the hapFLK method

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Outline

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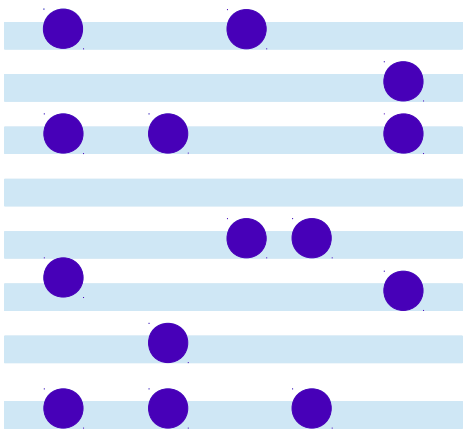
Genome scans for selection

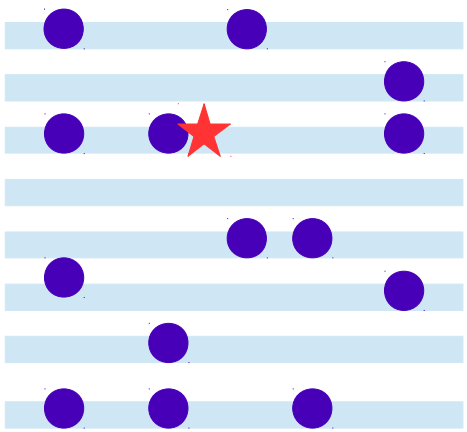
- Most genomic regions are neutral, but some of them are (or have been) under selection (natural or artificial).
- Detecting the regions under selection is important for theory (evolution) and applications (medicine, agronomy).
- Genome wide scans for selection now possible from dense genotyping (SNP chips) or sequencing (NGS) data.
- Focus on positive (adaptative) selection.

Genetic diversity around a positively selected mutation

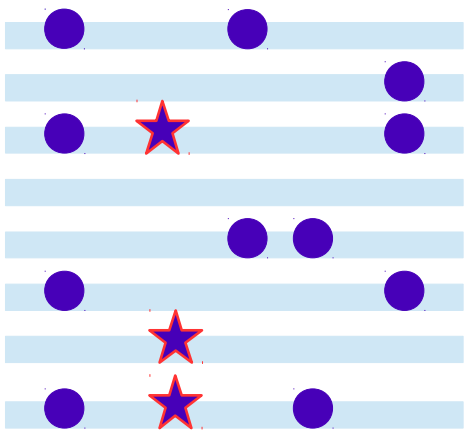
No selection



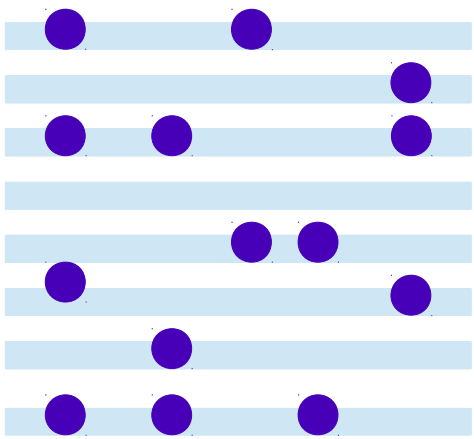
Selection on
a new variant



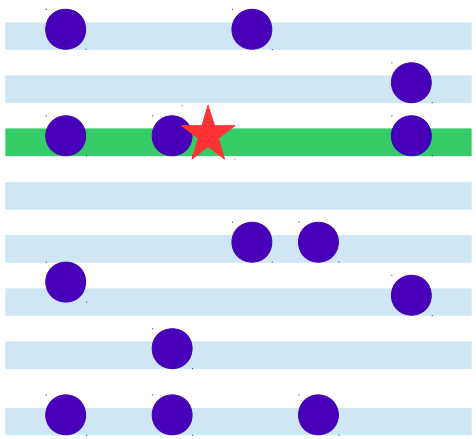
Selection on
standing variation



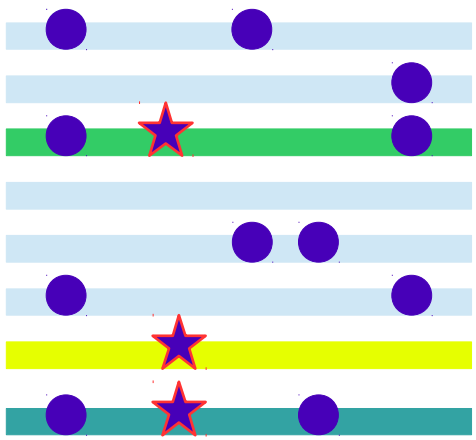
Genetic diversity around a positively selected mutation



One single haplotype



Several haplotypes

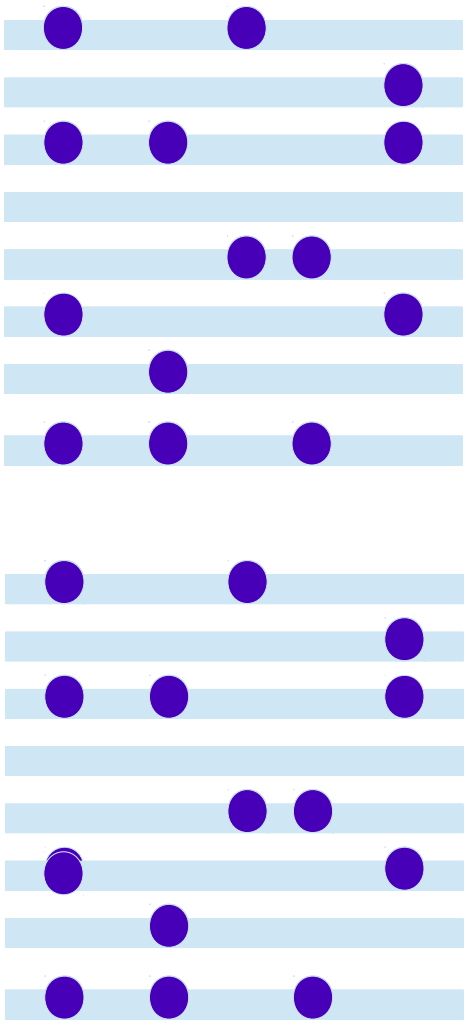


Genetic diversity around a positively selected mutation

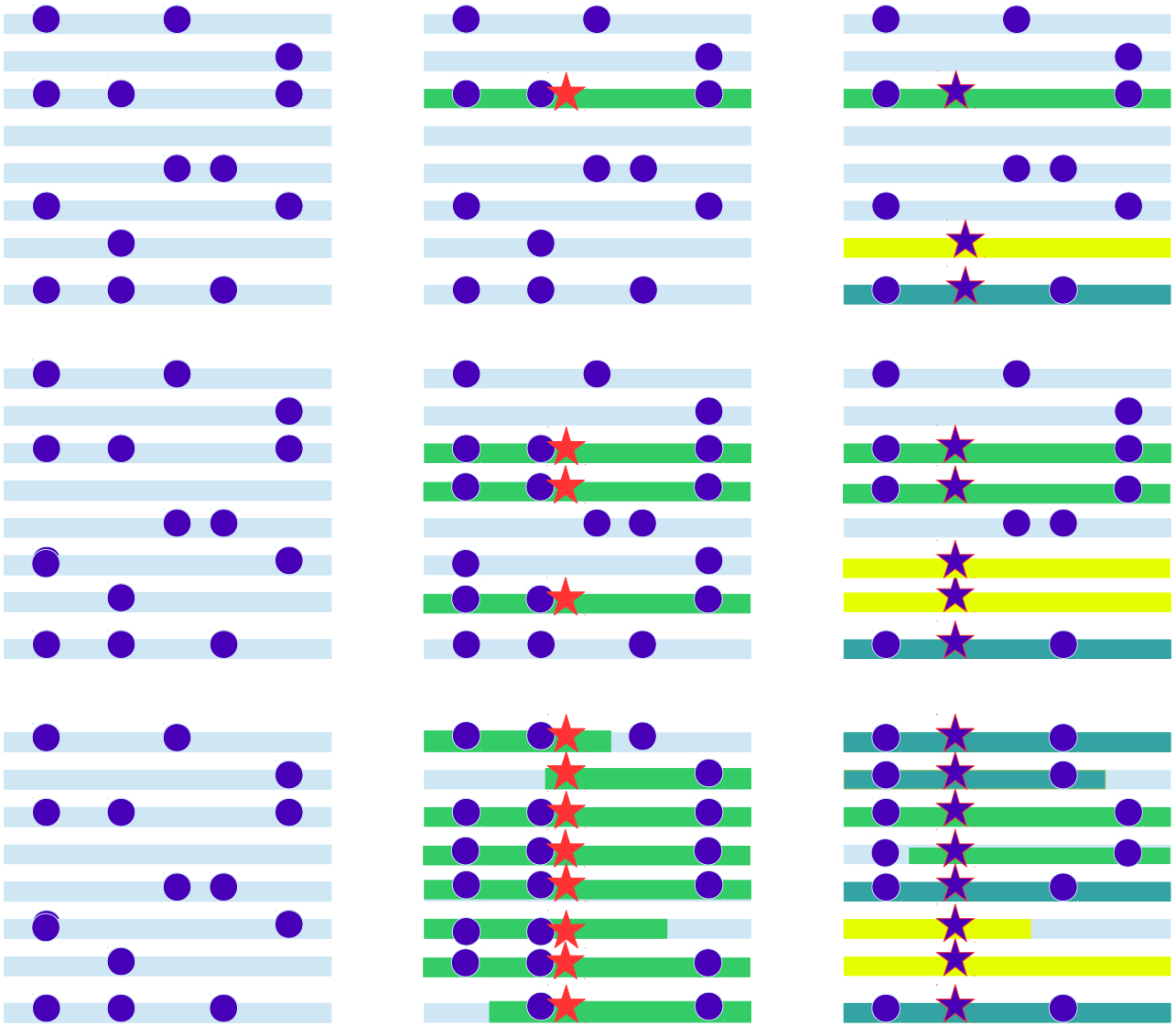
Random drift evolution

One haplotype increases in frequency

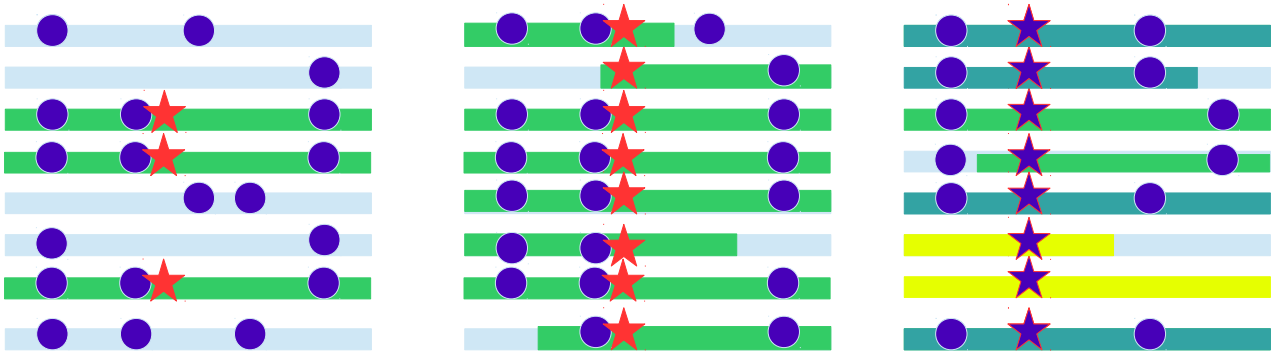
Several haplotypes increase in frequency



Genetic diversity around a positively selected mutation



Different sweep signatures



Sweep scenario	partial	hard	soft
Allele frequencies	elevated	extreme	intermediate / extreme
Haplotype frequencies	elevated for one haplotype	one fixed haplotype	elevated for several haplotypes

Detection of selective sweeps from genomic data

Detection power may be increased by:

- Comparing neutral vs selected populations.
- Account for population history, in particular their hierarchical structure (FLK).
- Using haplotype information (hapFLK).

Outline

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F_{ST} based tests

$p = (p_1, \ldots, p_i, \ldots, p_n)$: allele frequencies at one SNP in several populations.

$\overline{p}$ and s_p^2: observed mean and variance of p.

$$F_{ST} = \frac{s_p^2}{\bar{p}(1-\bar{p})}$$

- H_0 : "neutral evolution" (genetic drift)
  vs H_1 : "positive selection in one (or more) population ".
- H_0 rejected if F_{ST} too large.

Lewontin et Krakauer (LK) test (1973)

$$T_{LK}^{\ell} = \frac{n-1}{\bar{F}_{ST}} F_{ST}^{\ell}$$

- T_{LK} distribution under H_0 is χ^2 if :

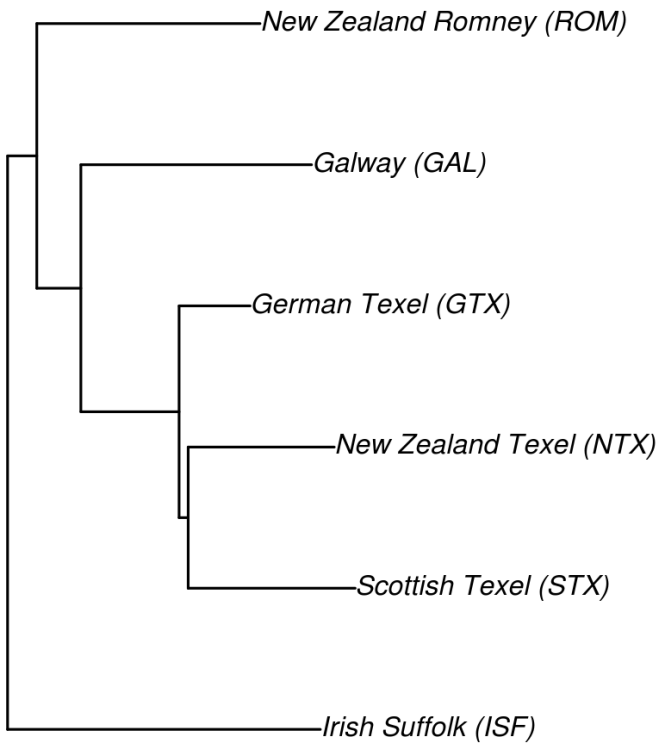
$$Var(p_i) = F_{ST} p_0 (1 - p_0), \quad Cov(p_i, p_j) = 0$$

- Only true if populations have a star like phylogeny with equal population sizes.

FLK test (Bonhomme *et al*, 2010)

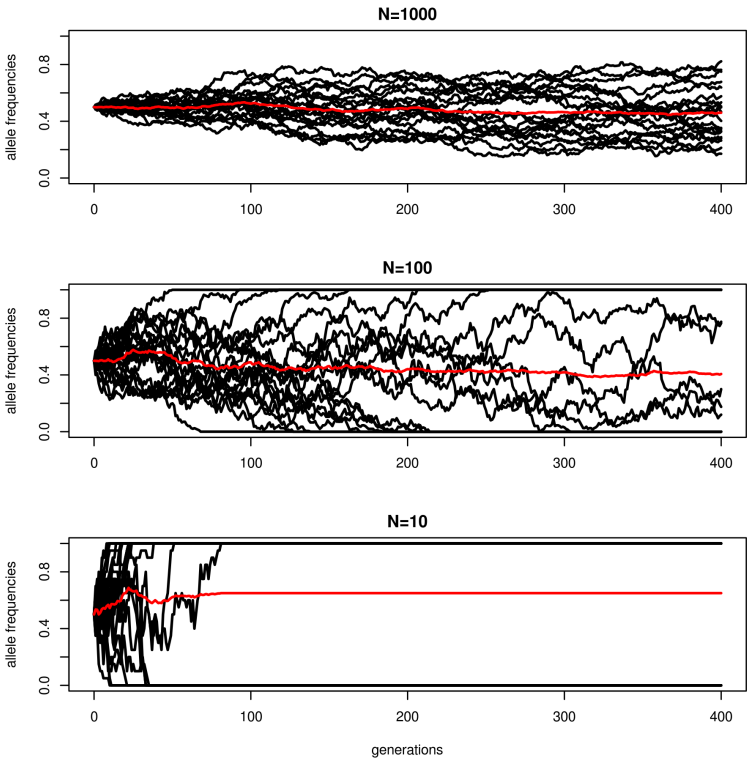
Extension of F_{ST} accounting for

- differences in effective size between populations.
- differences in correlations between population pairs.



(first estimated from genome wide data)

Genetic drift in one population



$$\mathbb{E}(p(t)) = p_0 \quad (1)$$

$$Var(p(t)) = F_t p_0(1-p_0) \quad (2)$$

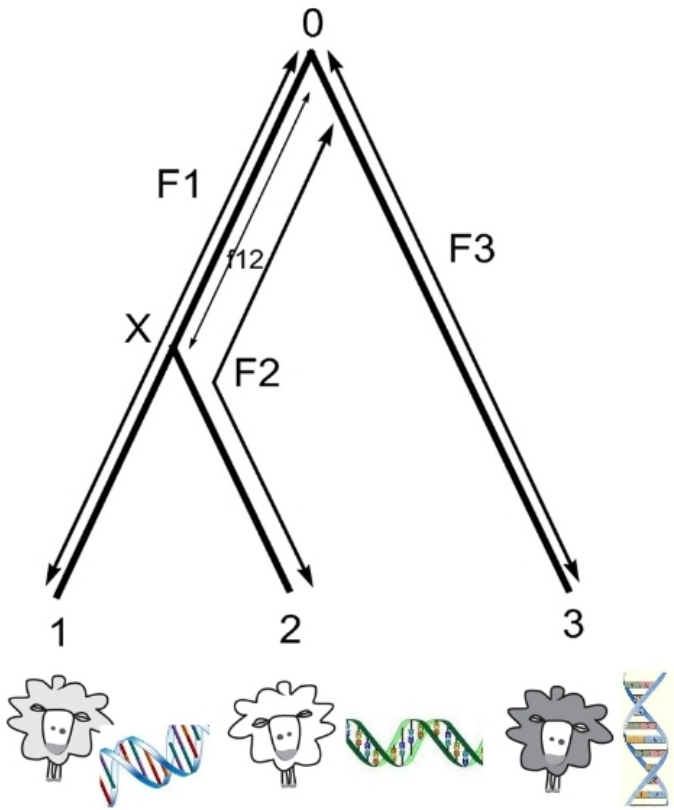
Wright-Fisher fixation index

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t \approx \frac{t}{2N}$$

N: effective population size

Extension to several populations with arbitrary phylogeny

The distribution of p under H_0 can be modelled using the kinship matrix F.



$$Var(p_i) = F_i p_0(1-p_0)$$
$$Cov(p_i, p_j) = f_{ij} p_0(1-p_0)$$

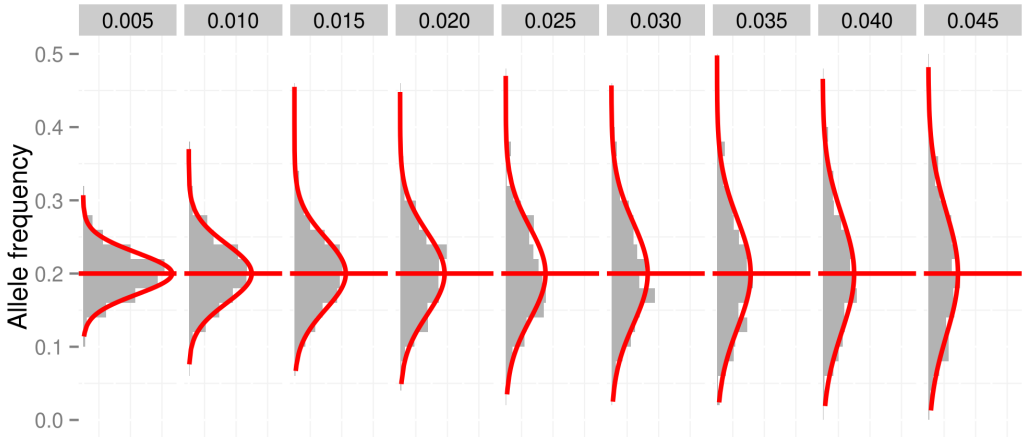
$$F_3 = 1 - \left(1 - \frac{1}{2N_3}\right)^t \approx \frac{t}{2N_3}$$
$$f_{12} = 1 - \left(1 - \frac{1}{2N_{12}}\right)^{t_{12}} \approx \frac{t_{12}}{2N_{12}}$$

Kinship matrix

$$F = \begin{pmatrix} F_1 & f_{12} & 0 \\ f_{12} & F_2 & 0 \\ 0 & 0 & F_3 \end{pmatrix}$$

$$\rightarrow Var(p) = F p_0(1-p_0)$$

Normal approximation



- Provided F_t is small, we can model:

$$p(t) \sim \mathcal{N}(p0, F_t p0(1 - p0))$$

See Nicholson *et al.* (2002).

FLK test (Bonhomme *et al.* 2010)

bi-allelic markers (SNP)

$$T_{F-LK} = (\mathbf{p} - \hat{p}_0 \mathbf{1}_n)' \widehat{Var(\mathbf{p})}^{-1} (\mathbf{p} - \hat{p}_0 \mathbf{1}_n)$$

$$\hat{p}_0 = \frac{\mathbf{1}_n' F^{-1} \mathbf{p}}{\mathbf{1}_n' F^{-1} \mathbf{1}_n}, \quad \widehat{Var(\mathbf{p})} = F \hat{p}_0 (1 - \hat{p_0})$$

multi-allelic markers

$A > 2$ allèles

- $\mathbf{p}_0$ vector of size A.
- $\mathbf{p}$ vector of size $n \times A$.
- $Var(\mathbf{p})$ can be written as a function of F and $\mathbf{p} - \mathbf{p}_0$

Distribution under H_0 is $\chi^2((A-1)(n-1))$

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Estimation of the population kinship matrix

- The **Reynolds genetic distance** $\mathcal{D}$ (Reynolds, Weir and Cockerham, 1983) between two populations i and j has expectation:

$$E(\mathcal{D}_{ij}) = \frac{F_i + F_j}{2}$$

  see Laval *et al.* (2002).
- The matrix of Reynolds distances is computed over many ($\sim 10^4$) SNPs. Assumes majority of them are neutral.
- The population tree is built using the neighbour joining algorithm on this matrix.

Outline

Accounting for correlation between SNPs

Cumulating single SNP tests

Windowing approach average / max of SNP statistics over genome windows (*Weir et al. 2005*)

Composite likelihood Product of SNP likelihoods within genome windows (XP-CLR : *Chen et al. 2010*)

Bayesian hierarchical models autoregressive component in the model (*Guo et al. 2009*)

Choice of window size? fixed?

Bayesian methods computer intensive (MCMC).

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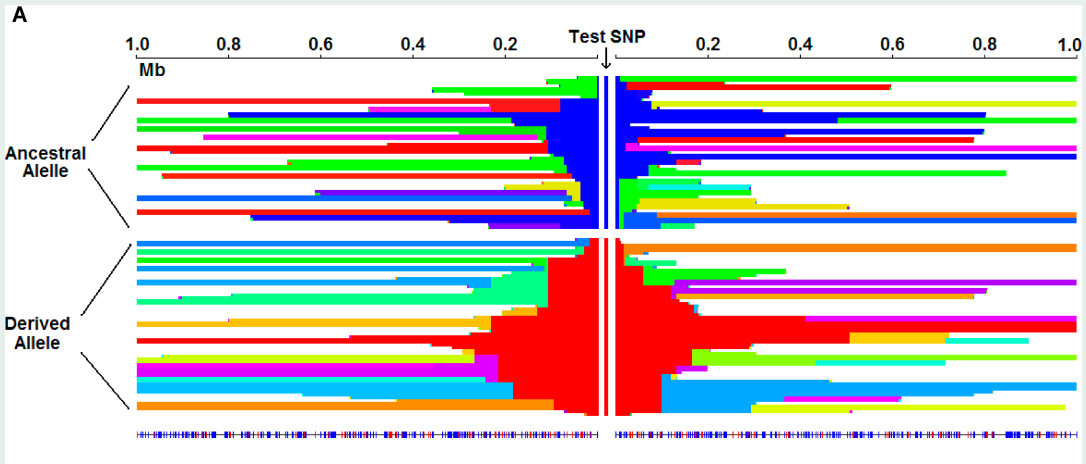
Accounting for correlation between SNPs

Using haplotype length

Within population EHH (*Sabeti et al. 2002*), iHS (*Voight et al. 2006*)



Between populations XP-EHH *(Sabeti et al. 2007)*

Limited to two populations.

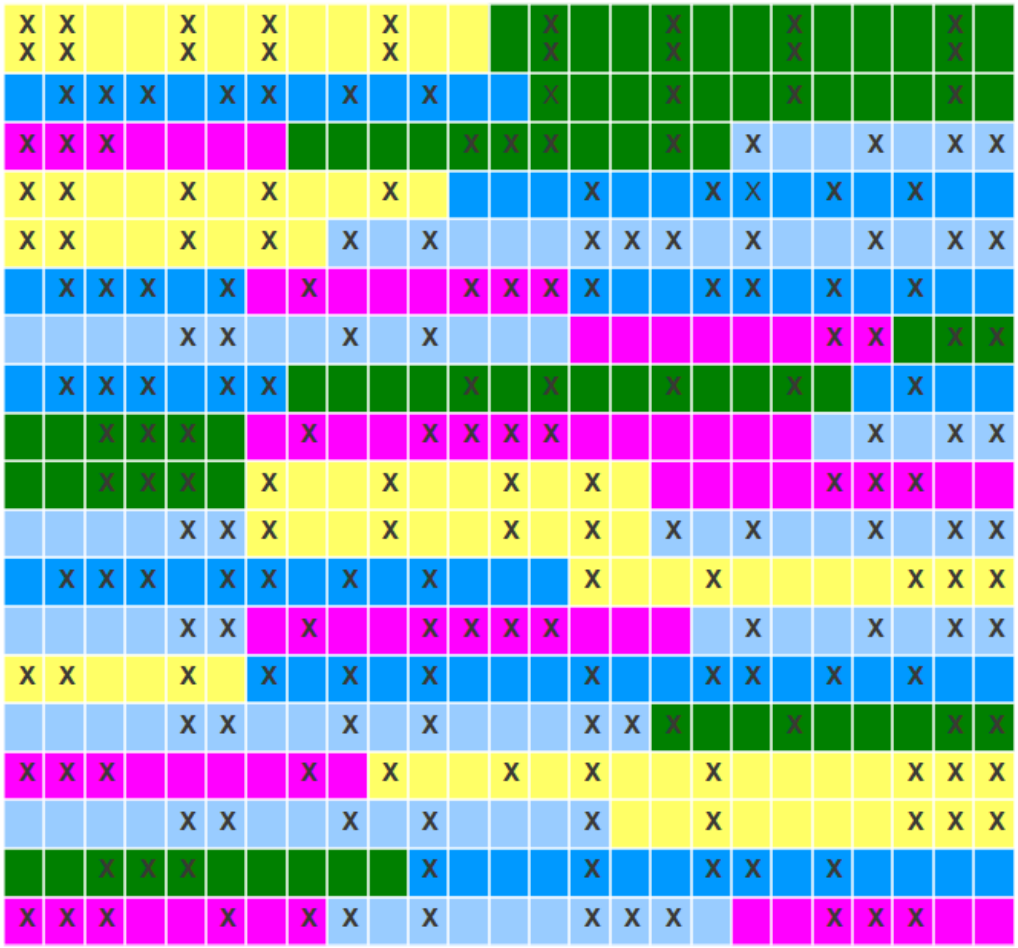
Our approach

FLK test at the haplotype level

1. Define haplotypes using a continuous model over the genome (no fixed window)
2. FLK test from these haplotypes (multi-allelic version)

Local haplotype clustering (*Scheet and Stephens* 2006)

Genetic similarity between individuals evolves continously over the genome due to ancestral recombinations.

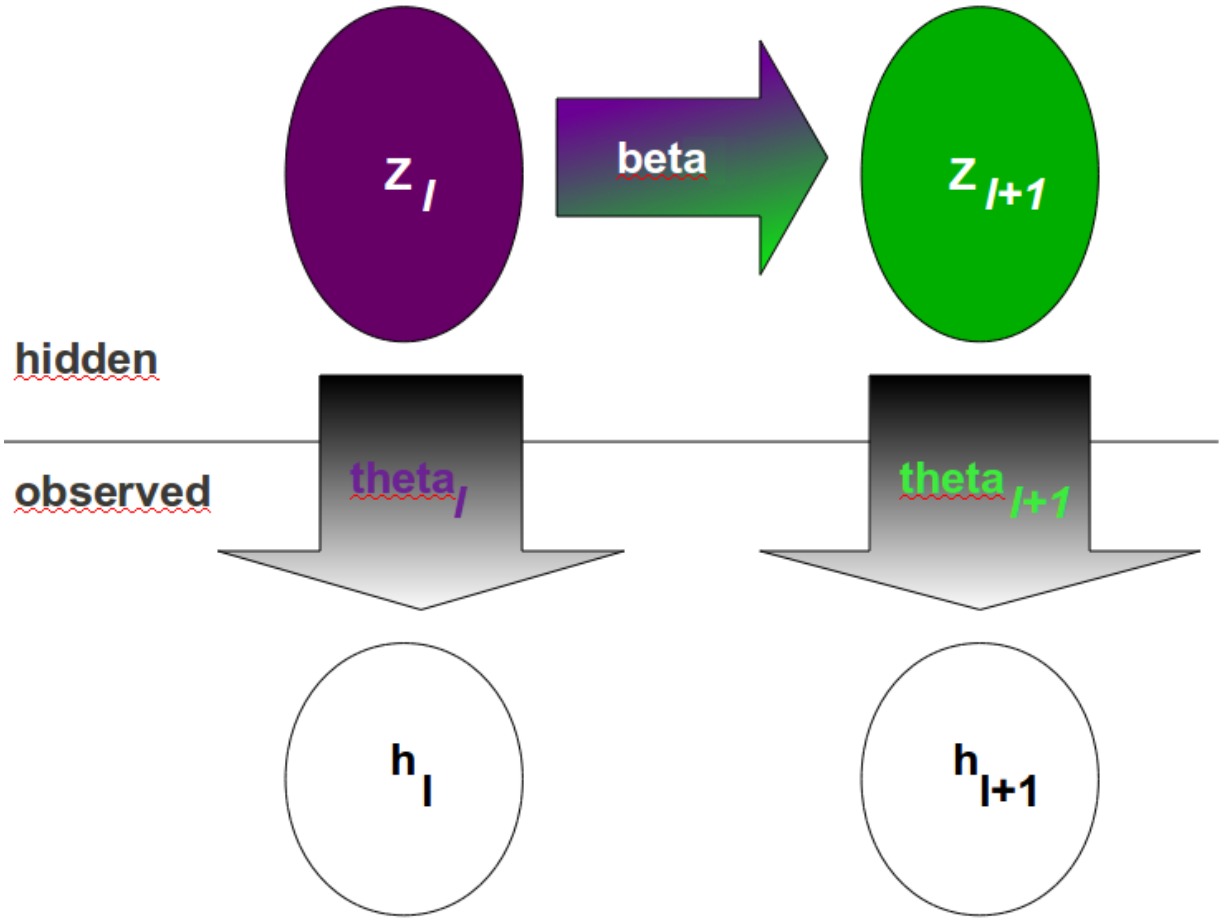


Example: 10 individuals

lines: haplotypes

columns: SNPs

Hidden Markov Model

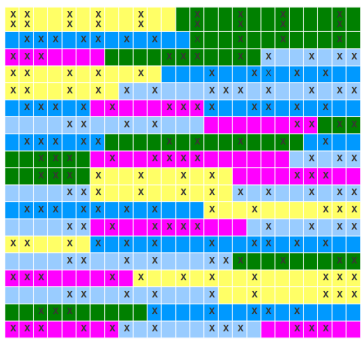


$z_{i\ell}$ cluster of haplotype i at SNP ℓ (hidden state)

$h_{i\ell}$ allele of haplotype i at SNP ℓ (observed state)

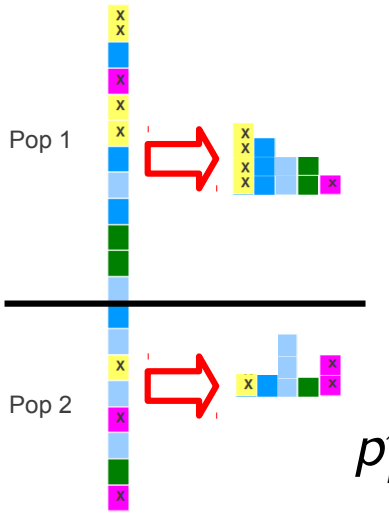
hapFLK test (Fariello *et al*, 2013)

Cluster estimation



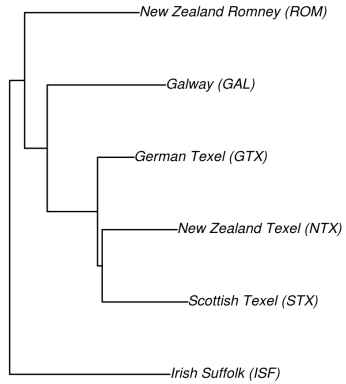
EM algorithm

Estimation of cluster frequencies for each SNP ℓ and population j:



$$p_{kj}^{\ell} = \frac{1}{N_j} \sum_{i=1}^{N_j} \mathbb{P}(z_{ik}^{\ell} | \Theta)$$

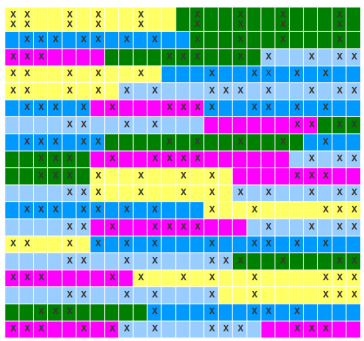
Computation of T_{F-LK}, using clusters as alleles.



Average of T_{F-LK} over EM iterations provides hapFLK

hapFLK test (Fariello *et al*, 2013)

Cluster estimation



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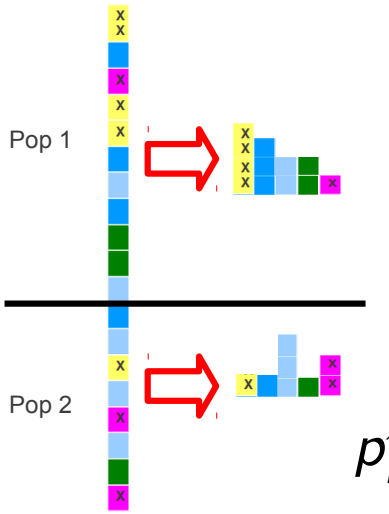

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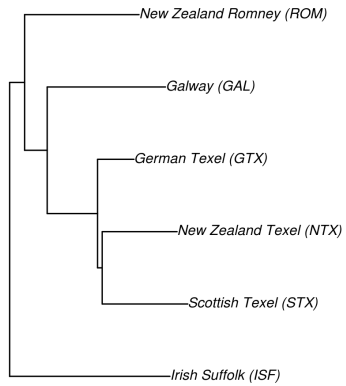

Average of T_{F-LK} over EM iterations provides hapFLK

Our approach

FLK test at the haplotype level

1. Define haplotypes using a continuous model over the genome (no fixed window)
2. FLK test from these haplotypes (multi-allelic version)

- Any number of populations.
- Accounts for populations hierarchical structure / unequal population sizes.
- Genotype data allowed.
- Missing data allowed.

Outline

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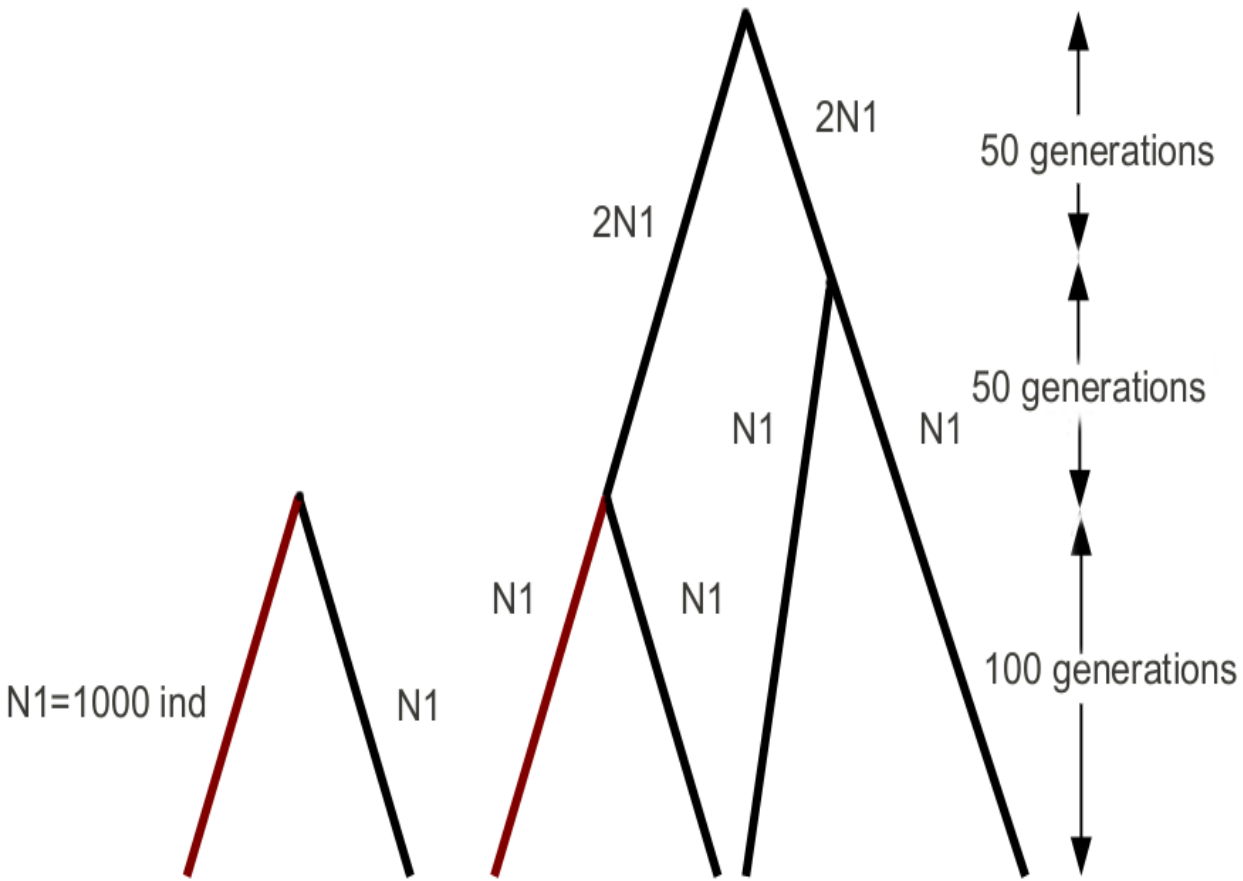
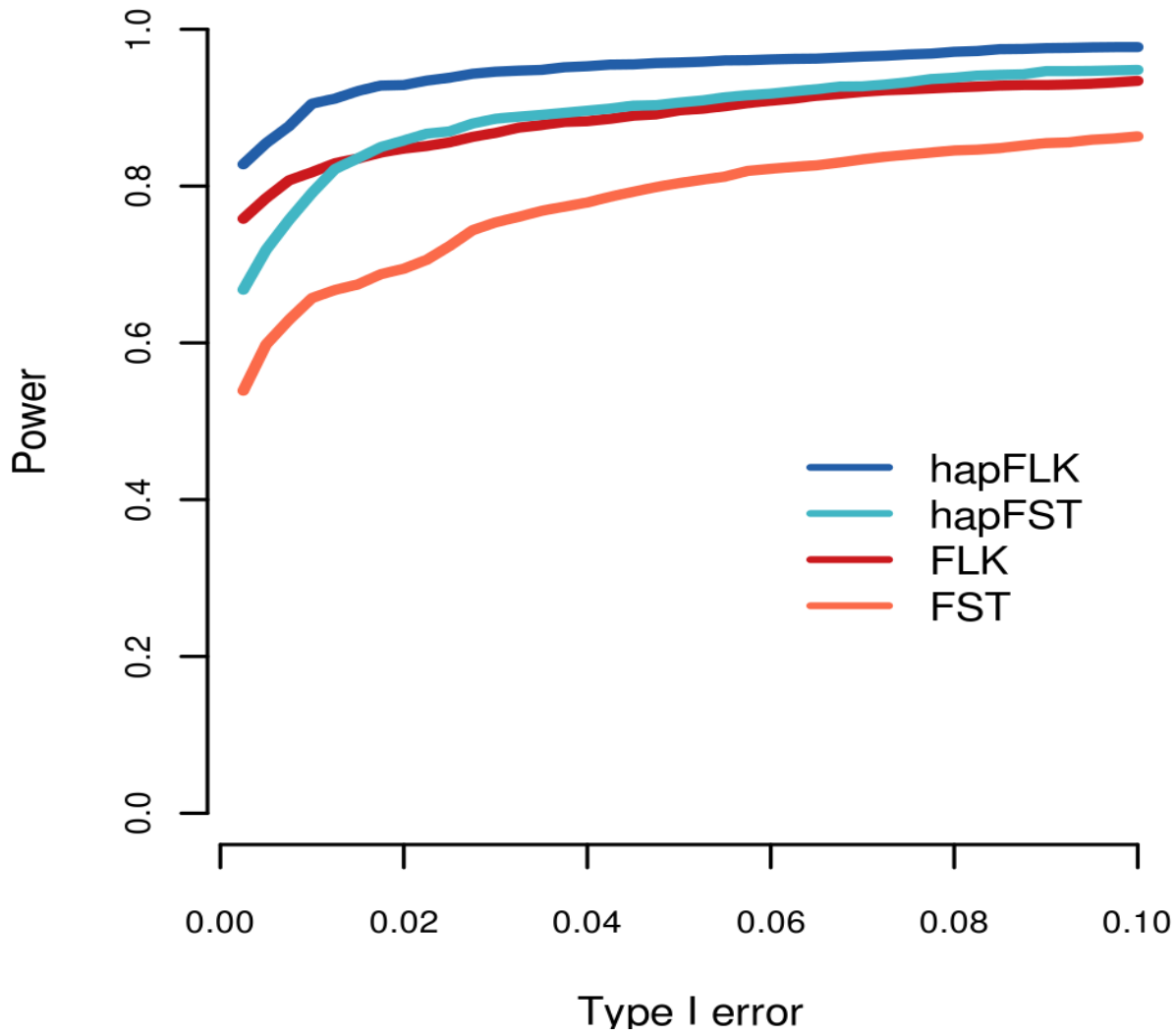
Simulation results

Simulation of 5Mb segments with 100 SNPs (dense genotyping) or 300 SNPs (full sequencing) with MAF $> 5\%$.

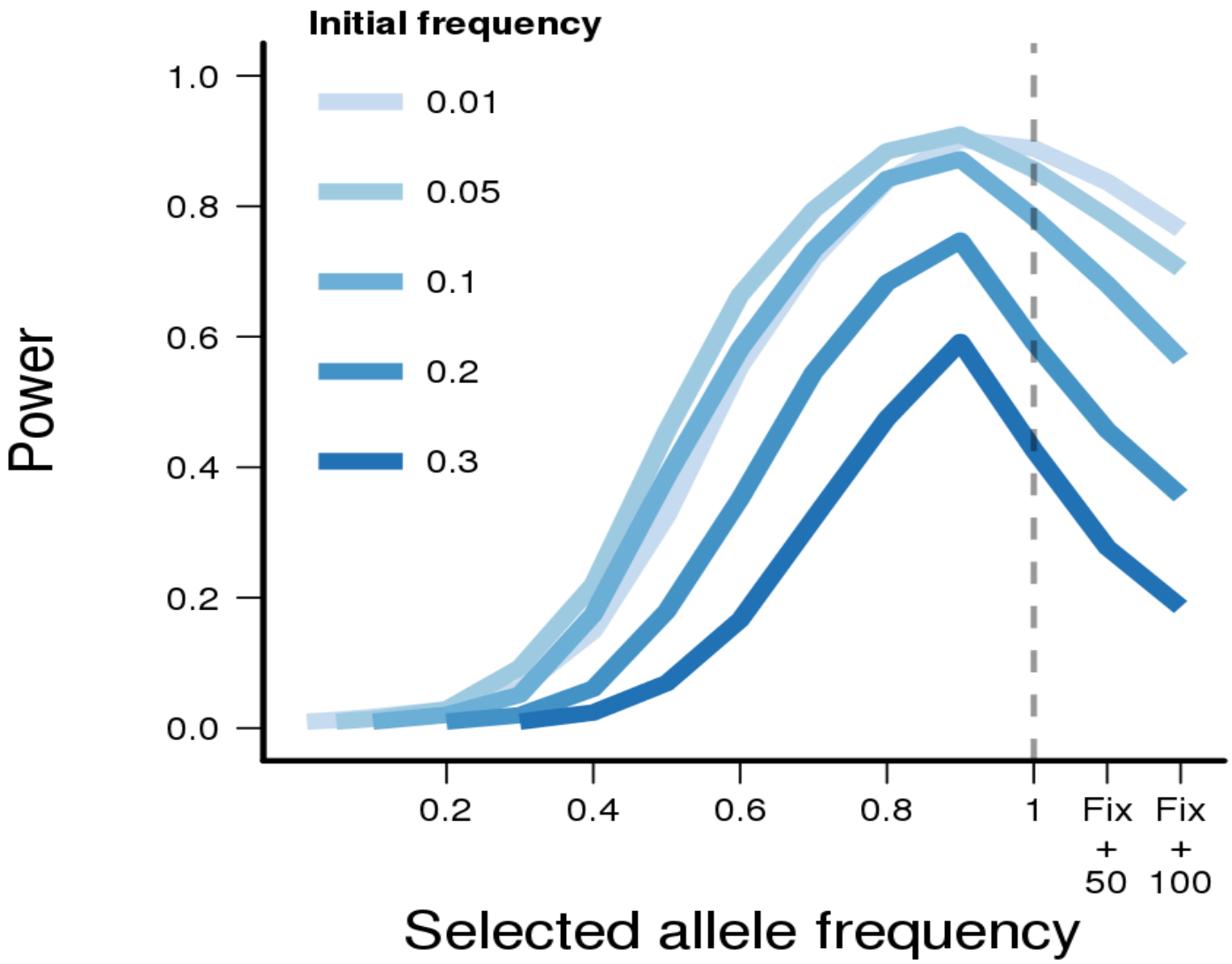


Increased power for haplotype based tests



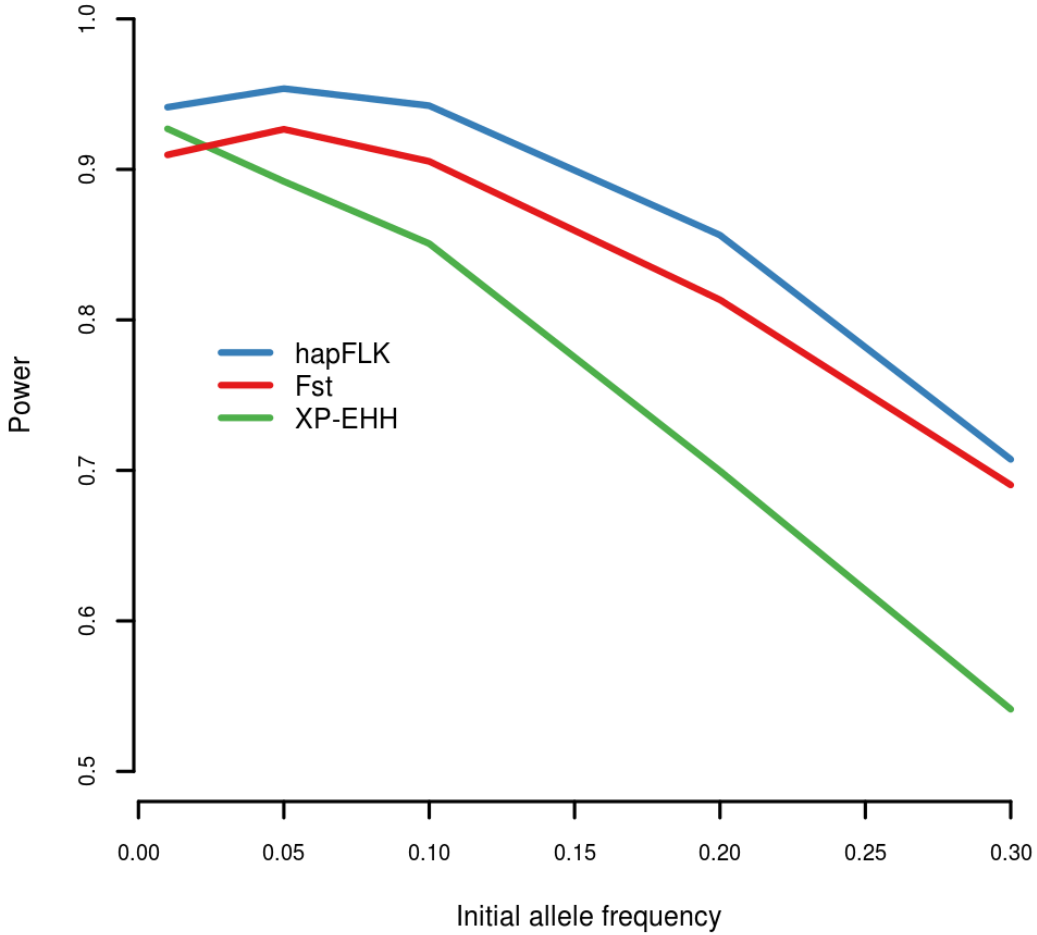
Hard sweep scenario, 4 populations, genotyping data.

Soft and incomplete sweeps can be detected



2 populations, genotyping data.

Increased power for soft sweeps compared to XP-EHH

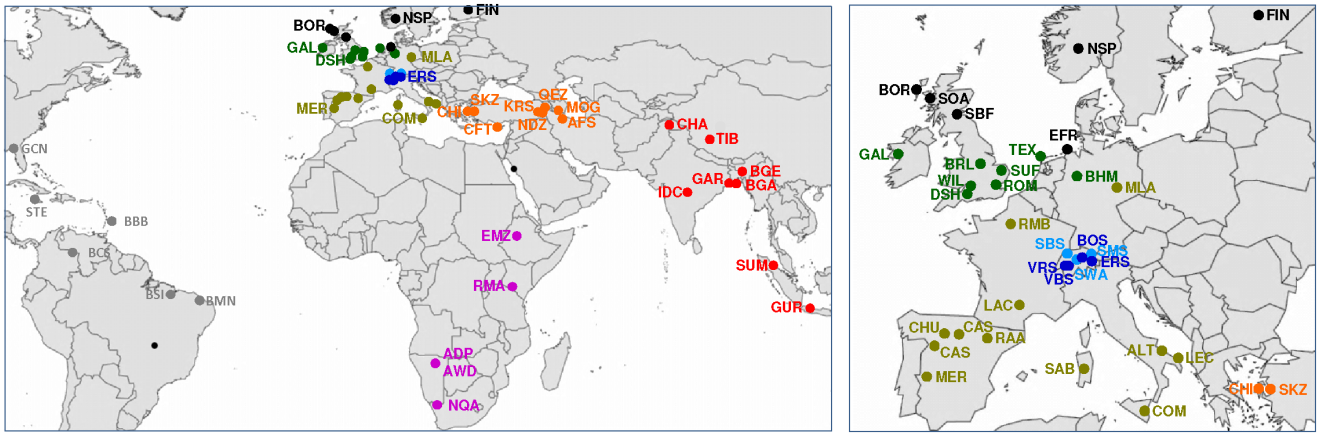


2 populations, genotyping data, type I error $= 5\%$.

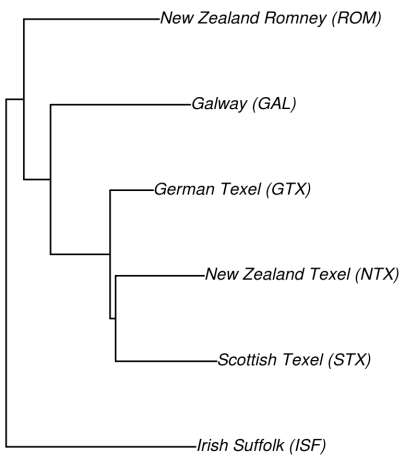
Outline

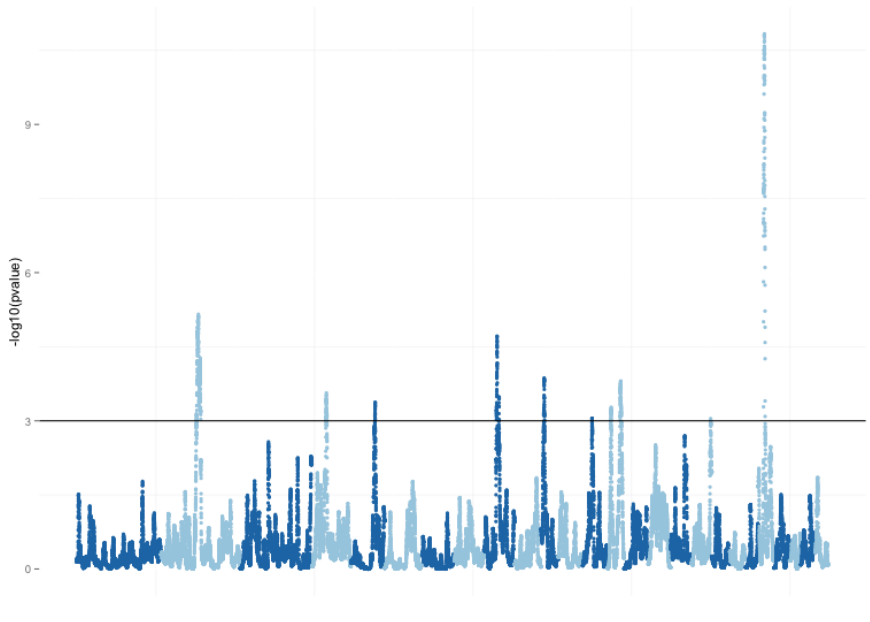
The Sheep HapMap data

74 populations, 50K SNPs (Kijas *et al*, 2012)

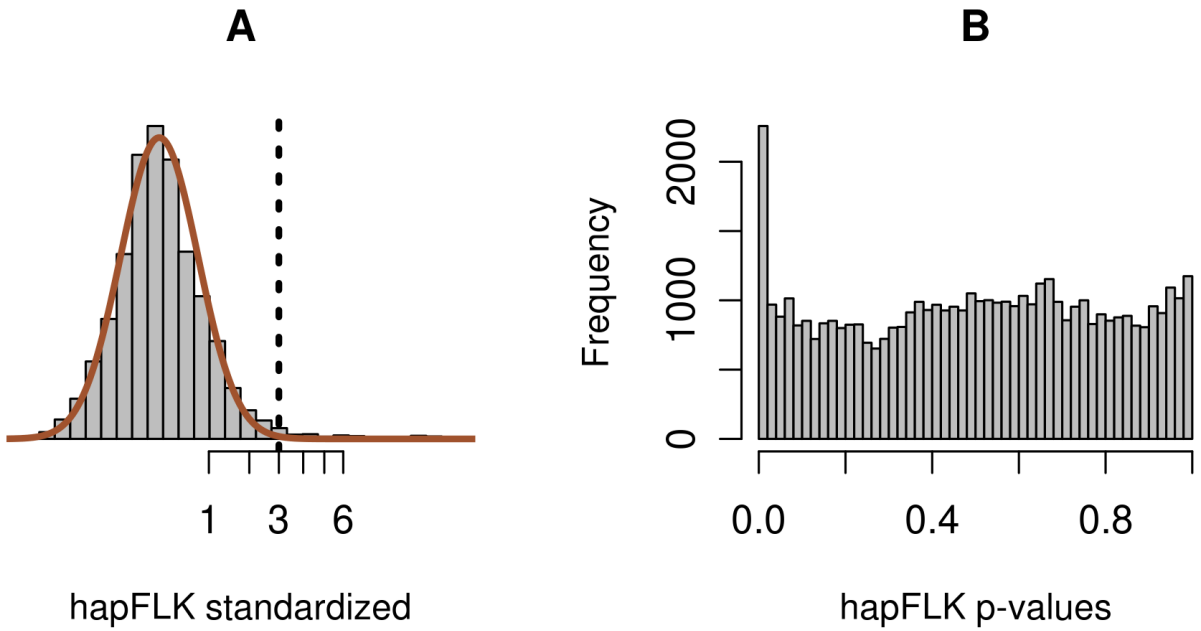


Focus on northern Europe





Computation of p-values

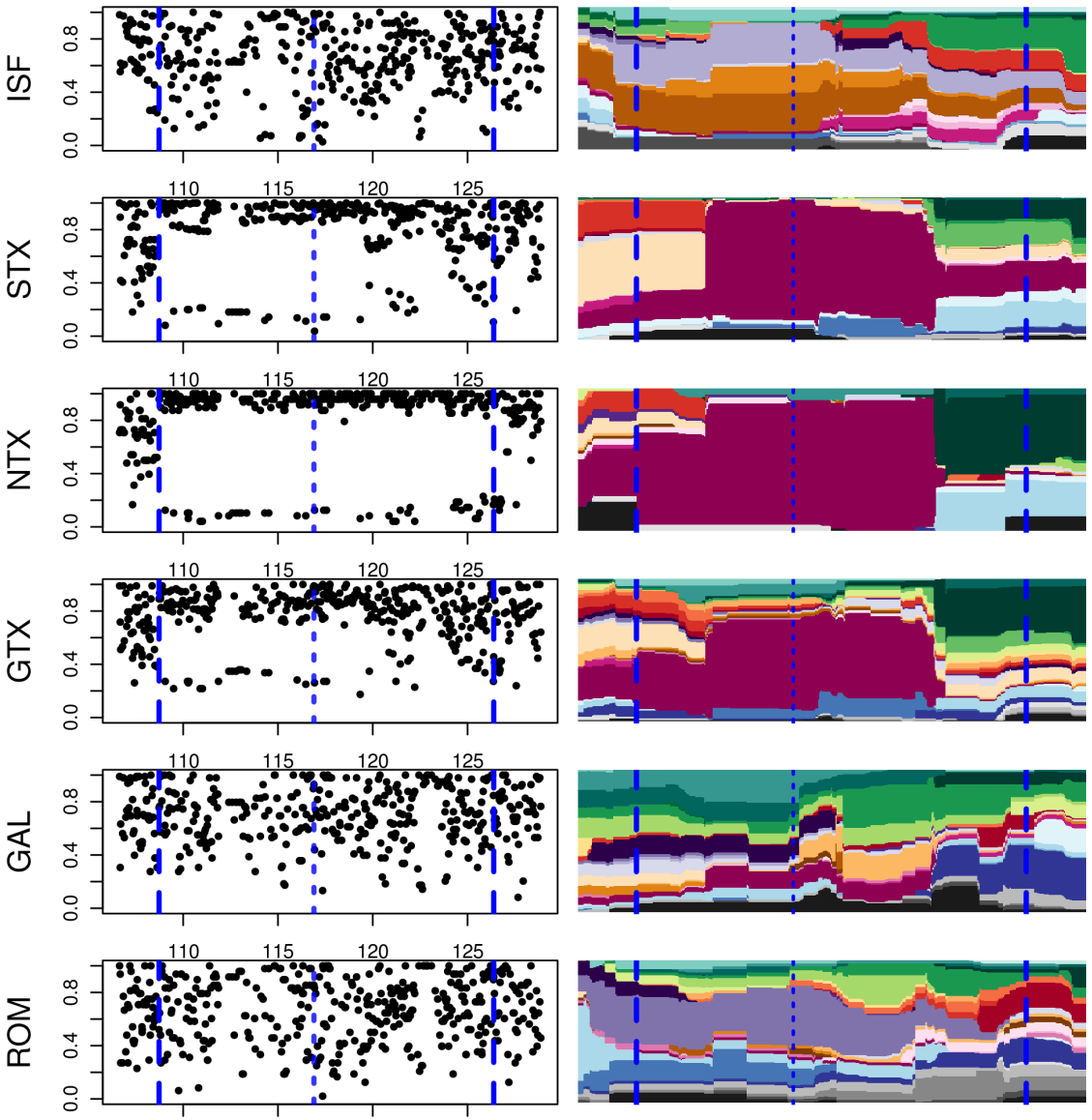


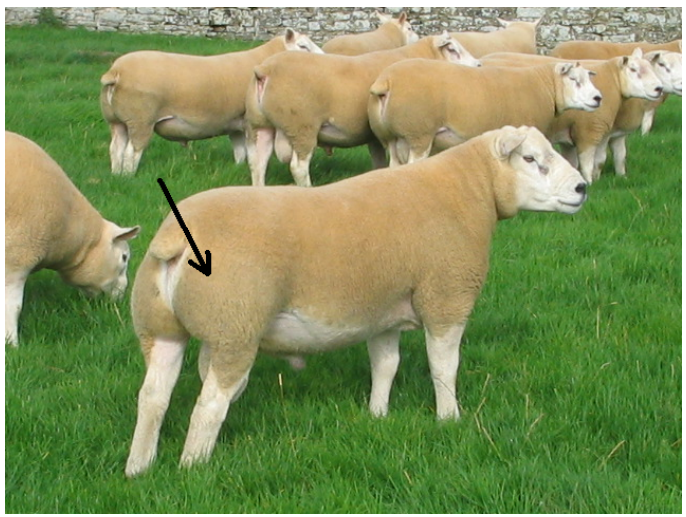
- *hapFLK* approximately gaussian, with some outliers.

$\Rightarrow$ Normalization using robust mean and variance estimates (function *rlm* in R)

Hard sweep signal in Texel Sheep

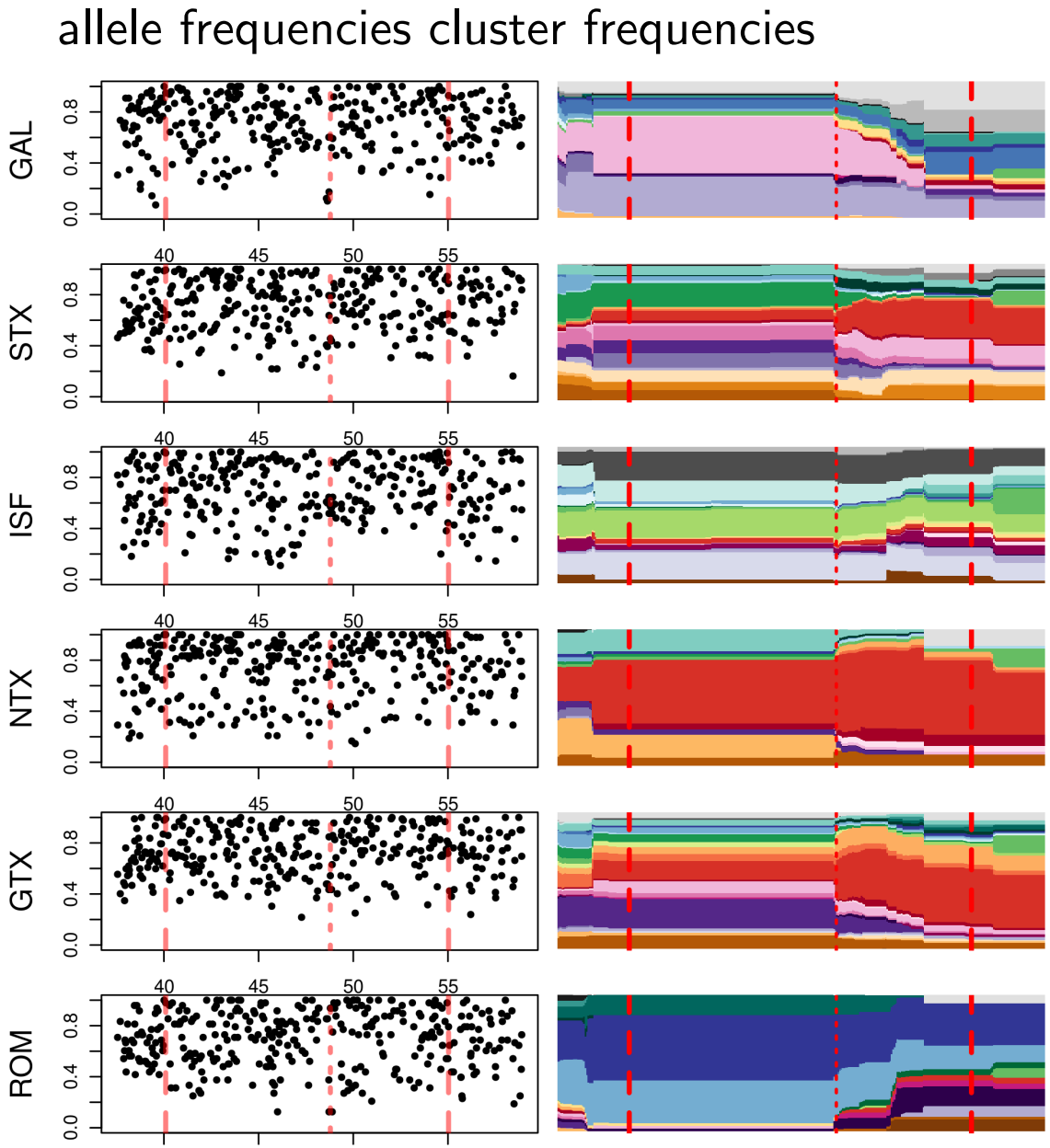
allele frequencies cluster frequencies





Candidate mutation in MSTN

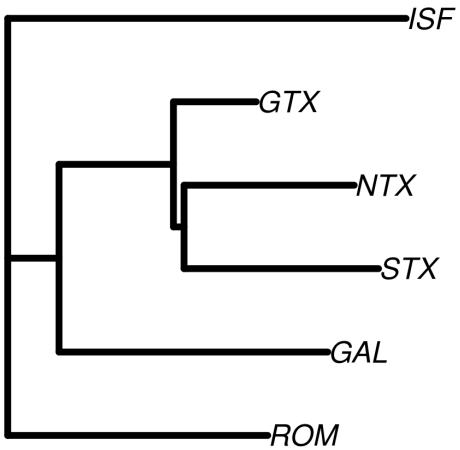
Soft / Incomplete sweep signal in New Zealand Sheep



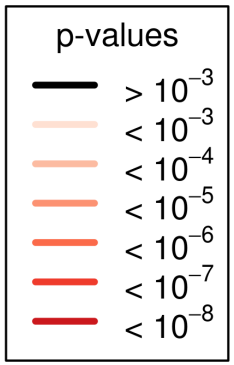
- One cluster at relatively high frequency in New Zealand Texel (NTX)
- Two clusters at fixation in New Zealand Rommey (ROM).

New Zealand breeds are the ones under selection

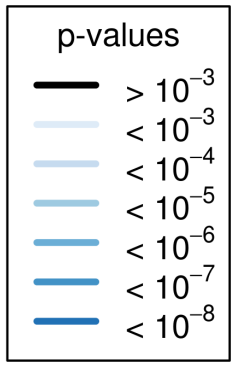
Whole genome tree



SNP trees



Haplotype trees



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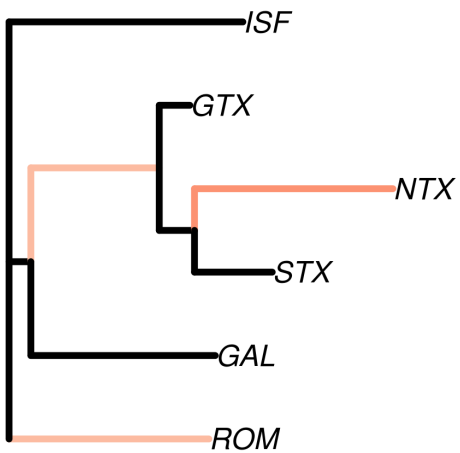
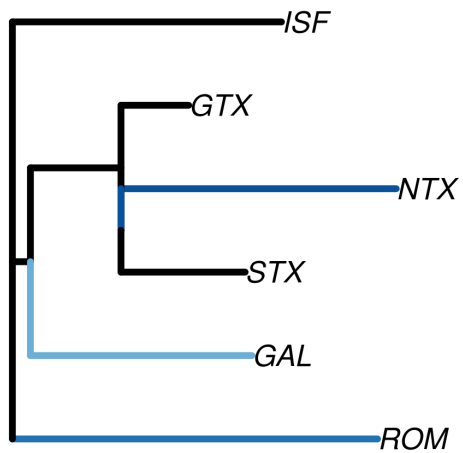
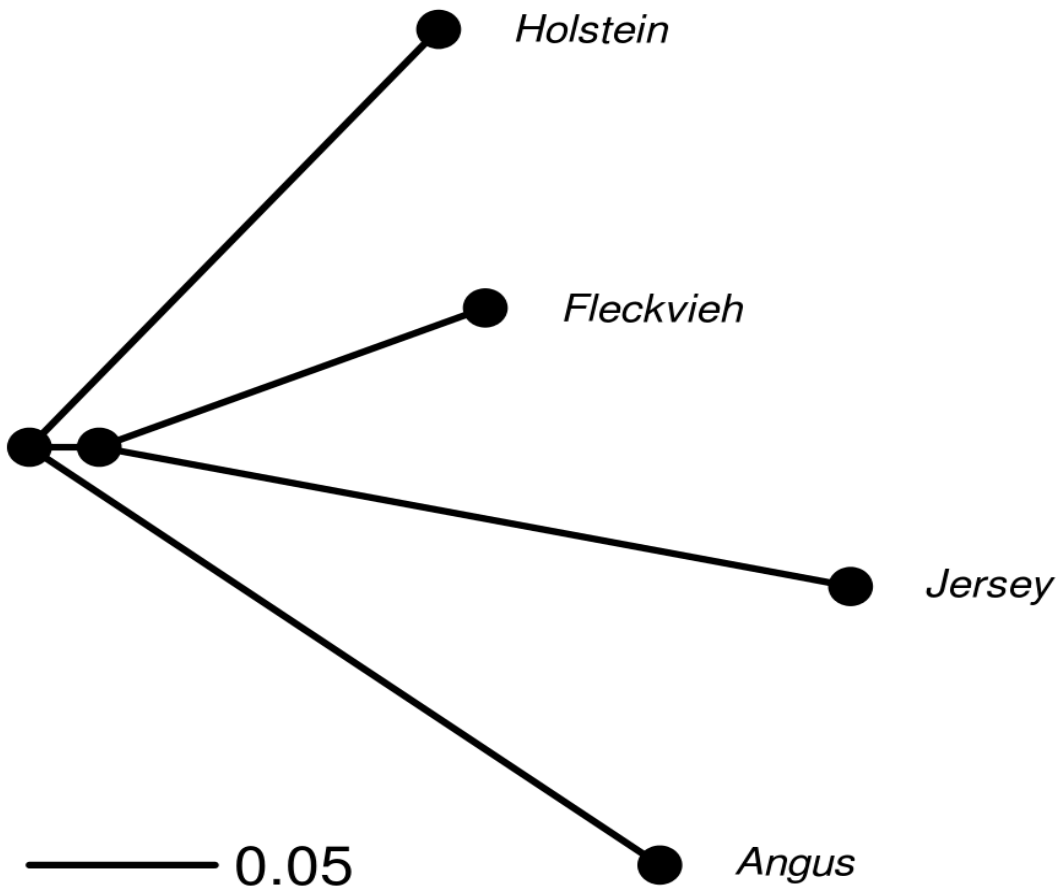




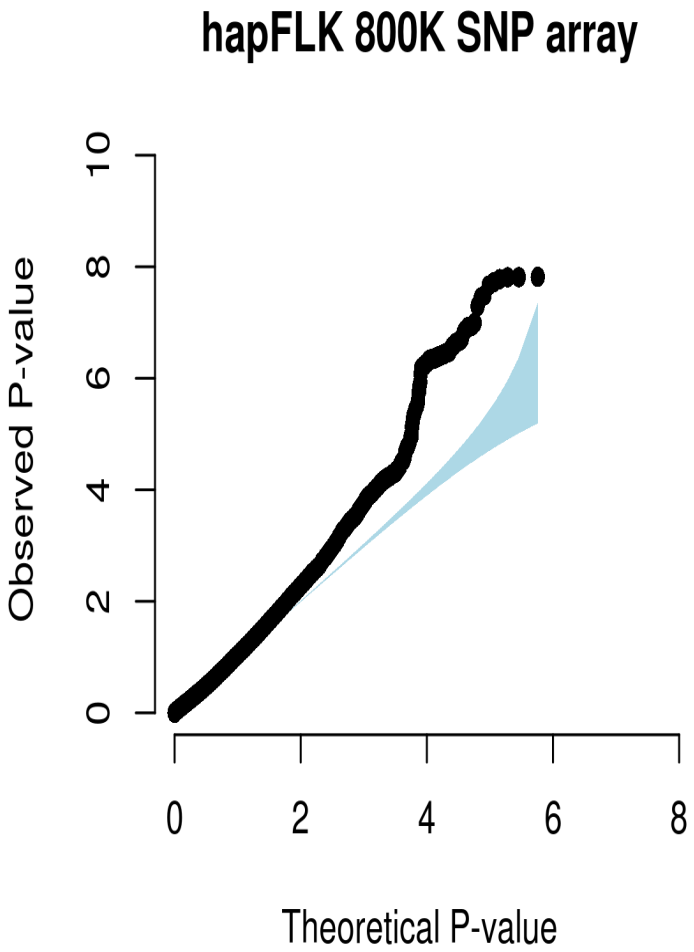
Outline

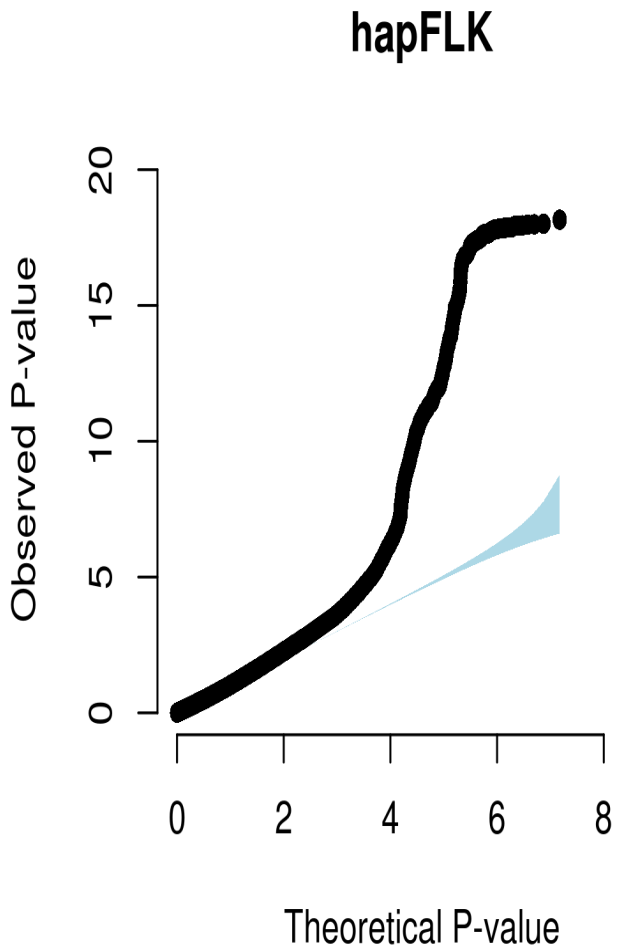
The 1000 bulls genome project, run2

- 234 sequences from 4 breeds (90 used).
- 29 millions bi-allelic variants (SNPs and indels)

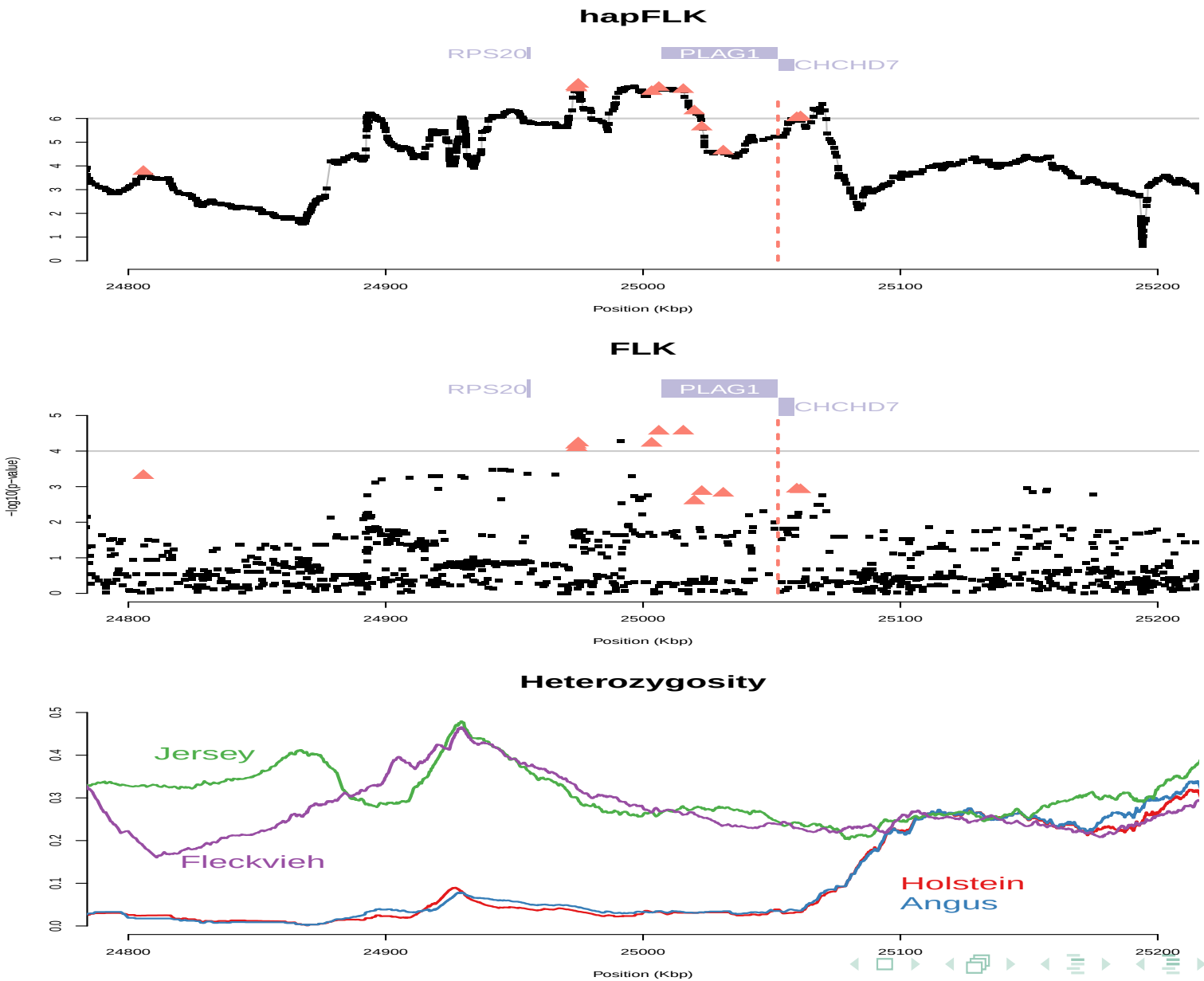


Increased power from NGS data





PLAG1 region - locate causal mutation



Outline

Conclusion

- The hapFLK approach:
  - Detection of positive selection from multi-population samples.
  - Accounts for population size heterogeneity and hierarchical structure of populations.
  - Uses haplotype information.

  $\rightarrow$ increased detection power
- Other advantages:
  - No need for sliding window.
  - Run from unphased genotype data, missing data allowed.
  - Soft and incomplete sweeps can be detected.
- Limitations:
  - Pure drift model.
  - Outlier approach.

References

- Methods:
  - M. Bonhomme, C. Chevalet, B. Servin, S. Boitard, J. Abdallah, S. Blott, M. San Cristobal (2010). Detecting selection in population trees: the Lewontin and Krakauer test extended. *Genetics* 186: 241-26.
  - M. I. Fariello, S. Boitard, H. Naya, M. SanCristobal, B. Servin (2013). Detecting signatures of selection through haplotype differentiation among hierarchically structured populations. *Genetics* 193: 929-941.
- Applications:
  - M.I. Fariello, B. Servin, G. Tosser-Klopp, R. Rupp, C. Moreno, International Sheep Genome Consortium, M. San Cristobal and S. Boitard (2014). Selection Signatures in Worldwide Sheep Populations. *PLoS ONE* 9(8), e103813.
  - P.F. Roux, S. Boitard, Y. Blum et al (2015). Combined QTL and Selective Sweep Mappings with Coding SNP Annotation and Cis-eQTL Analysis Revealed PARK2 and JAG2 as New Candidate Genes for Adiposity Regulation. *G3* 5(4) 517-529.
  - S. Boitard, M. Boussaha, A. Capitan, D. Rocha and B. Servin (2016). Uncovering Adaptation from Sequence Data: Lessons from Genome Resequencing of Four Cattle Breeds. *Genetics* 203: 433-450.

Outline

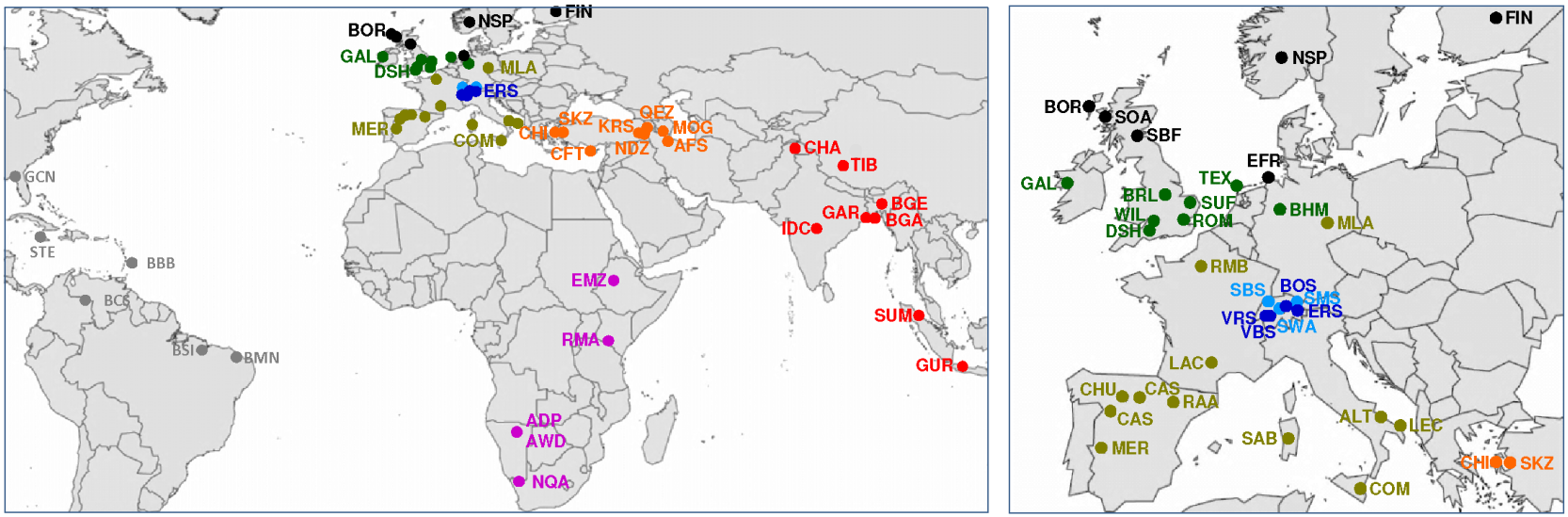
The `hapflk` software

- Builds and plots population trees (using `R` packages)
- Performs FLK and hapFLK tests on SNP data
- Computes associated p-values
- Plots cluster frequencies

Technical information

- Webpage: `https://forge-dga.jouy.inra.fr/projects/hapflk`
- Free software (GPL).
- Binaries for Linux 64bits and MacOSX 10.6+
- Source package available (requires `python`, `numpy`, `scipy`, `C` compiler).
- Other needs for additional routines: `R` with `ape`, `phangorn` and `ggplot2` packages, `python statsmodels` package.

Example data: sheep from Northern Europe



Kijas *et al.* (2012) PLoS Biology
6 Populations + Outgroup (Soay), 388 individuals, 50K SNPs
Available at `http://www.sheephapmap.org`

Before diving in ...

- Remember assumptions underlying the neutral model:
  - Population tree
  - Pure drift model (no mutations, no admixture)
  - Small F_i (say < 0.2)
- This means
  - Discard strongly bottleneck-ed or admixed populations
  - Discard low frequency variants (at the meta-population level), likely to have appeared after population spit.
- Perform a diversity analysis before:
  - Population structure (STRUCTURE, PCA, treemix ...) to remove outliers.
  - Within population kinship between individuals to identify a set of "unrelated" individuals

Main steps of the analysis

1. Compute kinship matrix and FLK.
2. Perform hapFLK test chromosome by chromosome.
3. Merge hapFLK results in a single file.
4. Compute hapFLK p-values.
5. Call significant regions and plot p-values.
6. Annotate interesting regions (e.g. myostatin region on chr 2).
   - Plot haplotype cluster frequencies.
   - Plot local population trees.