Population stratification

Background & PLINK practical

Variation between, within populations

- Any two humans differ ~0.1% of their genome (1 in ~1000bp)
- ~8% of this variation is accounted for by the major continental racial groups
- Majority of variation is within group
  - but genetic data can still be used to accurately cluster individuals
  - although biological concept of “race” in this context controversial

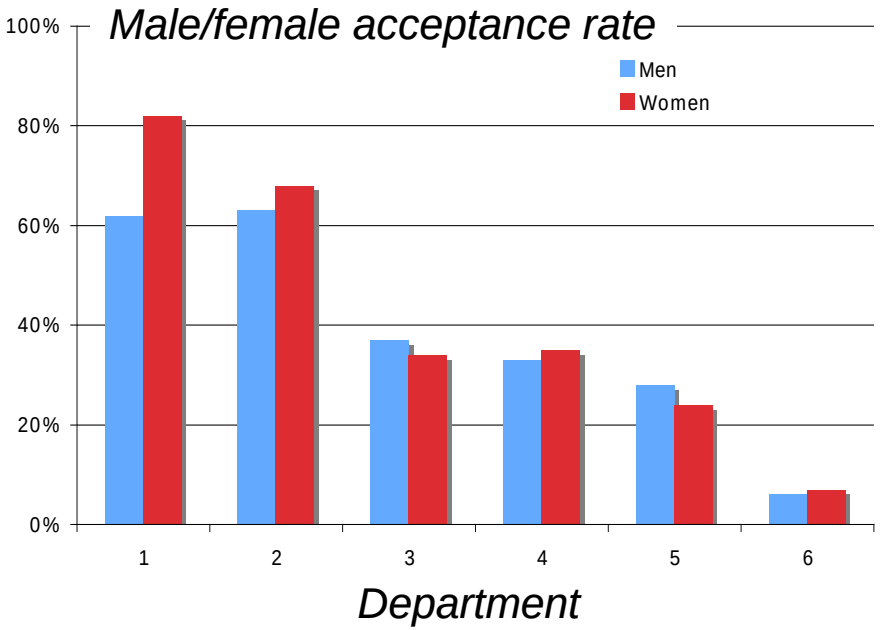
Stratified populations: Wahlund effect

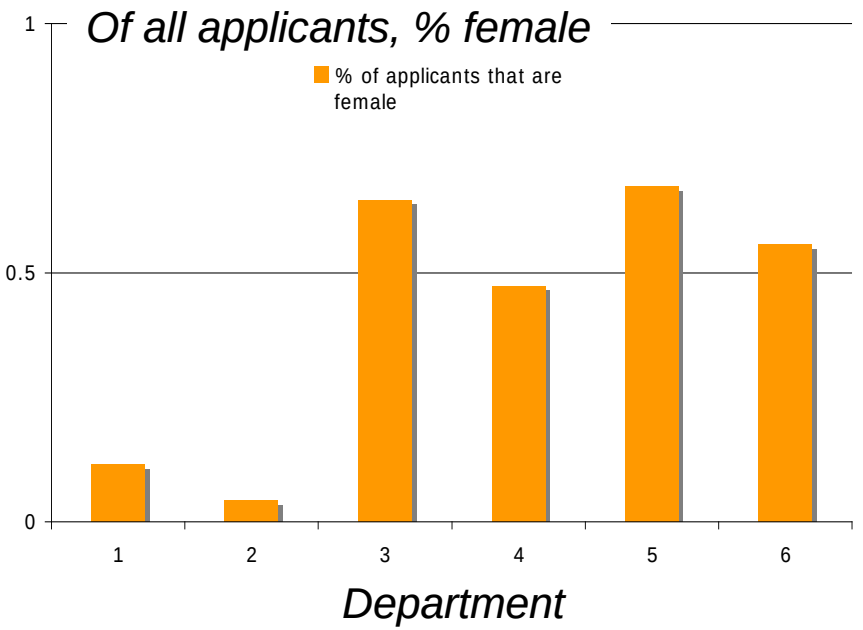
	Sub-population		
	1	2	1+2
A_1	0.1	0.9	*0.5*
A_2	0.9	0.1	*0.5*
A_1A_1	0.01	0.81	*0.41 (0.25)*
A_1A_2	0.18	0.18	*0.18 (0.50)*
A_2A_2	0.81	0.01	*0.41 (0.25)*

Quantifying population structure

- Expected average heterozygosity
  - in random mating subpopulation (H_S)
  - in total population (H_T)
    - from the previous example,
      - $H_S = 0.18$, $H_T = 0.5$
- Wright's fixation index
  - $F_{ST} = (H_T - H_S) / H_T$
    - $F_{ST} = 0.64$
  - 0.01 - 0.05 for European populations
  - 0.1 - 0.3 for most divergent populations

- Confounding due to unmeasured variables is a common issue in epidemiology
  - "Simpson's paradox"
- Berkley sex bias case
  - claim that female graduate applicants were prejudiced against
  - 44% men accepted, 35% women
  - *but*, stratified by department, no intra-department differences (see figure)
  - i.e. women more likely to apply to departments that were harder to get into (for both males and females)
- In genetic association studies,
  - "accepted or not" → disease or not
  - "male/female" → genetic variant
  - "department" → ancestry
- Happens when both outcome and genotype frequencies vary between different ethnic groups in the sample

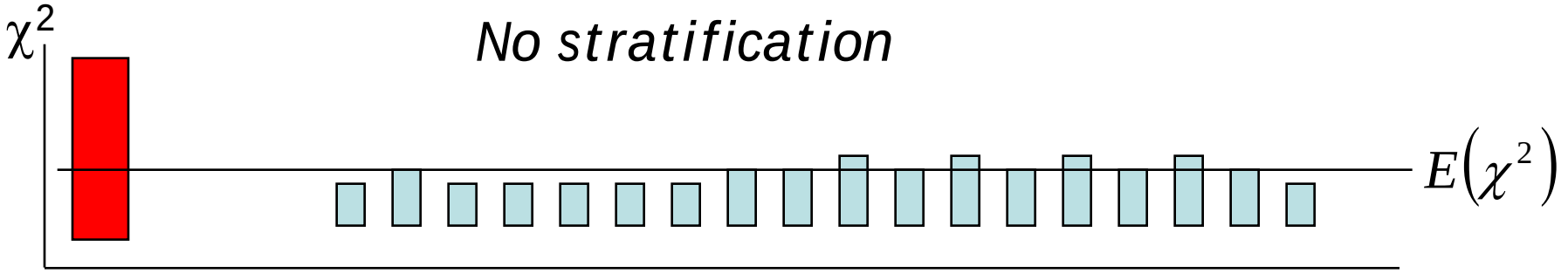


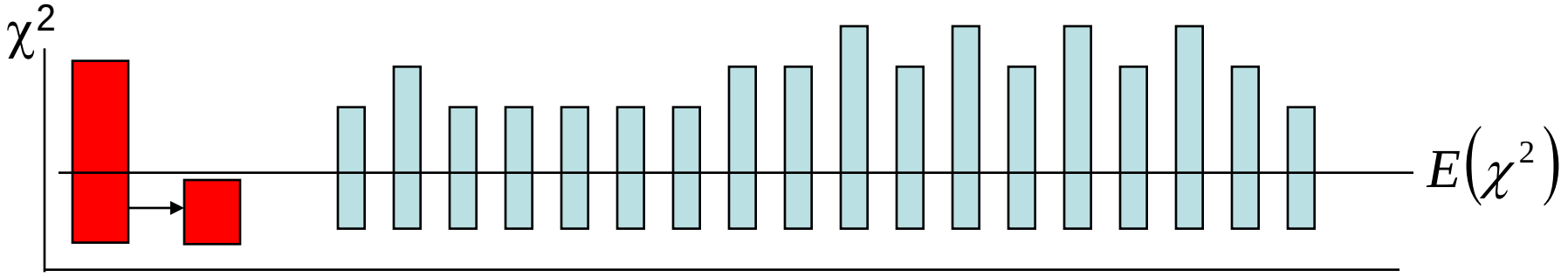


Approaches to detecting stratification using genome-wide SNP data

- Genomic control
  - average correction factor for test statistics
  - ratio of median chi-sq to expectation under null (0.456 for 1df)
- Clustering approaches
  - assign individuals to groups
  - model based and distance based
- Principal components analysis, multidimensional scaling
  - continuous indices of ancestry

Genomic control

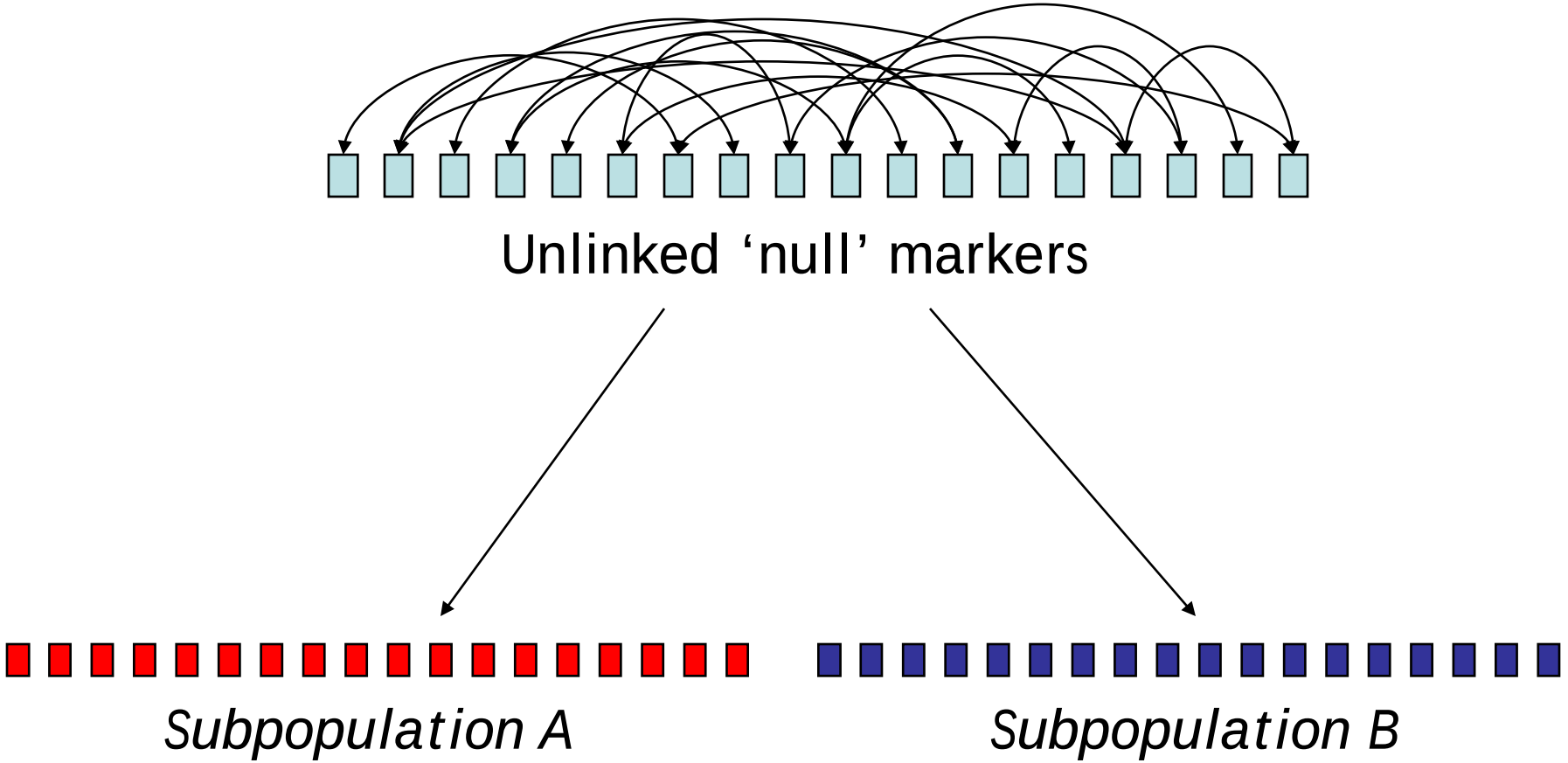




Stratification → adjust test statistic

Structured association

LD observed under stratification



Discrete subpopulation model

- *K* sub-populations, “latent classes”
  - Sub-populations vary in allele frequencies
  - Random mating within subpopulation
- Within each subpopulation
  - Hardy-Weinberg and linkage **equilibrium**
- For population as a whole
  - Hardy-Weinberg and linkage **disequilibrium**

Worked example

- Look at Excel spreadsheet ~pshaun/pop-strat.xls
- **Scenario:** two sub-populations, of equal frequency in total population. We know allele frequencies for 5 markers unlinked markers
- **Problem:** For a given individual with genotypes on these 5 markers, what is the probability of belonging to population 1 versus population 2?
- Allele frequencies:

Population	**M1**	**M2**	**M3**	**M4**	**M5**
P_1	0.05	0.3	0.4	0.2	0.15
P_2	0.3	0.9	0.3	0.05	0.6

Steps:

1) Class-specific allele frequencies $\rightarrow$ class-specific genotype frequencies (HWE)
2) Single locus $\rightarrow$ multi-locus (5 marker) genotype frequencies (LE), P(G|C)
3) Prior probability of class, P(C). Hint: we are given this above.
4) Bayes theorem to give P(C|G) from P(G|C) and P(C)

Statistical approaches to uncover hidden population substructure

- **Goal** : assign each individual to class *C* of *K*
- **Key** : conditional independence of genotypes, *G* within classes (LE, HWE)

P(C)	prior probabilities
P(G | C)	class-specific allele/genotype frequencies
P(C | G)	posterior probabilities

Bayes theorem:

$$P(C \mid G) = \frac{P(G \mid C)P(C)}{\sum_{j} P(G \mid C)P(C)}$$

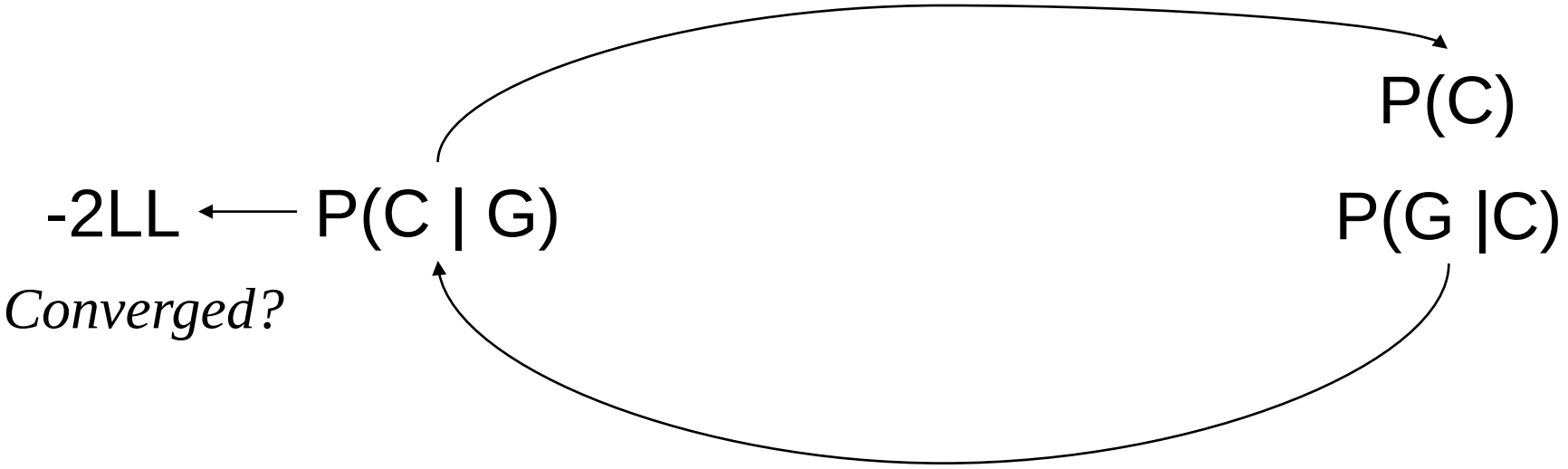
Sum over j = 1 to K classes

Problem: in practice, we don't know P(G|C) or P(C) either!

Solution: EM algorithm (LPOP), or Bayesian approaches (STRUCTURE)

E-M algorithm

E step:
counting individuals and alleles in classes



M step:
Bayes theorem, assume conditional independence

Stratification analysis in PLINK

- Calculate IBS sharing between all pairs
  - “--genome” command; can take long time, but can be parallelized easily
  - generates (large) .genome file
  - can be used to spot sample duplicates
  - also contains IBD estimates: these are only meaningful within a ~homogeneous sample
- Given IBS data, perform clustering
  - complete linkage clustering
  - can specify various constraints, e.g. PPC test, cluster size (e.g. 1:1 matching) or # of clusters
- Given IBS data, perform MDS
  - extract first K components, e.g.4-6
  - plot each component, each pair of components

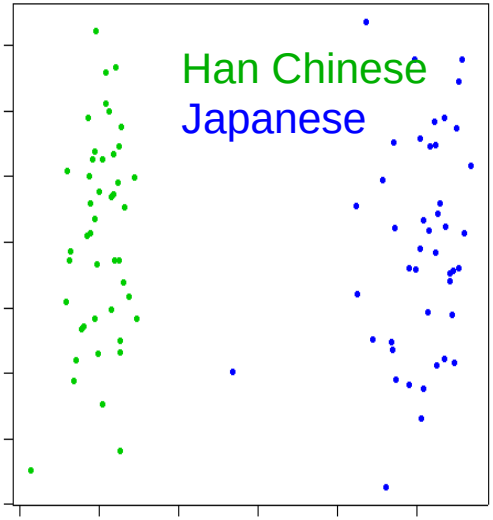
Pairwise allele-sharing metric

Reference

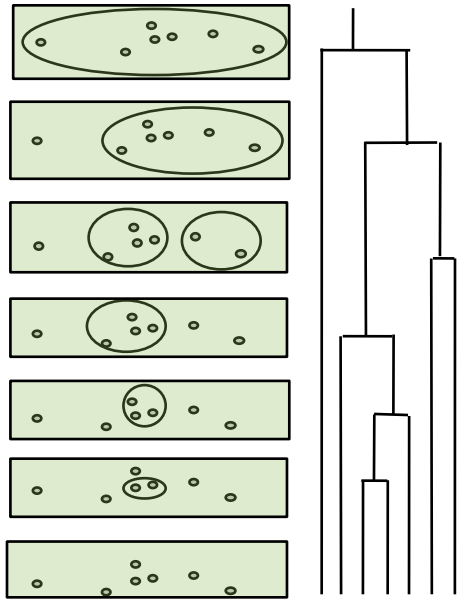
Same population

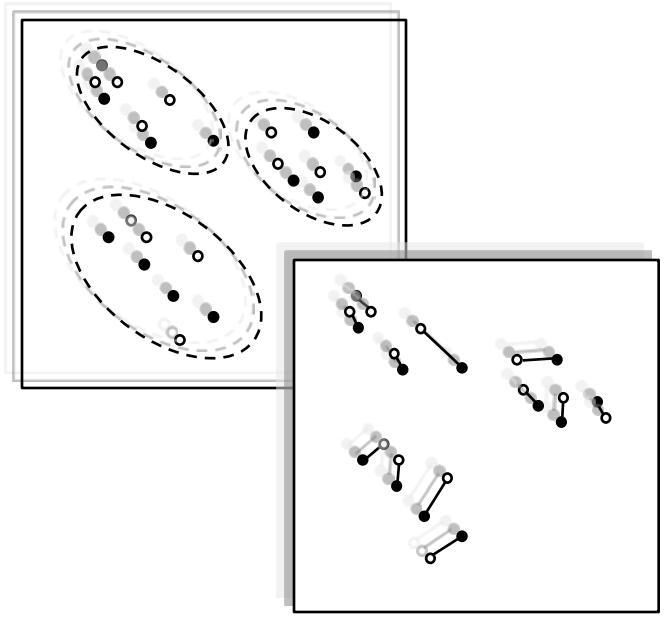
Different population

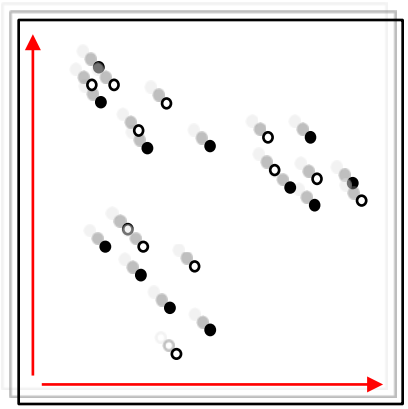
Multidimensional scaling/PCA



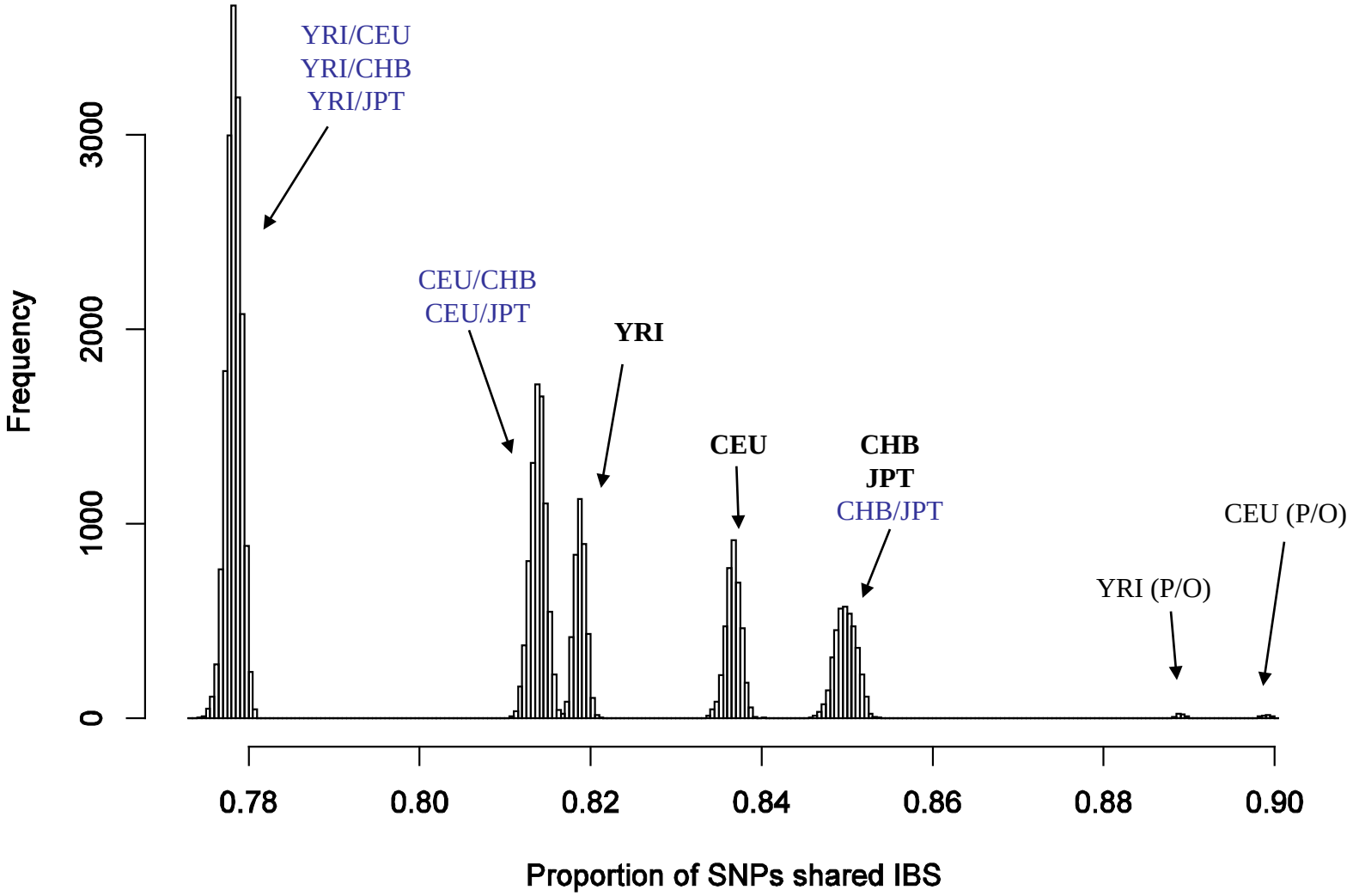
Hierarchical clustering





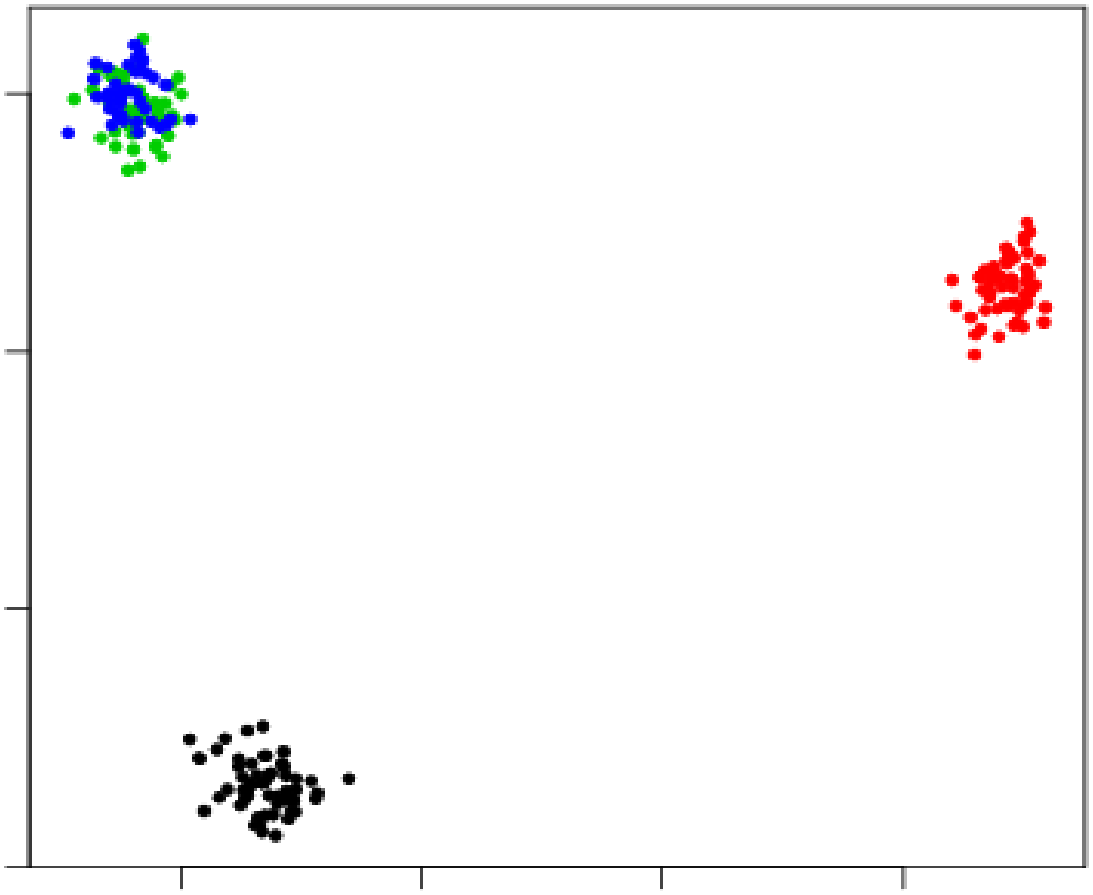


Distribution of IBS between and within HapMap subpopulations



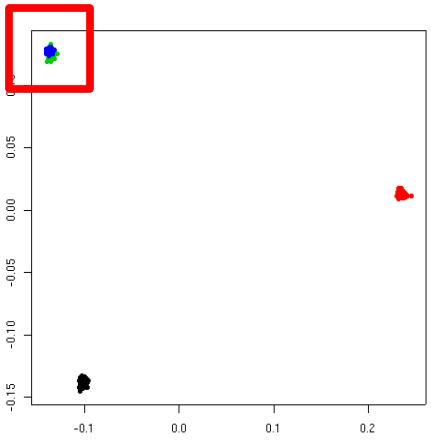
Multidimensional scaling (MDS) analysis HapMap data (equiv. to PCA)

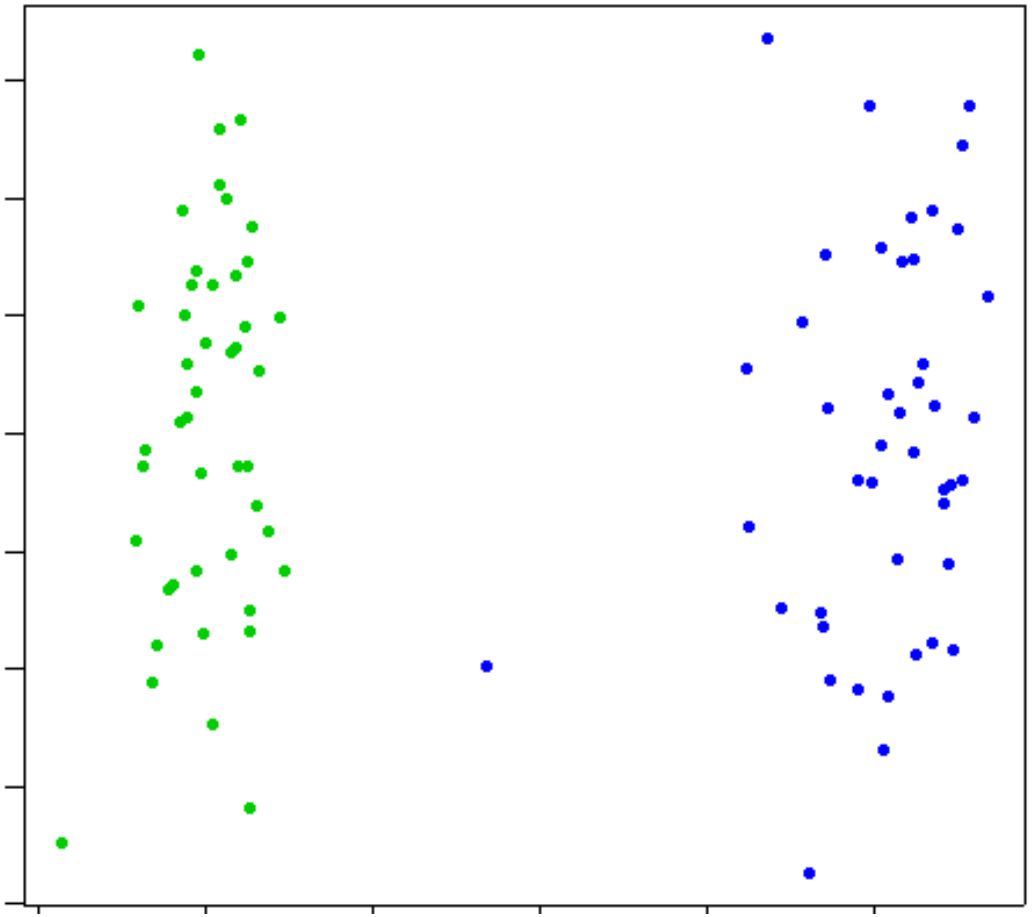
CEPH/European
Yoruba
Han Chinese
Japanese



~2K SNPs

Han Chinese
Japanese





~10K SNPs

PPC (pairwise population concordance) test

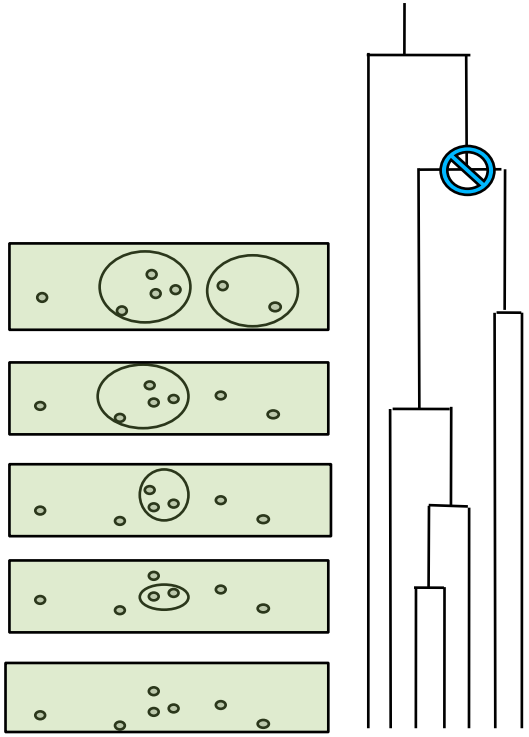
{ AA , BB } : { AB , AB }

{ individual 1 , individual 2 }

1 : 2

Expected 1:2 ratio in individuals from same population
Significance test of a binomial proportion

<u>Note:</u> Requires analysis to be of subset of SNPs in approx. LE within sub-population. Would also be sensitive to inbreeding



Two example pairs: (50K SNPs with 100% genotyping)

Ind1	*Ind2*	{ AA , BB }	{ AB , AB }	Ratio	*p*-value
CHB	CHB	3451	6927	1 : 2.007	0.569
JPT	CHB	3484	6595	1 : 1.892	0.004

Proportion of all CHB-CHB pairs significant = 0.076
Proportion of all CHB-JPT pairs significant = 0.475

(Power for difference at p=0.05 level)

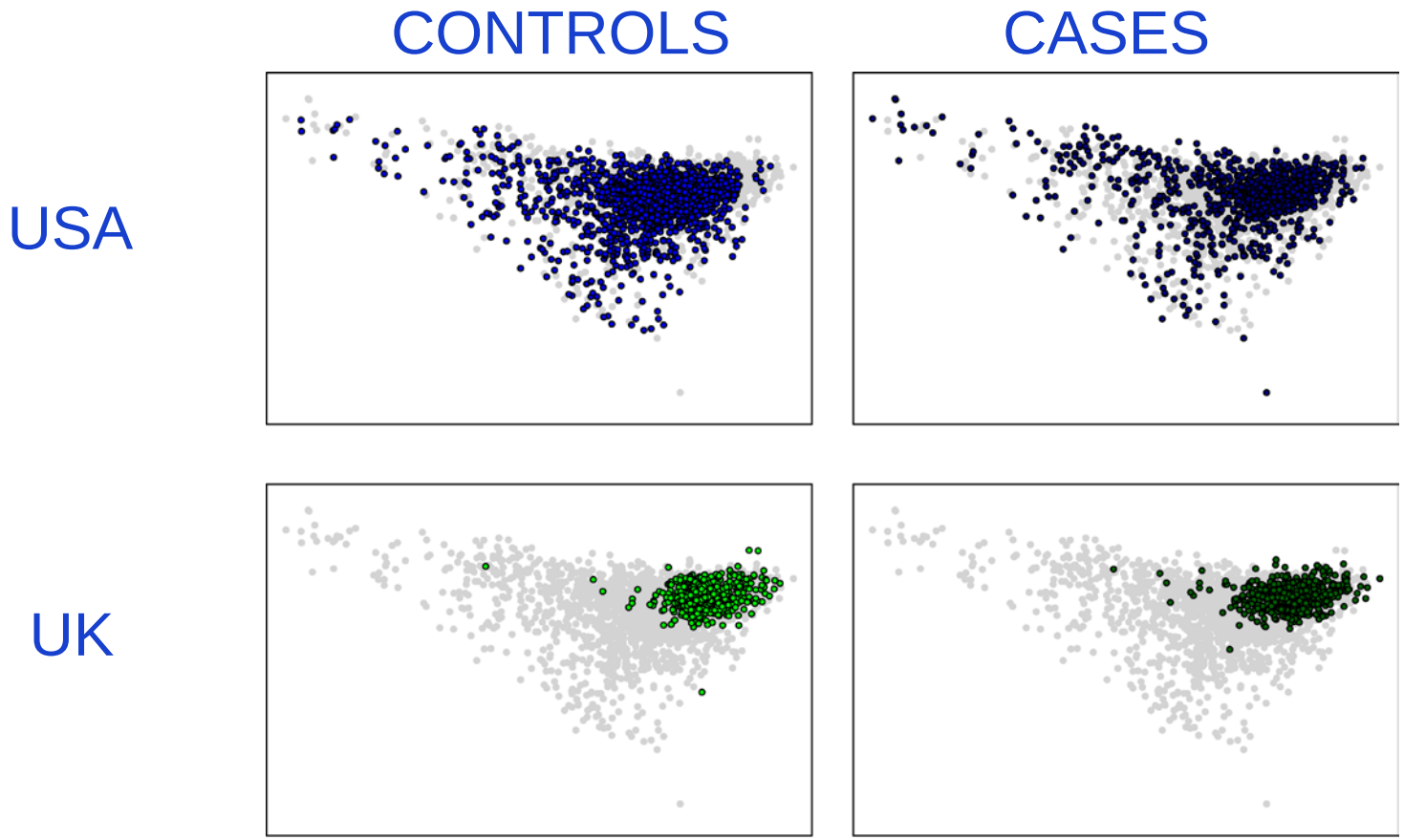
```
1        HCB1 HCB8 HCB26 HCB5 HCB15
2        HCB2 HCB45 HCB12
3        HCB3 HCB14 HCB32 HCB18 HCB27 HCB23 HCB30
4        HCB4 HCB38 HCB39 HCB20
5        HCB6 HCB21 HCB43
6        HCB7 HCB29 HCB31 HCB11 HCB40 HCB24 HCB33
7        HCB9 HCB16 HCB22
8        HCB10 HCB44 HCB19 HCB41 HCB42 HCB35 HCB36
9        HCB13 HCB17 HCB34 HCB25 HCB28 HCB37

10       JPT1 JPT19 JPT13 JPT16 JPT29 JPT36
11       JPT2 JPT28
12       JPT3 JPT17 JPT38 JPT44 JPT8 JPT23
13       JPT4 JPT18 JPT21 JPT27 JPT41 JPT43
14       JPT5 JPT30 JPT39 JPT42 JPT9
15       JPT6 JPT37 JPT24
16       JPT7 JPT12 JPT10 JPT25 JPT14 JPT26 JPT34 JPT33
17       JPT11 JPT31 JPT40 JPT15 JPT22
18       JPT20
19       JPT32*
20       JPT35
```

MDS analysis

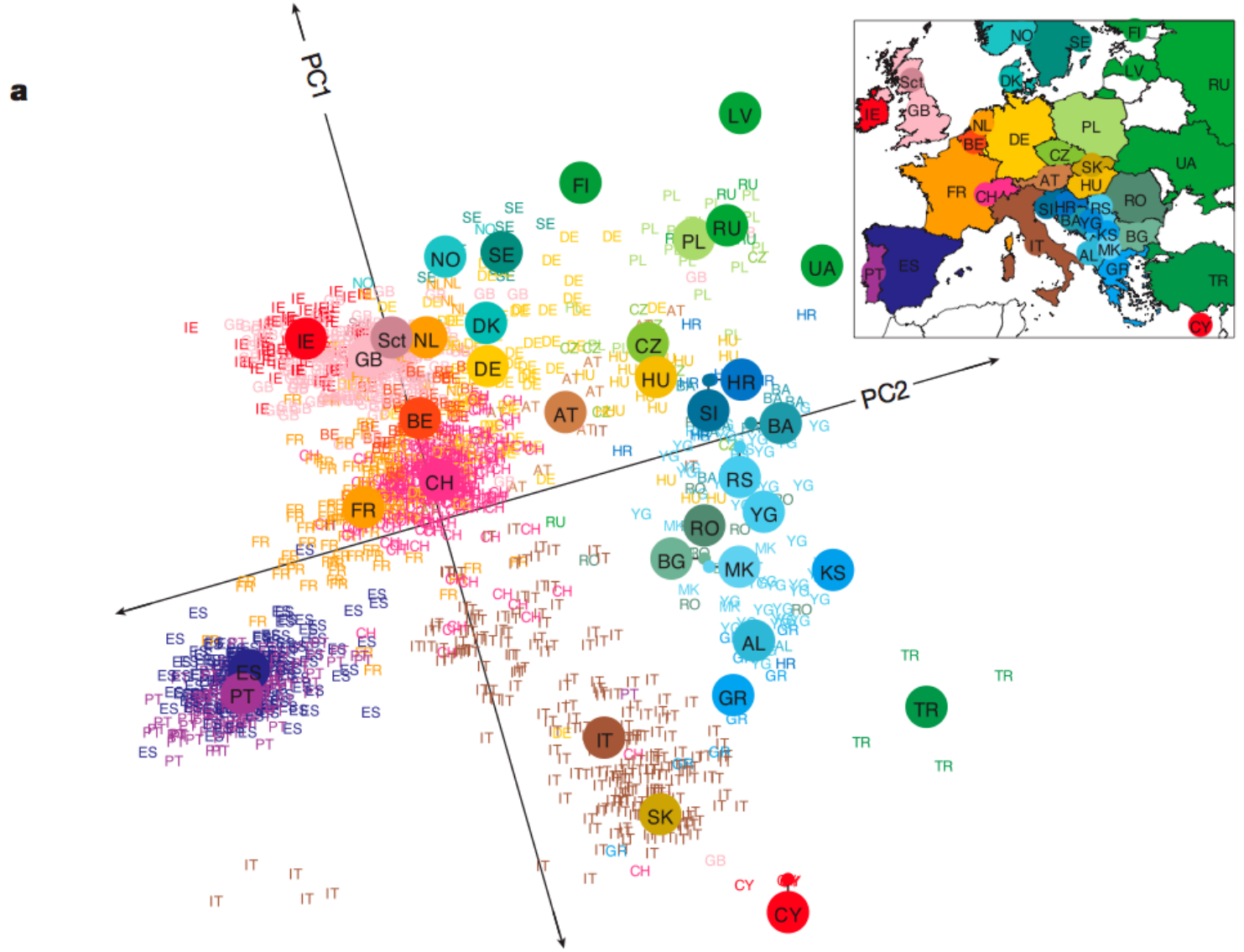
- Often useful to treat each MDS component as a QT and perform WGAS (regress it on all SNPs), to ask:
  - what is the genomic control lambda? If not >>1, then the component probably does not represent true, major stratification
  - which genomic regions load particularly strongly on the component (i.e. which regions show largest frequency differences between the groups the component is distinguishing?)

Practical example: bipolar GWAS



Evaluated via permutation that within site the average case is equally similar to the average control as another case

Fine-scale genetic variation reflects geography



Novembre *et al, Nature* (2008)