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