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Alice Mouton, Christine Fournier-Chambrillon, Pascal Fournier, Jakub Skorupski, Przemyslaw Śmietana, Benoît Vacherie, Thomas Guérin, Emilie Payen, Laurie Bertrand, Ghislaine Magdelenat, et al.

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European mink (*Mustela lutreola*) reference genome and population genomics

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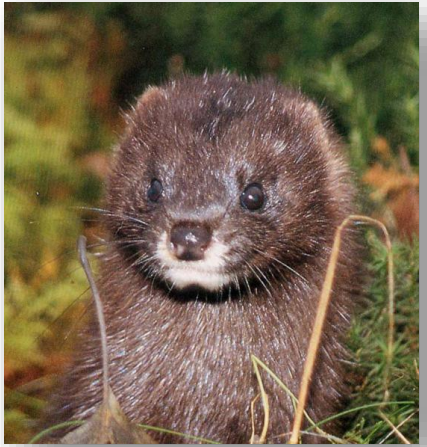
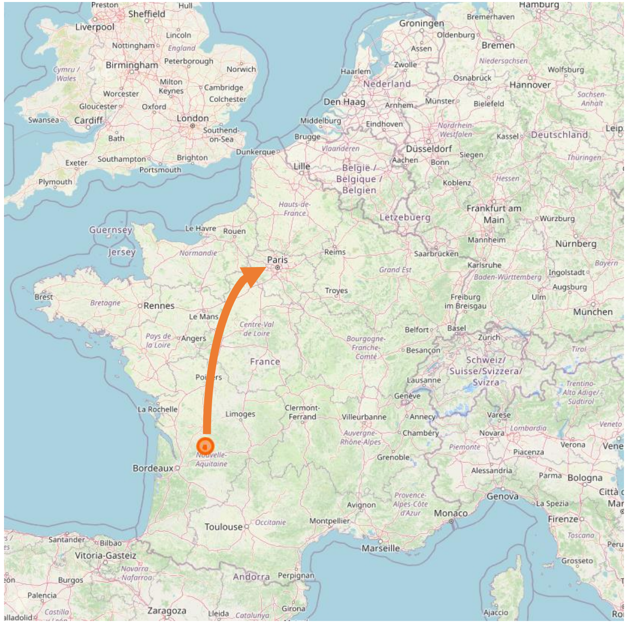


Mouton Alice, University of Liege

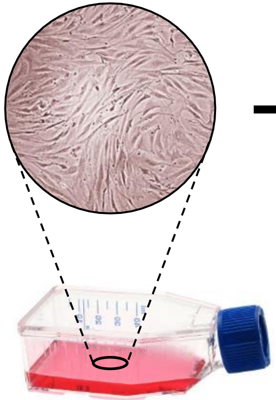
34th European Mustelid Colloquium September 2022

Reference genome : individual selected

- February 2006 : skin biopsy taken from a male captured by GREGE in SW France, in the framework of PNA (National Action Plan)
- Sent to MNHN (National Museum of Natural History, Paris) for establishment of cell culture in flasks
- March 2006 : cryopreservation in liquid nitrogen (-196°C) of cryotubes containing mink cells ("suspended life")



OpenStreetMap



Culture flask

Cryotube

Liquid nitrogen tank

Long term cryopreservation

eRGA
EUROPEAN REFERENCE GENOME ATLAS
Pilot Project

34th European Mustelid Colloquium
14-16 September 2022
Online Conference

EUROPEAN REFERENCE GENOME ATLAS

The European Reference Genome Atlas (ERGA) initiative is a pan-European scientific response to current threats to biodiversity. Reference genomes provide the most complete insight into the genetic basis that forms each species and represent a powerful resource in understanding how biodiversity functions. With approximately one fifth of the ~200,000 European species at risk of extinction, we need to act fast and together to generate high-quality complete genome resources in large scale.

Science needs genomes to understand biodiversity,
biodiversity needs to be understood to be protected.

https://www.erga-biodiversity.eu/

Reference genome : ERGA pilot project

- ERGA (European Reference Genome Atlas) Pilot projet
  European mink selected as a critically endangered species

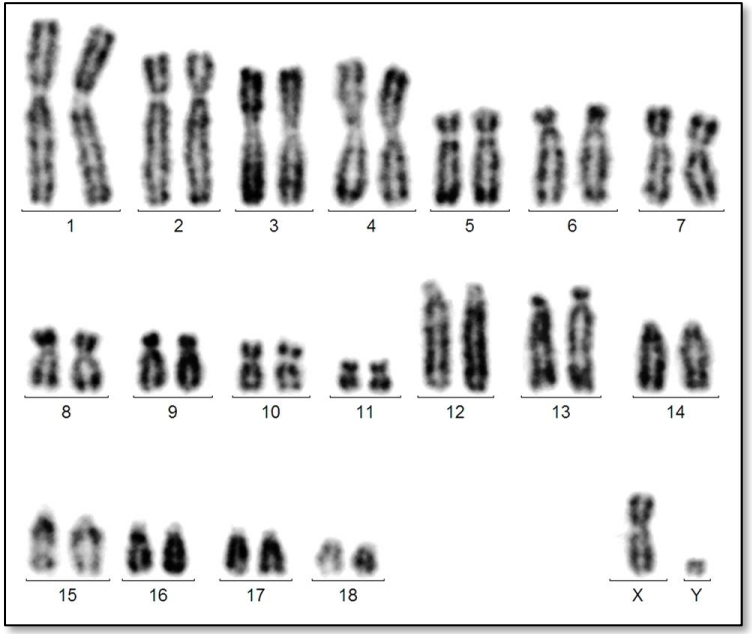
- June 2021 : New cell cultures initiated from samples cryopreserved in 2006
  Control of karyotype, barcode

- July 2021 : 27 million cells produced and transferred at Genoscope (sequencing center) for :
  - DNA & RNA extraction
  - sequencing (long reads, short reads, Hi-C…)
  - genome assembly

- Mars 2022 : Assembly v1

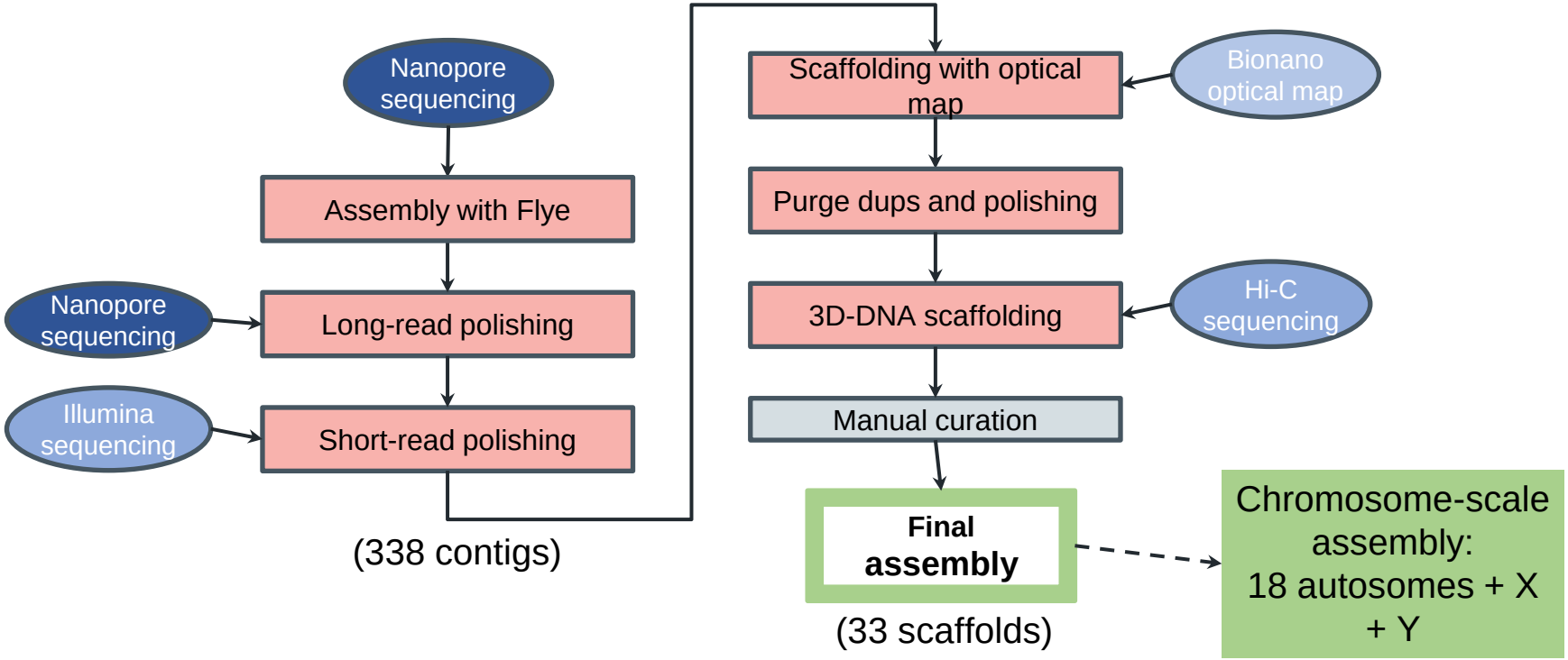
- May 2022 : Assembly v2

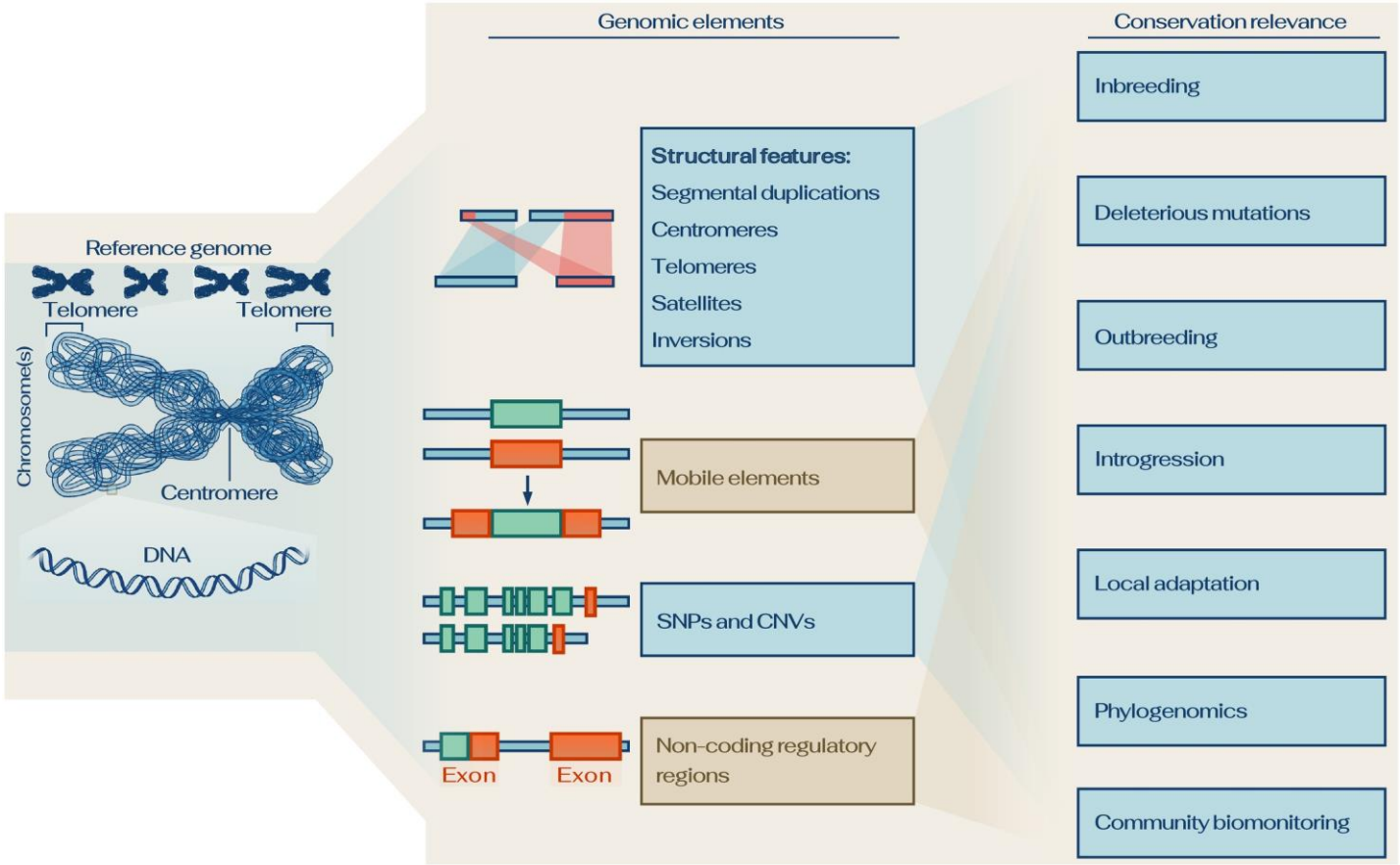
https://www.erga-biodiversity.eu/pilot-project



European mink karyotype (2N=38)
Bertrand Bed'hom (MNH Paris)

Reference genome : assembly process (Genoscope)



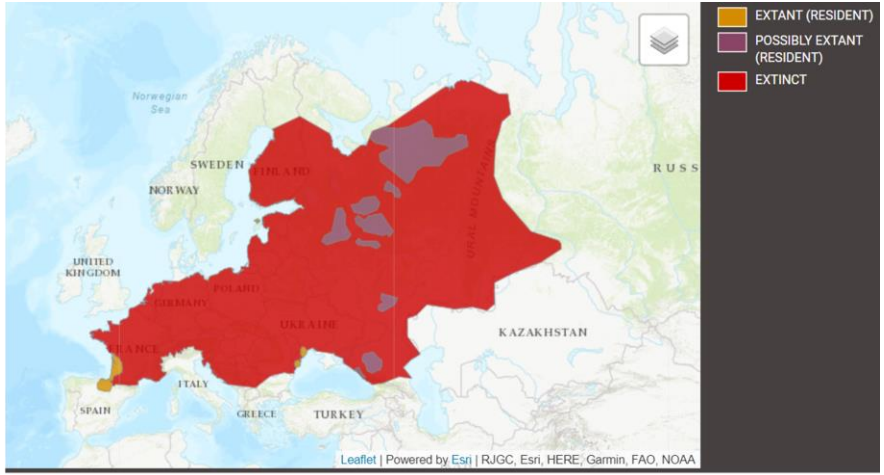


Trends in Ecology & Evolution

European mink (Mustela lutreola) is critically endangered (IUCN)

- Main threats: habitat loss and fragmentation, road collision, animal bites, confusion with american mink, rat poison.

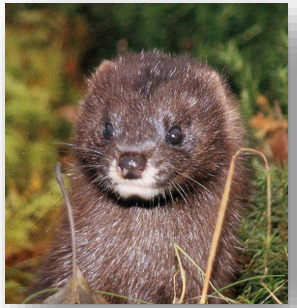
- Current distribution into three isolated areas
- High risk of inbreeding depression in western Europe (~ 500 individuals estimated)



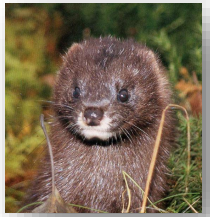
IUCN (International Union for Conservation of Nature) 2016. Mustela lutreola. The IUCN Red List of Threatened Species. Version 2019-3

=> Level of genomic diversity in Europe? Using 3RAD and Whole-genome resequencing

- large sets of markers that increase the ability to detect and quantify genetic variation in the population
- Identify chromosomal tracts of ancestry
- Demographic modeling
- Deleterious mutation



Whole Genome Resequencing



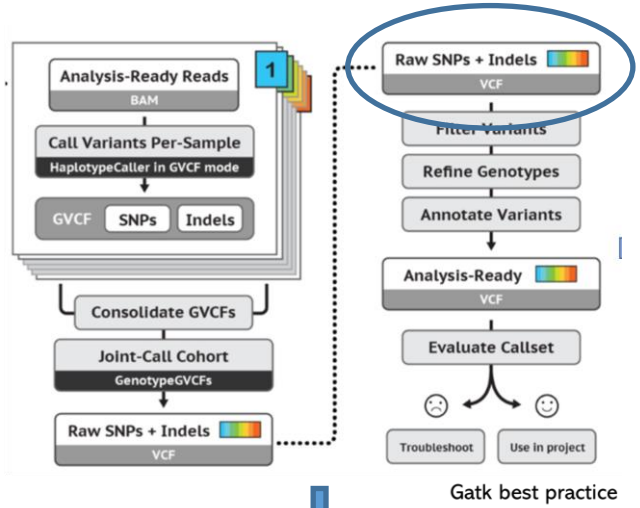
Two E.Minks from France (M,M)
Two E.Minks from Spain (M;F)
Two E.Minks from Romania (F,F)
Two E.Minks from Estonia

DNA sequencing
(Novogen, GIGA Uliege)
150 PE ~ 25X Coverage

→ Mapping Mustela V.2

ERGA
European Reference Genome Atlas

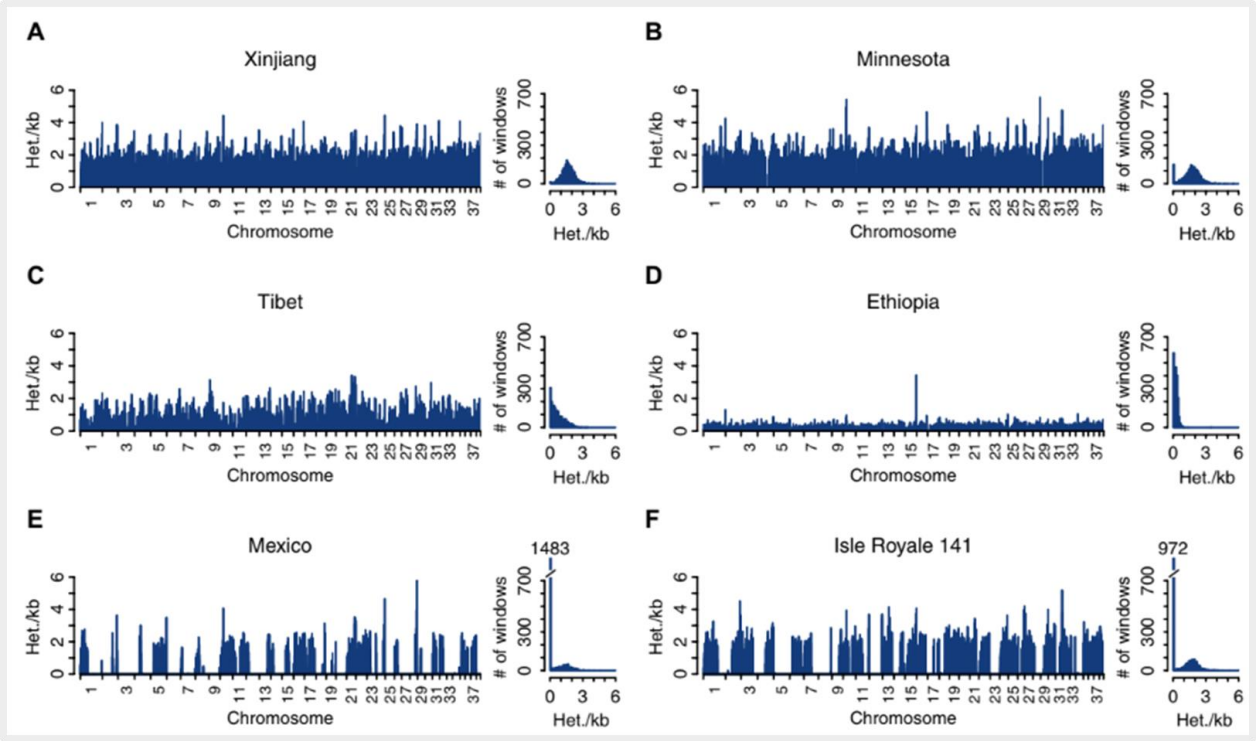
→ Mark Duplicate & Sort
(Picard)



Gatk best practice

↓

Downstreams analyses
(demography, admixture,
deleterious mutations,
ROH,…)



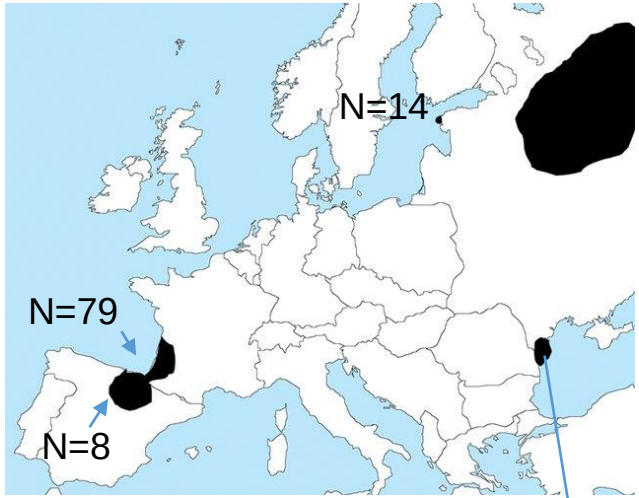
Distribution of GWH in wolves populations Robinson et al., Sci. Adv. 2019; 5 : eaau0757

How is the distributions of heterozygosity across the genome? Outbred (A,B), small isolated population (C,D) or inbred populations (E,F) ?

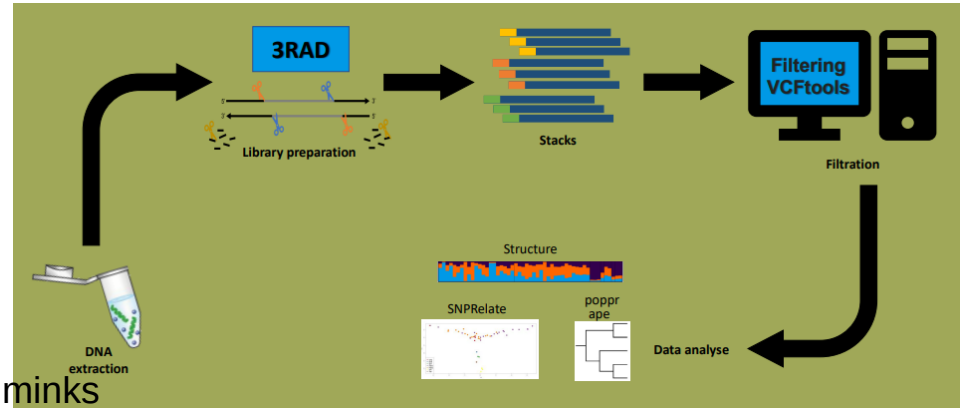
3RAD

Adapterama III: Quadruple-indexed, double/triple-enzyme RADseq libraries (2RAD/3RAD)

Natalia J. Bayona-Vásquez[1,2,3,*], Travis C. Glenn[1,3,4,5,*], Troy J. Kieran[1], Todd W. Pierson[1,8], Sandra L. Hoffberg[4,9], Peter A. Scott[6,10], Kerin E. Bentley[4,11], John W. Finger[1,5,12], Swarnali Louha[3], Nicholas Troendle[4,13], Pindaro Diaz-Jaimes[2], Rodney Mauricio[4] and Brant C. Faircloth[7]



Map from DOI: 10.13140/RG.2.1.4409.5528



134 minks

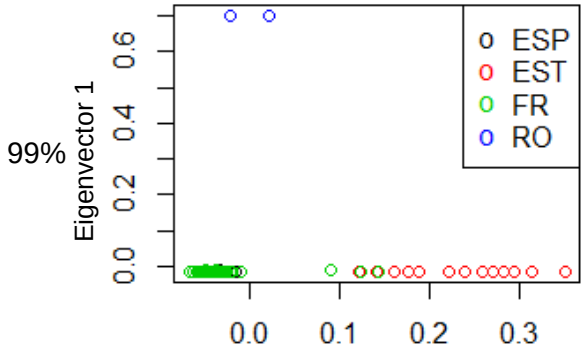
103 minks

3577 SNPs

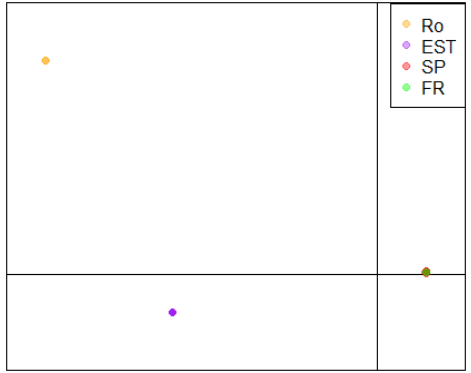
3RAD Preliminary results

3577 SNPs

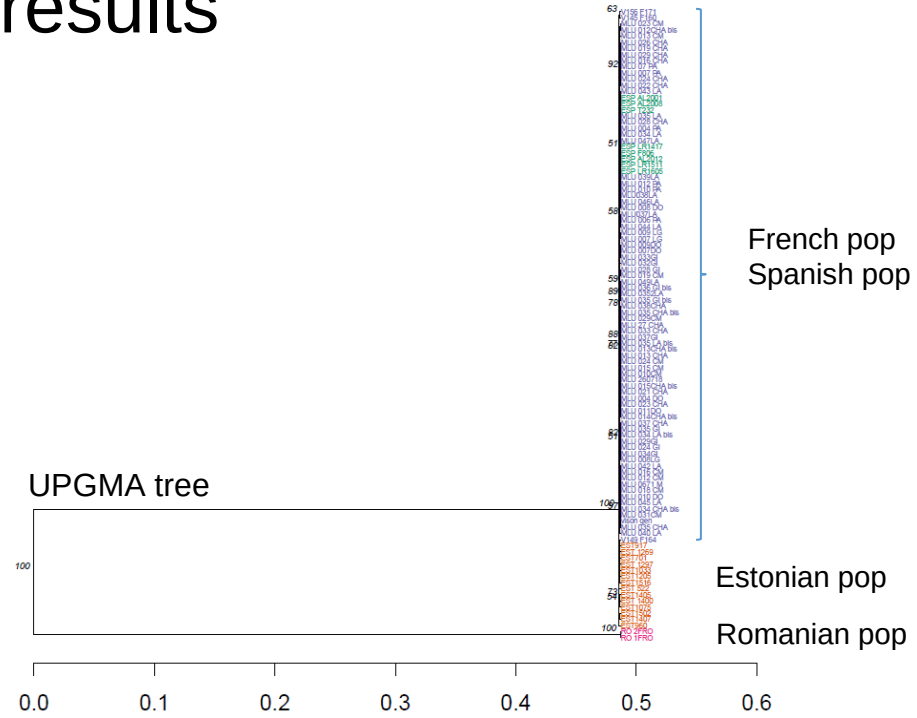
SNPrelate



DAPC



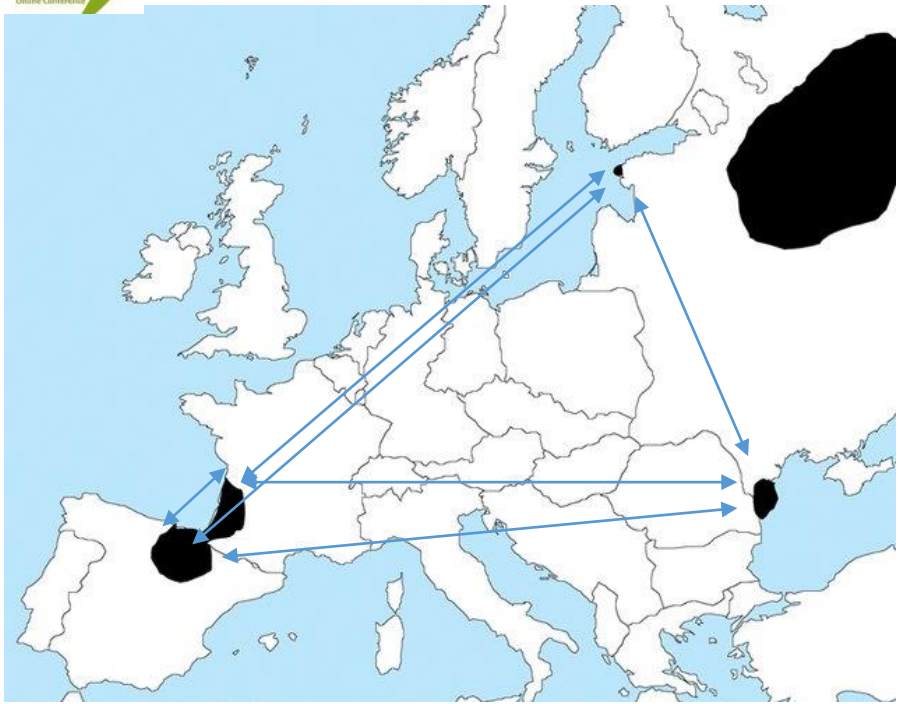
UPGMA tree



Genetic distance (proportion of loci that are different)

High genetic differentiation between the Romanian samples and the other populations

3RAD Preliminary results



Map geographic distribution of the european mink from DOI: 10.13140/RG.2.1.4409.5528

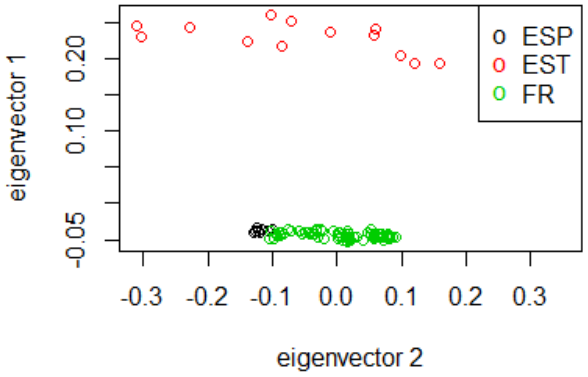
DiveRsity package

Fst \ Djost	Spain	Estonia	France	Romania
Spain		0	0	0.9824
Estonia	0.3242		0	0.9815
France	0.1459	0.4076		0.9825
Romania	0.9995	0.9977	0.9989	

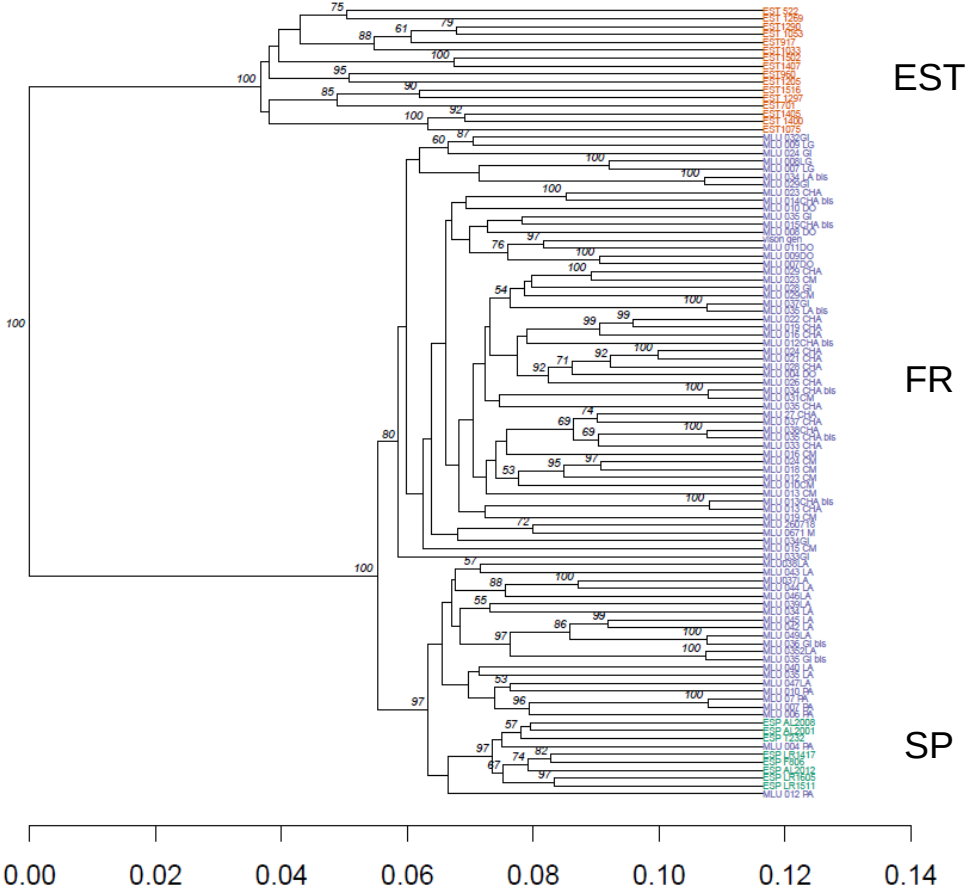
Djost index is very low except for the Romanian samples

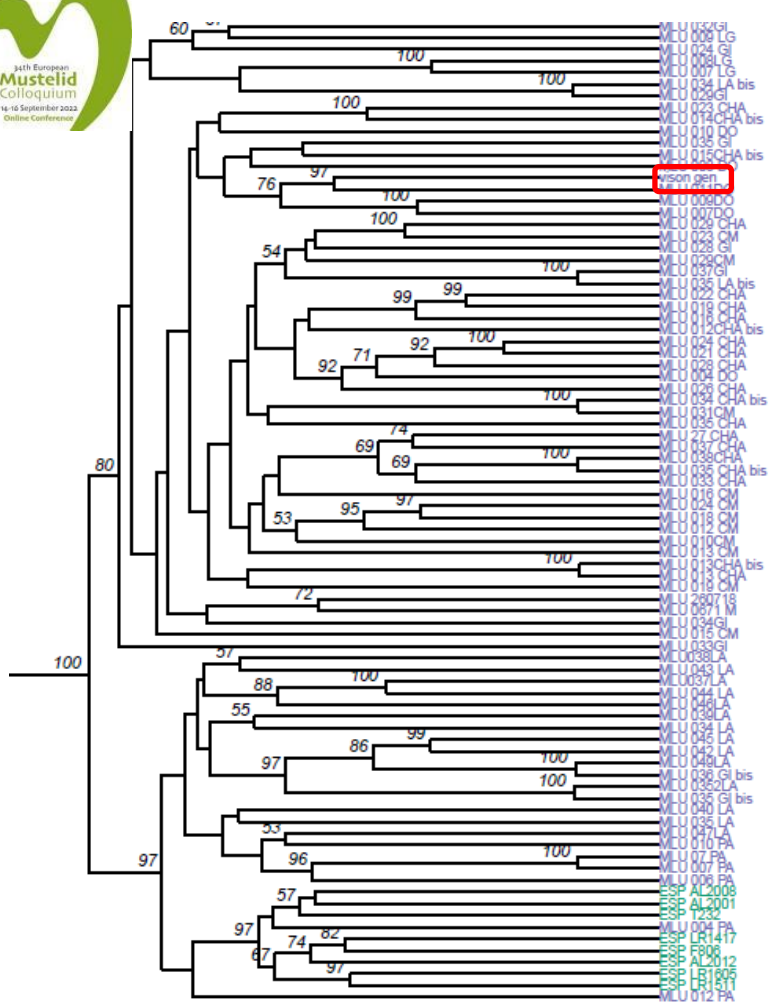
Fst indexes are moderate to really high between the populations

3RAD Preliminary results: What happen if we remove the romanian samples ?



1736 SNPs
N=100





=> Geographic structure of the French population

Perspectives

1. Reference Genome

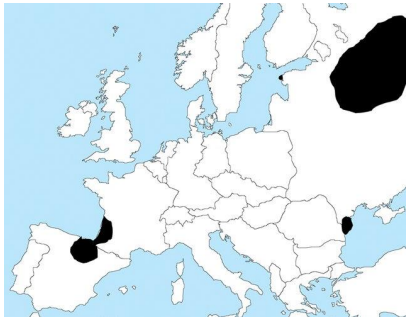
Finalize the reference genome assembly and start the annotation => *Mustela lutreola* v.3

2. Whole Genome resequencing

Eventually add two more individuals (EST) and continue the downstreams analyses with the final version + annotation

3. 3RAD analyses

Samples from Romania are highly divergent
French pop is more inbred than the Spanish and the Estonian pop
Pop gen on local populations



Map geographic distribution of the european mink from DOI: 10.13140/RG.2.1.4409.5528

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- Genoscope labs (Patrick Wincker)

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Elodie Brun, Odette Beluche, Sandrine Lebled, Claire Milani, Caroline Belser

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