

Sheep along the trading routes: what may we tell from their demographic history? A case study from Hungary

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BACKGROUND

In Hungary agriculture is a sector that plays a crucial role in the national economy especially in rural areas: sheep are the third largest livestock population species.

In this study we analysed four local Hungarian sheep breeds: Tsigai, Hortobágy Racka, Cikta and Babólna Tetra. Except for Babólna Tetra (which originated by crossbreeding the Hungarian Merino, the Romanov, and the Finnish Landrace), the origin and evolution of the other breeds is still unclear.





OBJECTIVE

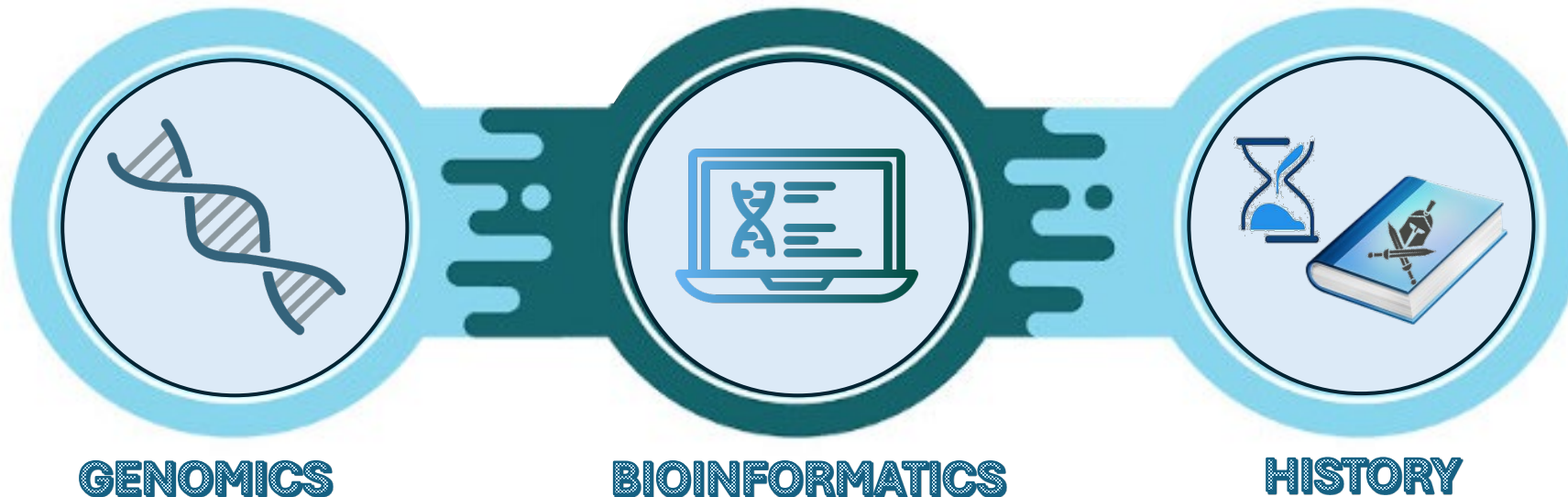


To assess the genetic diversity of four local Hungarian sheep breeds.

Since Hungary has been the convergence point of different expansion and trading routes (such as “the Varangians to the Greek”, a mediaeval trade route that connected Scandinavia, Kievan Rus' and the Eastern Roman Empire), we explored the pattern of admixture searching for some trace of Scandinavian ancestry in Hungarian sheep or *vice versa*.

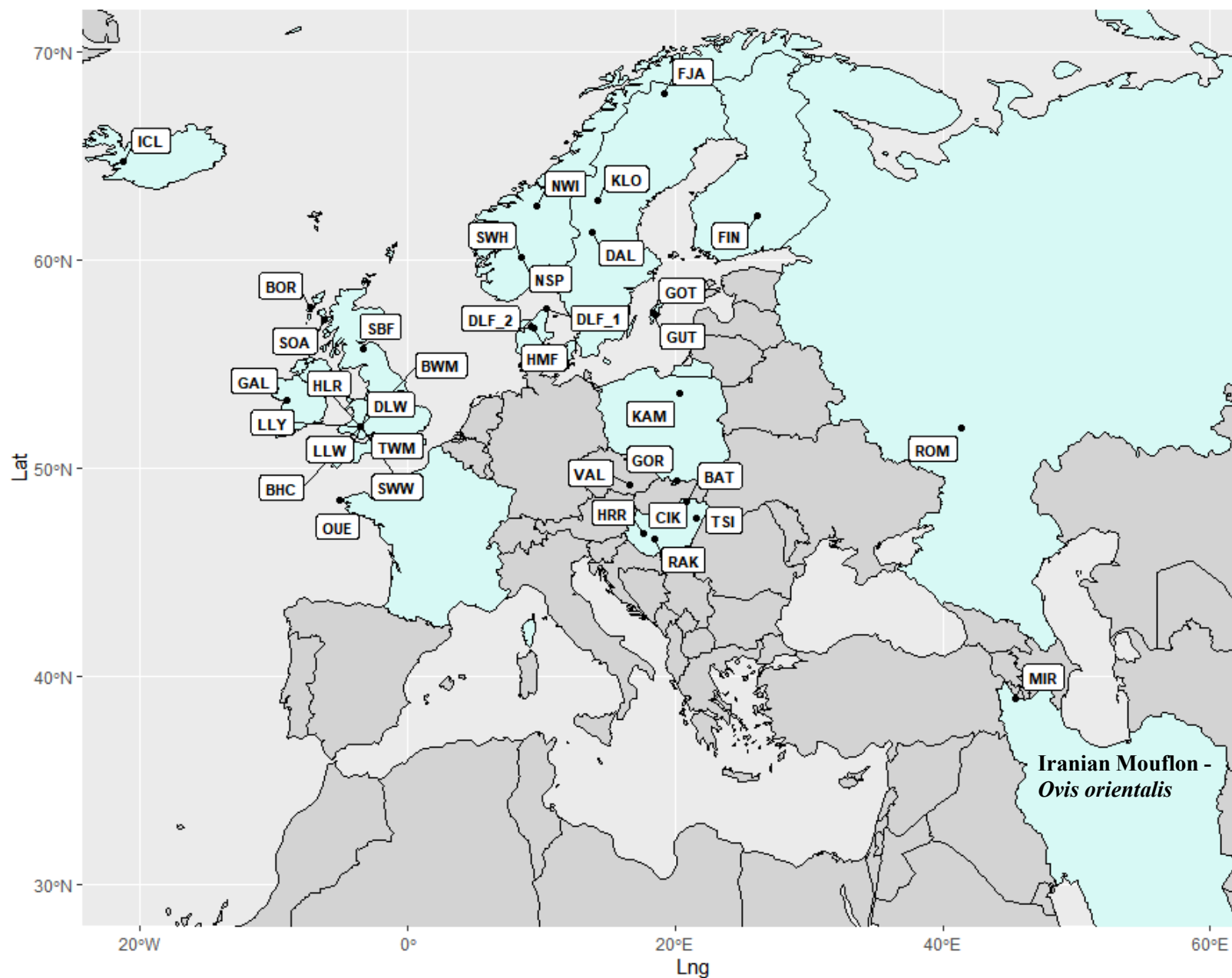
We included other local breeds, spanning from North-western Europe to the Volga river, to evaluate the possibility of gene flow among them.

MULTIDISCIPLINARY APPROACH





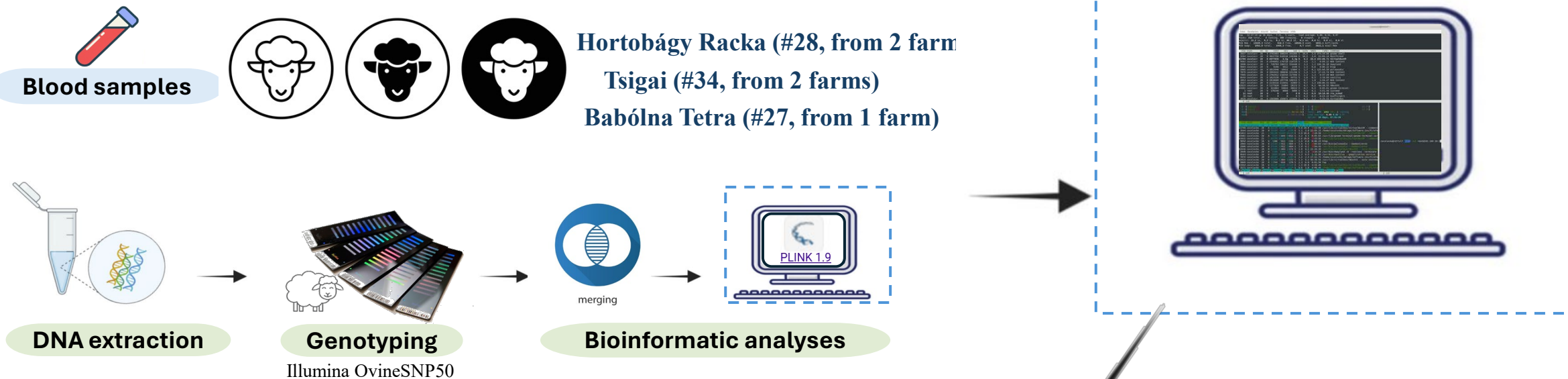
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Breed	Breed code
Valachian	VAL
Danish landrace (Hulsig-Vesterbølle)	DLF_1
Danish landrace (Ertebølle)	DLF_2
Headed Marsh sheep	HMF
Finnship	FIN
Ouessant	OUE
Bábolna Tetra	BAT
Cikta	CIK
Racka	RAK
Tsigai	TSI
Hortobágy Racka	HRR
Icelandic landrace	ICL
Spael-white	SWH
Old Norwegian Spaelsau	NSP
Norwegian White Spaelsau	NWI
Kamieniec	KAM
Romanov	ROM
Dalapäls	DAL
Fjällnäs	FJA
Gotland	GOT
Gute	GUT
Klövsjö	KLO
Brecknock Hill Cheviot	BHC
Boreray	BOR
Black Welsh Mountain	BWM
Hill Radnor	HLR
Llanwenog	LLW
Lleyn	LLY
Scottish Black face	SBF
Soay	SOA
South Wales Welsh Mountain	SWW
Talybont Welsh Mountain	TWM
Dolgellau Welsh Mountain	DLW
Galway sheep	GAL
Polish Mountain	GOR



Sampling and data preparation



Dataset building and data analyses

- After merging
 - Quality Control procedure
 - maf 0.01
 - geno 0.05
 - mind 0.05
 - Check heterozygosity
 - Check LD
 - Balancing the #animals
- 763 individuals were included for the following analyses

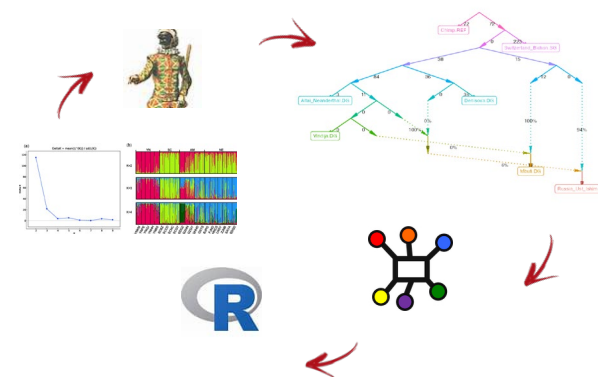
LD pruning

NO **YES**

Demographic
history
~ 35,688 markers

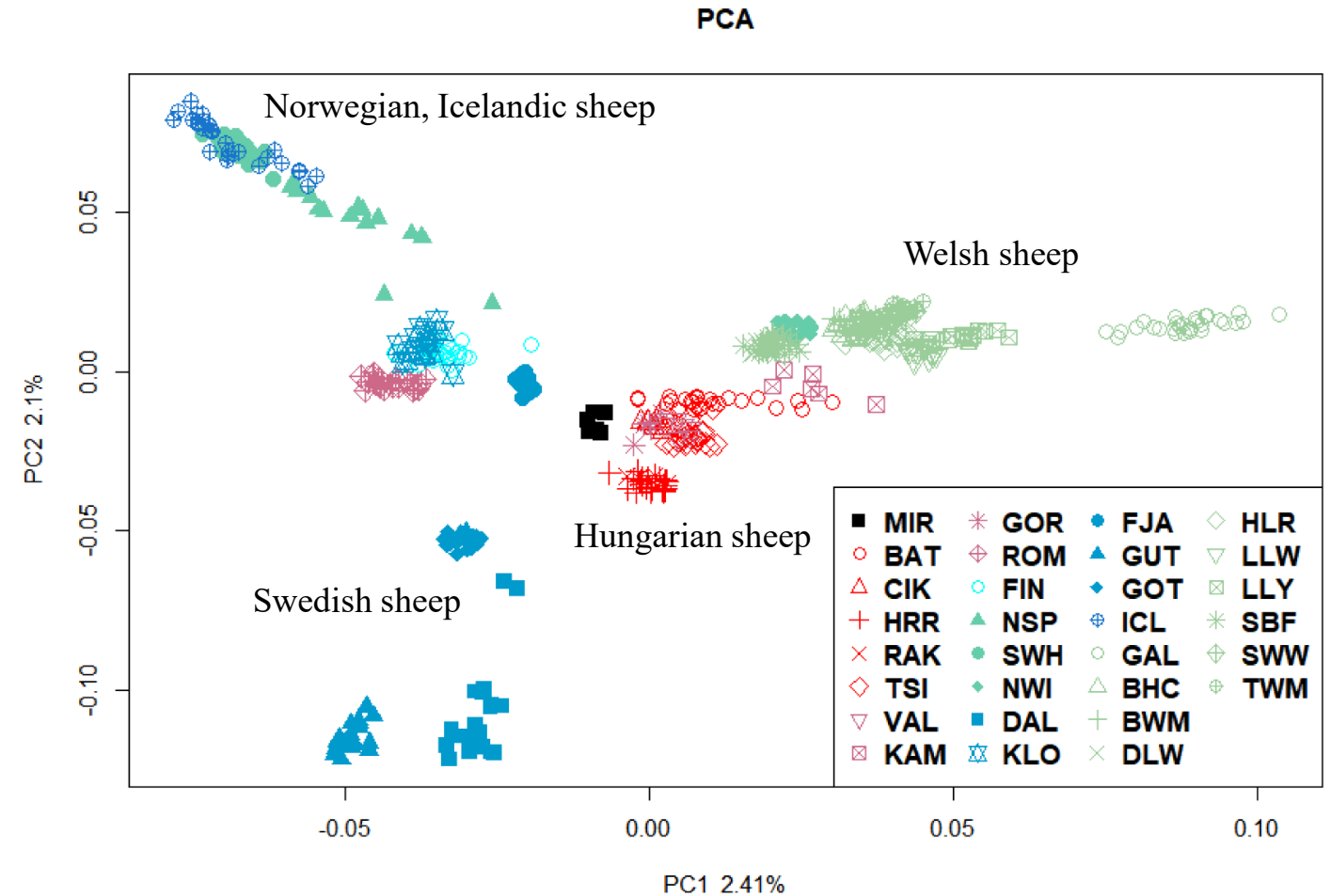
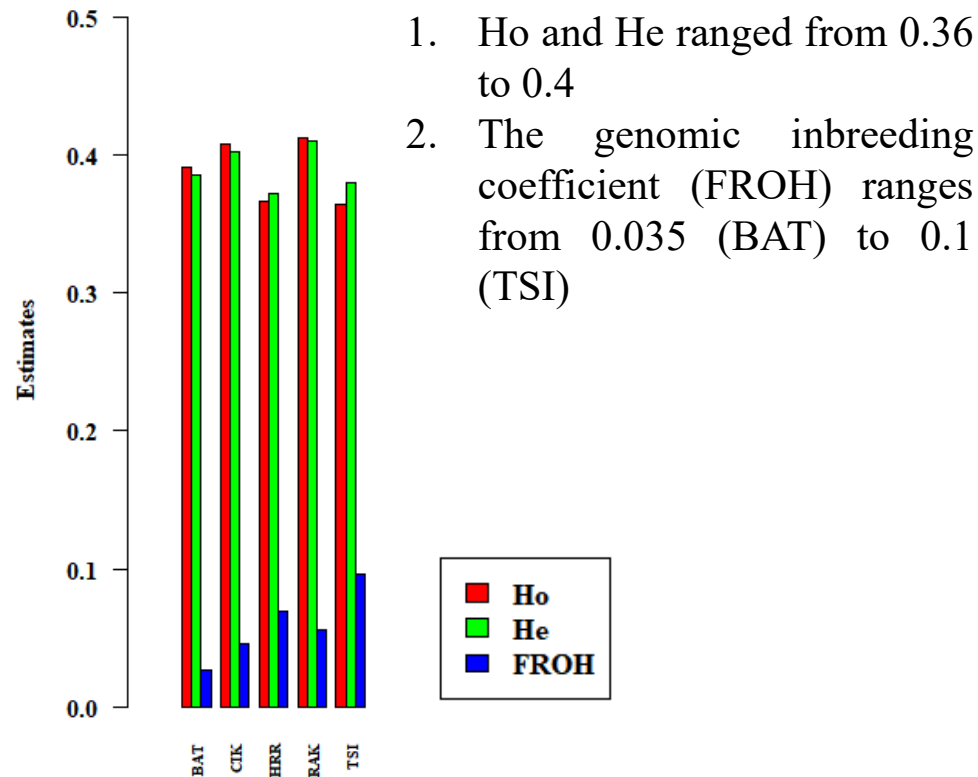
Genetic Structure
and Relationship
~ 31,010 markers

Softwares suite



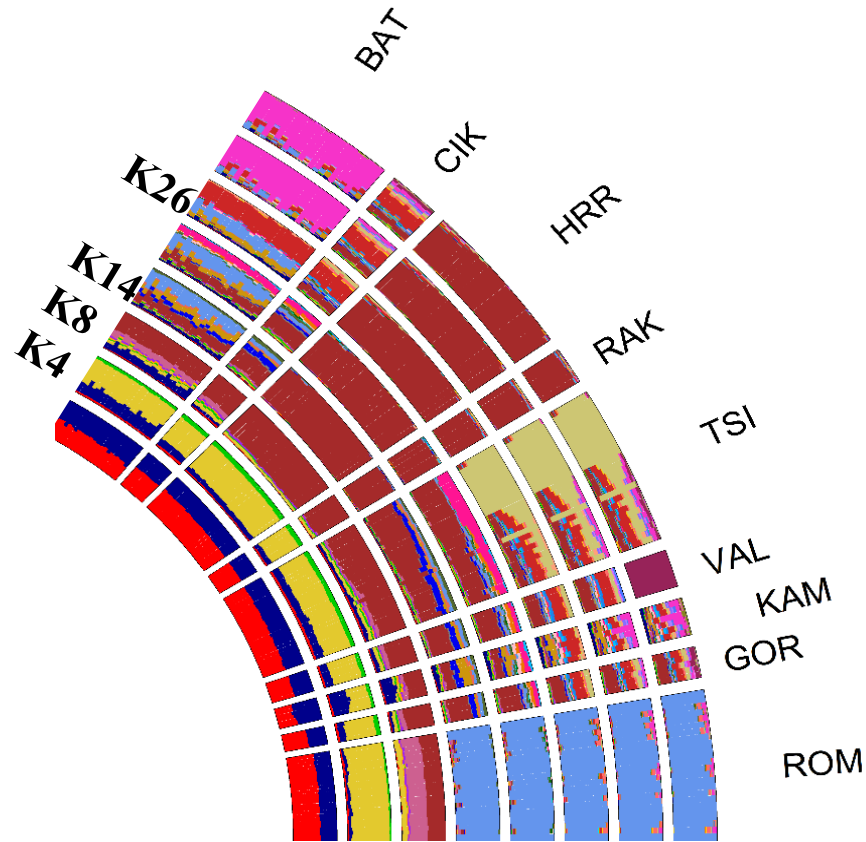


Observed and Expected Heterozygosity, FROH via ROHs

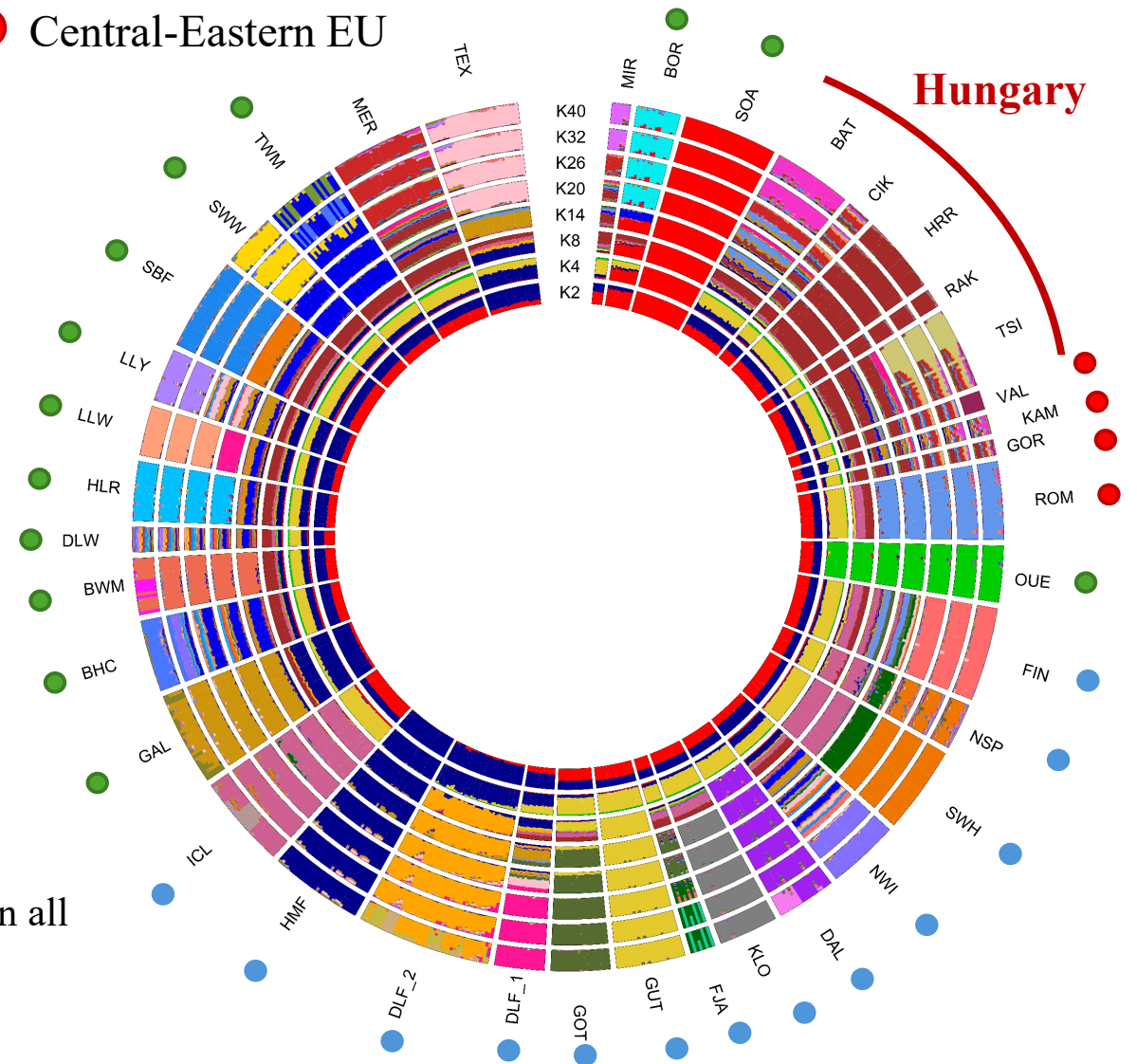


PCA

- (PC1) divides the breeds based on the type of tail (short and long tailed sheep)
- (PC2) separates most of the Swedish, Hungarian and other Central-Eastern EU sheep - such as VAL and GOR - from Norwegian, Icelandic and the Welsh sheep.



- N-Western EU
- Northern EU
- Central-Eastern EU



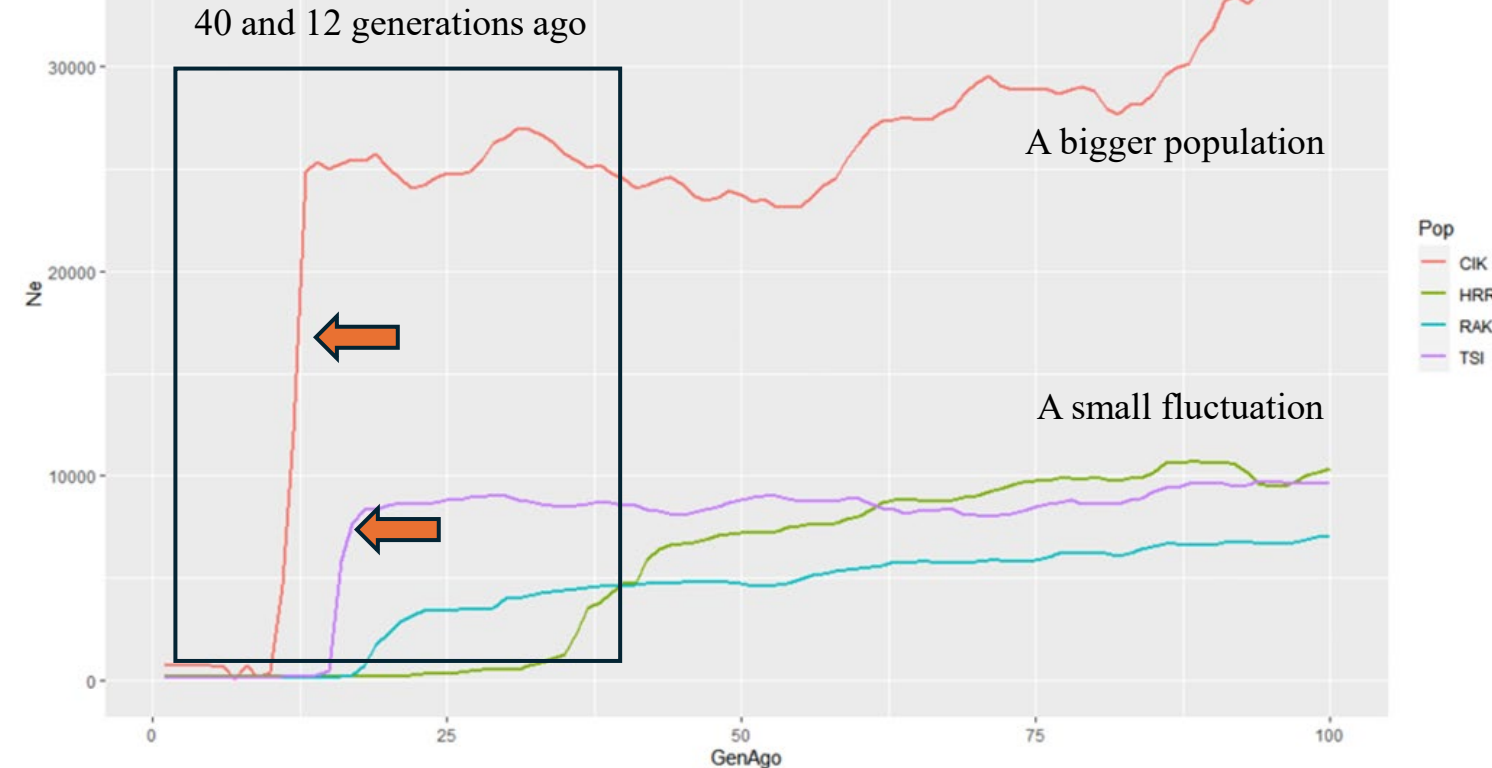
1. K4, yellow component (most prominent in the Swedish GUT)
2. K8, The indigenous component of the Hungarian Racka is observed in all the Central-Eastern European breeds
3. K14, ROM separated
4. K26, TSI divided into two groups, CIK and BAT admixed



Populations' demographic history and inbreeding

GONE: contemporary and historical estimates of the N_e (population size)

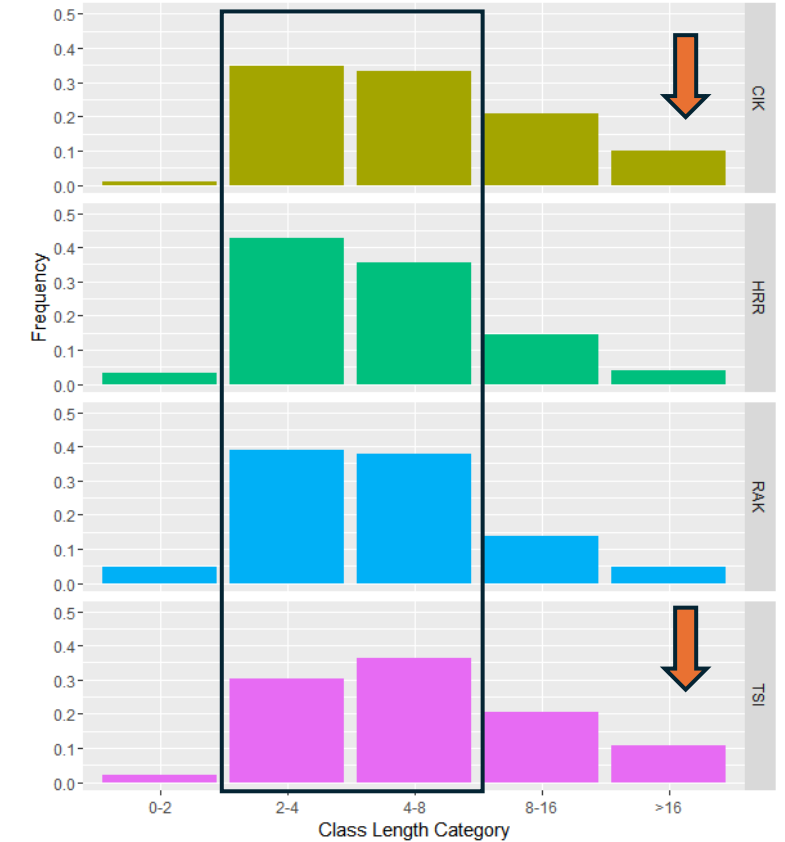
unphased genotypes, correction for sample sizes, number of internal replicates set to 100, assuming a generation time of 3 years for sheep per generation



1. The curves start to decline around 120-40 years ago according to historical bottlenecks
2. The reduction in N_e is more recent for TSI and CIK, according to previous studies based on STR and mtDNA (Gáspárdy et al., 2021, 2022; Kovács et al 2019)

% ROHs by class

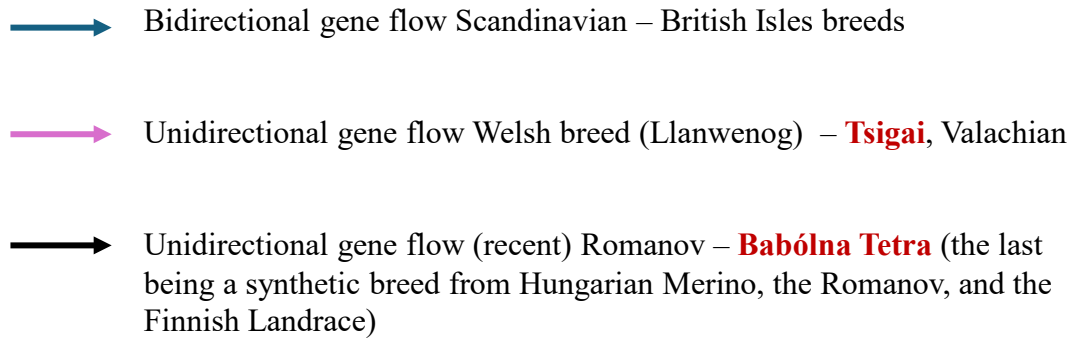
DetectRUNS: minimum length of ROH= 1Mb and the minimum number of SNP included in the runs = 30



1. A common pattern of distribution, with most of homozygous segments being between 2 and 8 Mb for all breeds.
2. In CIK and TSI around 10% of segments are longer than 16 Mb, suggesting a more recent inbreeding.



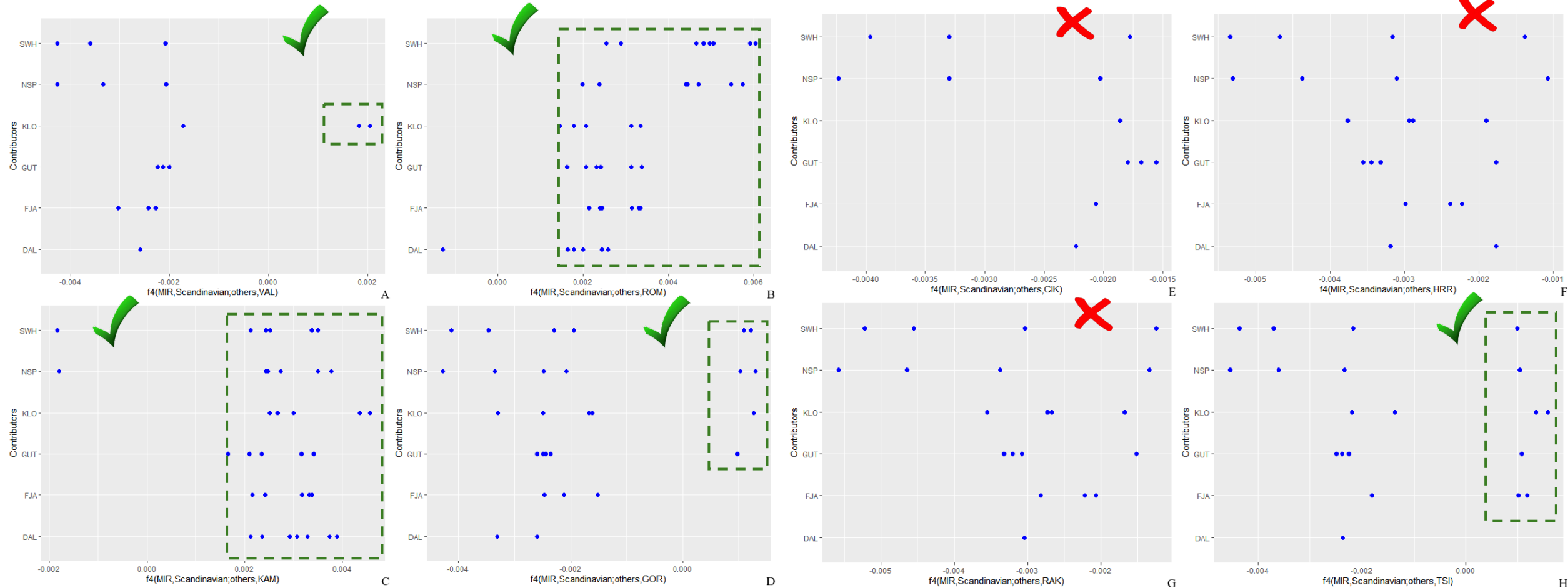
Optimal number of migration edges = 20 and 98.3% of the explained variance



At this point, our question is: can we find traces of old gene flow between Scandinavian and Central-Eastern EU breeds that can be related to any type of cultural influence/contact during the Viking and Medieval World (Vikings and Varangians/Rus') ?



f4-statistics generated by ADMIXTOOLS2 for Central Eastern European breeds



We tested all the possible tree topology combinations but retained only the most significant ones (f4-test) with a $|Z| > 3$

f4(Outgroup, B; C, D); to test for gene flow between B and D (estimate significantly positive), or B and C (estimate significantly negative) (Lipson et al, 2020)



Test pop	ref A	ref B	2-ref decay	timeframe	p-value
TSI	HMF	NSP	133.35 +/- 36.40	1000-1400 CE	0.022
	NSP	SWW	109.13 +/- 26.22	1200-1500 CE	0.027
	NSP	TWM	109.33 +/- 24.94	1200-1500 CE	0.046
VAL	DLF_2	FIN	86.64 +/- 40.08	1400-1700 CE	0.045
	GOR	NSP	73.68 +/- 28.25	1400-1700CE	0.016
ROM	FJA	HMF	150.88 +/- 53.52	800-1400 CE	0.01
	FJA	SBF	80.69 +/- 19.03	1400-1700 CE	0.048
	ICL	MIR	70.92 +/- 18.05	1400-1700 CE	0.022
	KLO	SBF	114.73 +/- 47.28	1100-1600 CE	0.044
	MIR	SBF	95.66 +/- 14.67	1300-1500 CE	0.0066

HRR	KAM	KLO	83.07 +/- 25.13	1400-1700 CE	0.0031
	BWM	KLO	72.21 +/- 28.83	1400-1700 CE	0.028
	ROM	VAL	155.18 +/- 38.87	900-1300 CE	0.044

Some event of admixture starting around 10th century

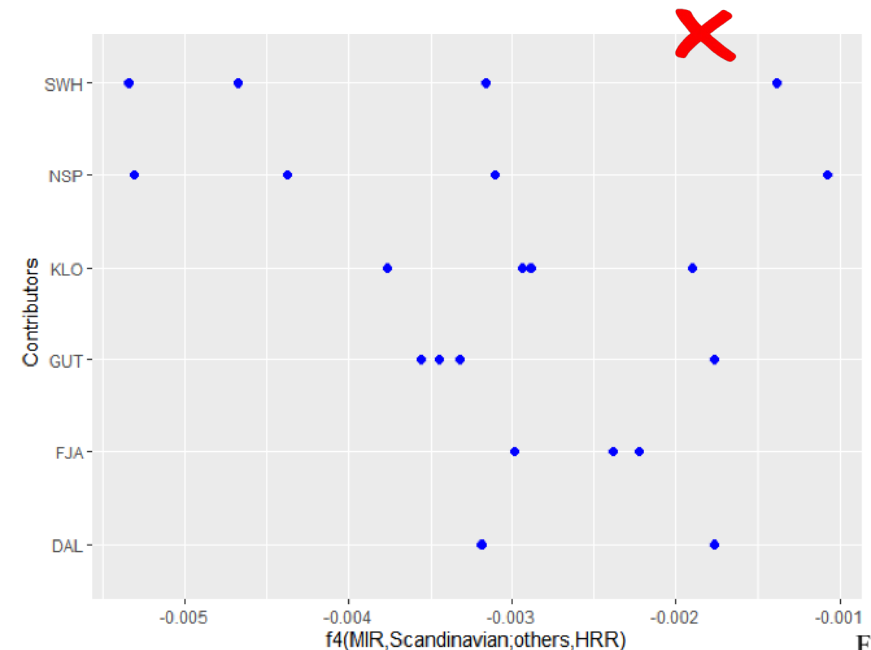
ALDER: LD decay curves and time of admixture

It computes weighted LD curves to infer the admixture time.

According to the **admixture events detected by f4-test** and involving Tsigai, Valachian and Romanov we obtained statistically significant signals of exponential decay from their decay curve weights.

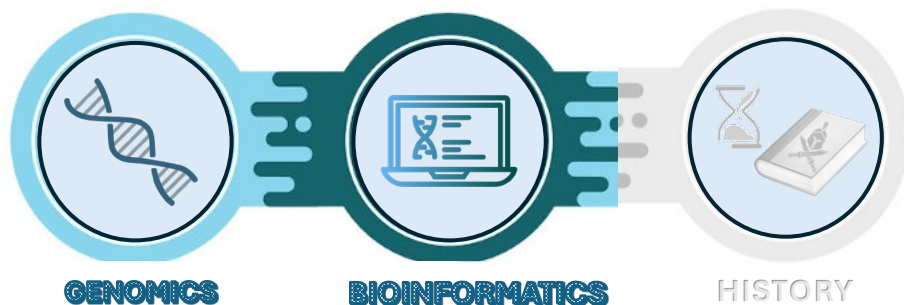
2-ref decay shows the estimate for admixture date, spanning from ~11th-18th century

F-statistics, which does not consider LD, can fail in capturing some admixture event.



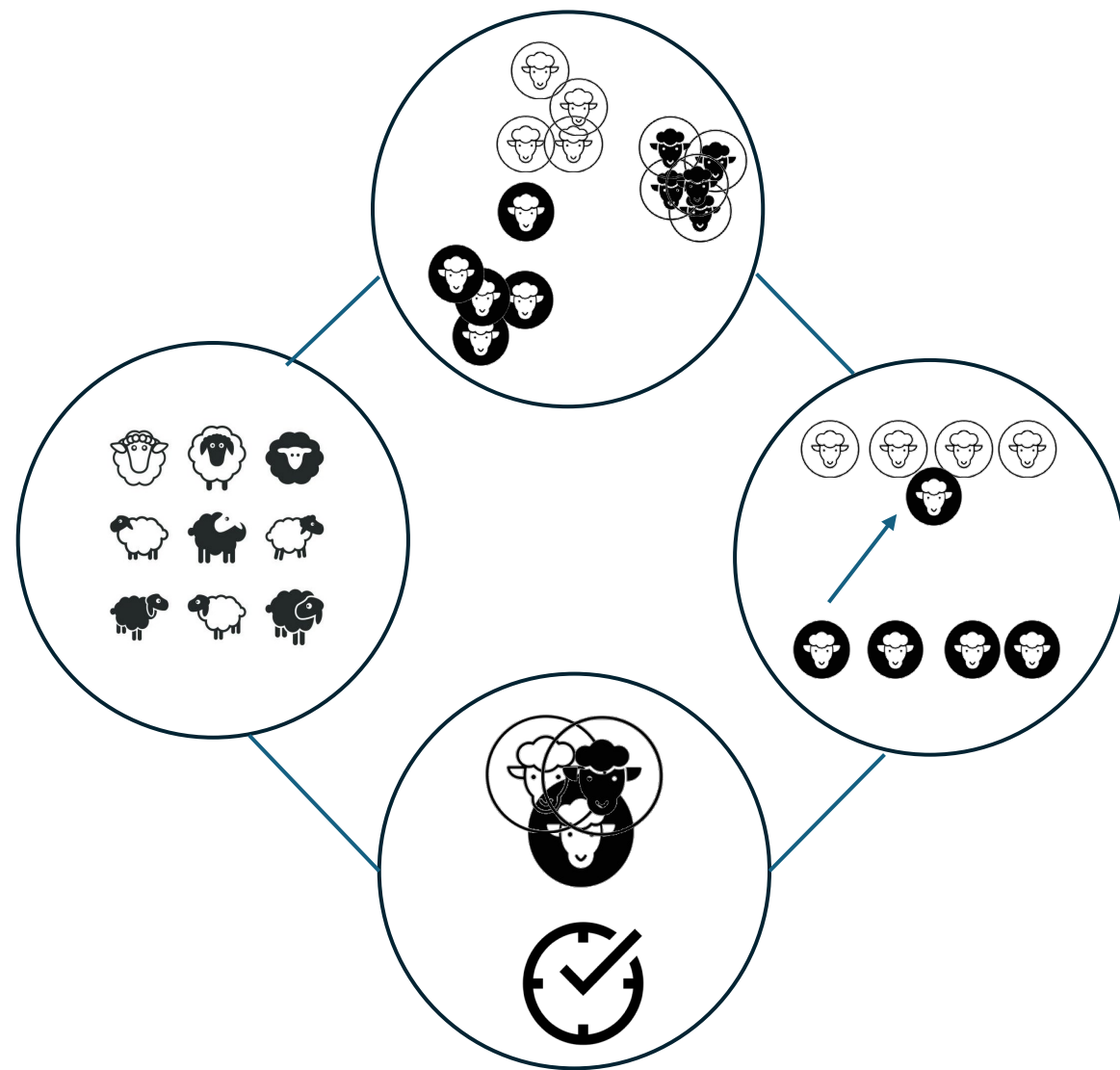


The complex demographic history of sheep: still under continuous revision



Admixture graph, f4-tests and the estimates for admixture date, spanning from ~11th-18th century, are quite consistent and can find an historical support.

The Nordic-like genetic component in some Hungarian breeds could result from a recent introgression of Finnsheep to increase fertility or from the Varangians (Rus) displacement along the Eastern Europe (Medieval trade routes).



introgression of Finnsheep or historical contacts ???



While these results provided some clues that may suggest a different story, more analysis are needed to provide grounds for inspiring future studies and opening new windows into the past.

Magyars (ancestors of present-day Hungarians) **Historical evidence about previous contacts:**

- Linguistic: Magyar language is of Finno-Ugric origin
- Excavations at the Viking Age settlement of Birka (Sweden) have uncovered artefacts of possible Magyar origin.
- Hungarian coins are known from Viking Age contexts in Eastern Scandinavia, suggested possible trading contacts
- Both graves and settlement layers show evidence of possible Magyar contacts.
- Written sources and Magyar folk-traditions

Preservation of local breeds as an agrobiodiversity resource and a cultural identity.





Thank you for your attention

