Data handling and PCA

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Today

- Experimental design
- Data handling
- PCA
- Spatial and isolation-by-distance methods

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- **Experimental design**
- Data handling
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Real-life scenario

Let’s figure out the evolutionary history of X

YOU

Experimental design

Sample size	Per-sample depth
1,000	1X
500	2X
100	10X
20	50X

total depth is
1,000X

Fumagalli 2013

Experimental design

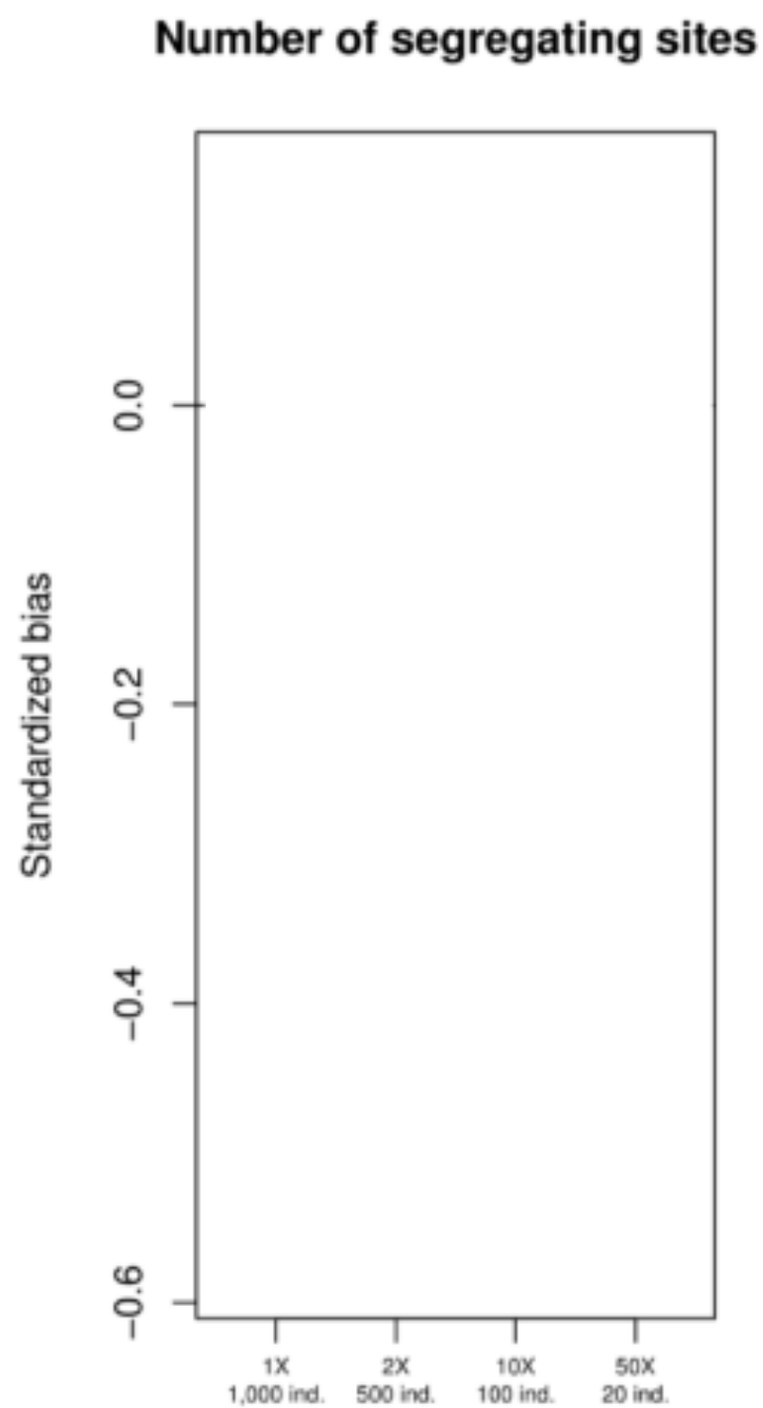
- Let's measure the bias of these set-ups for a set of summary statistics

- We'll compare the value we estimate from our data (S hat) against the true value of the **whole population** (S)

$$Bias(S) = \frac{\hat{S} - S}{S}$$

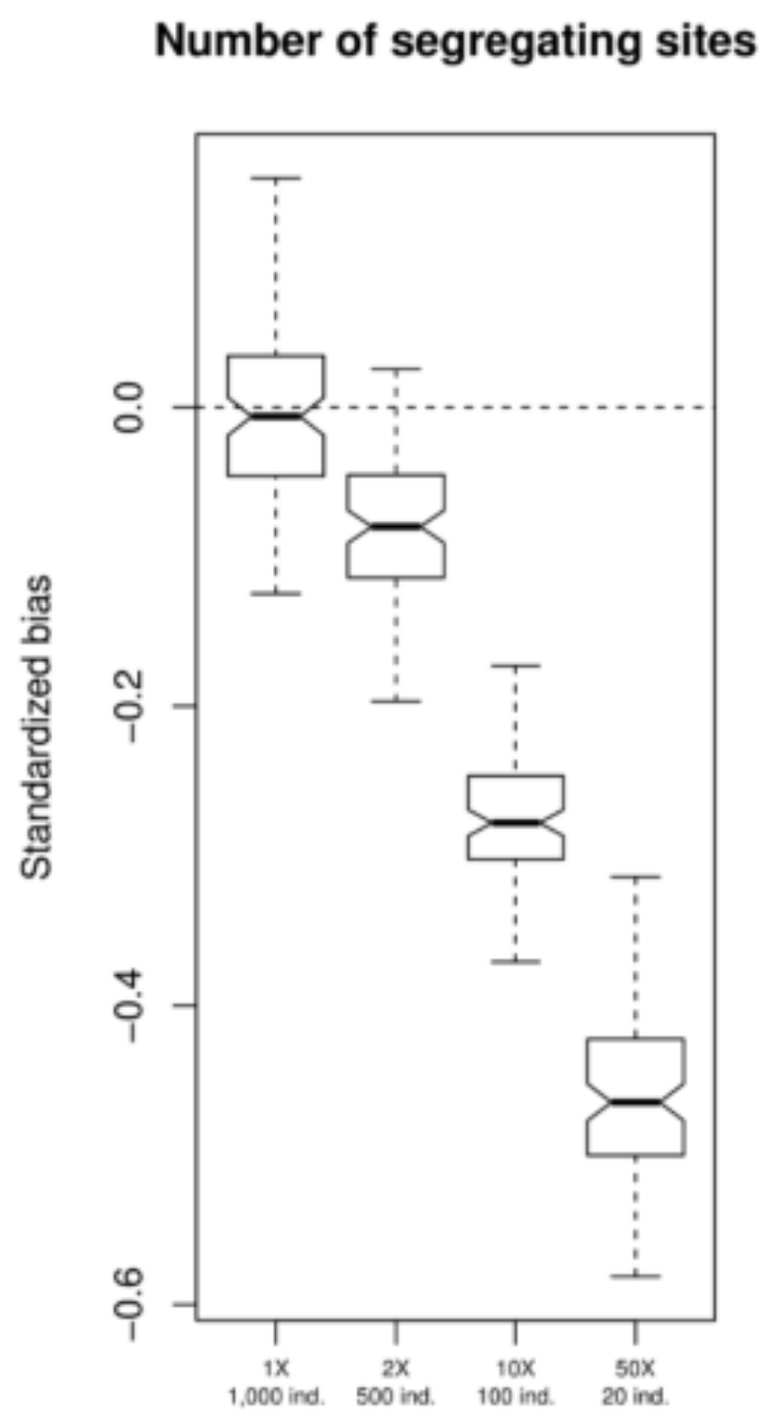
Fumagalli 2013

Experimental design



Fumagalli 2013

Experimental design



Fumagalli 2013

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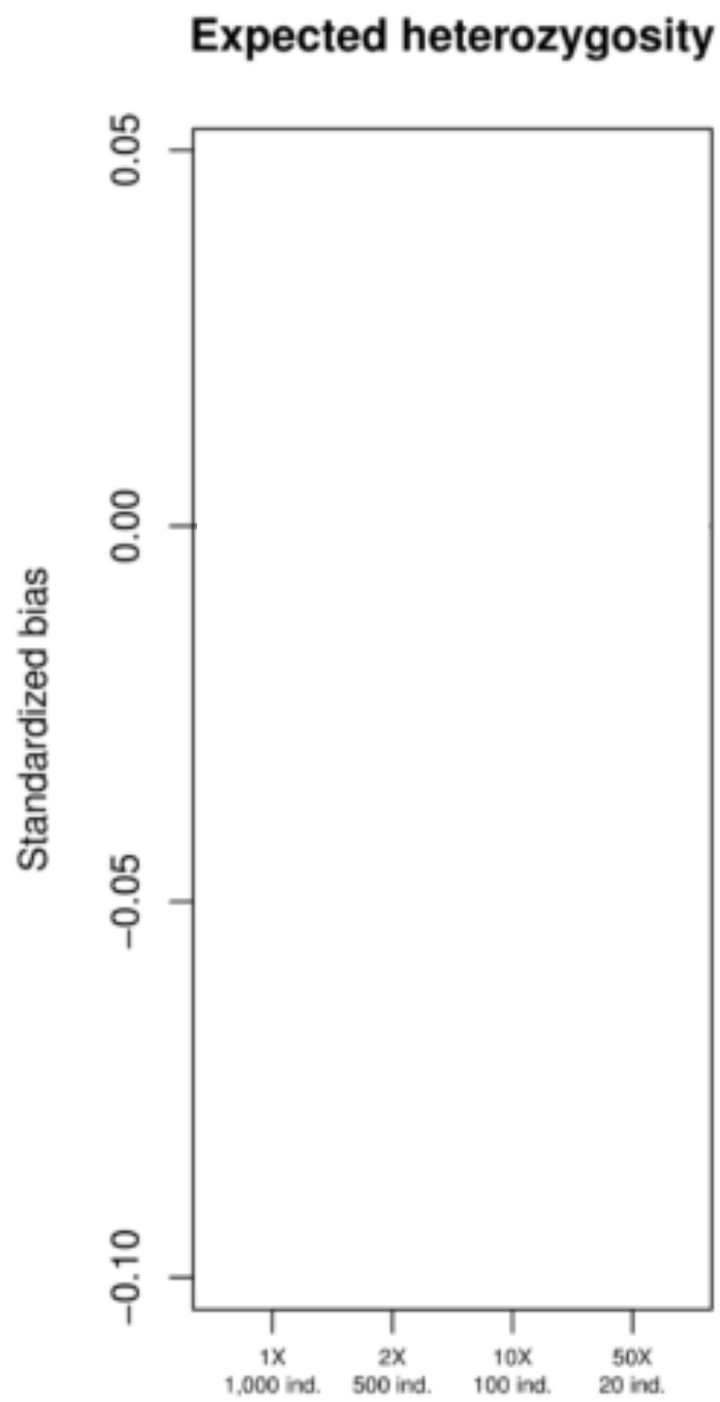

Fumagalli 2013

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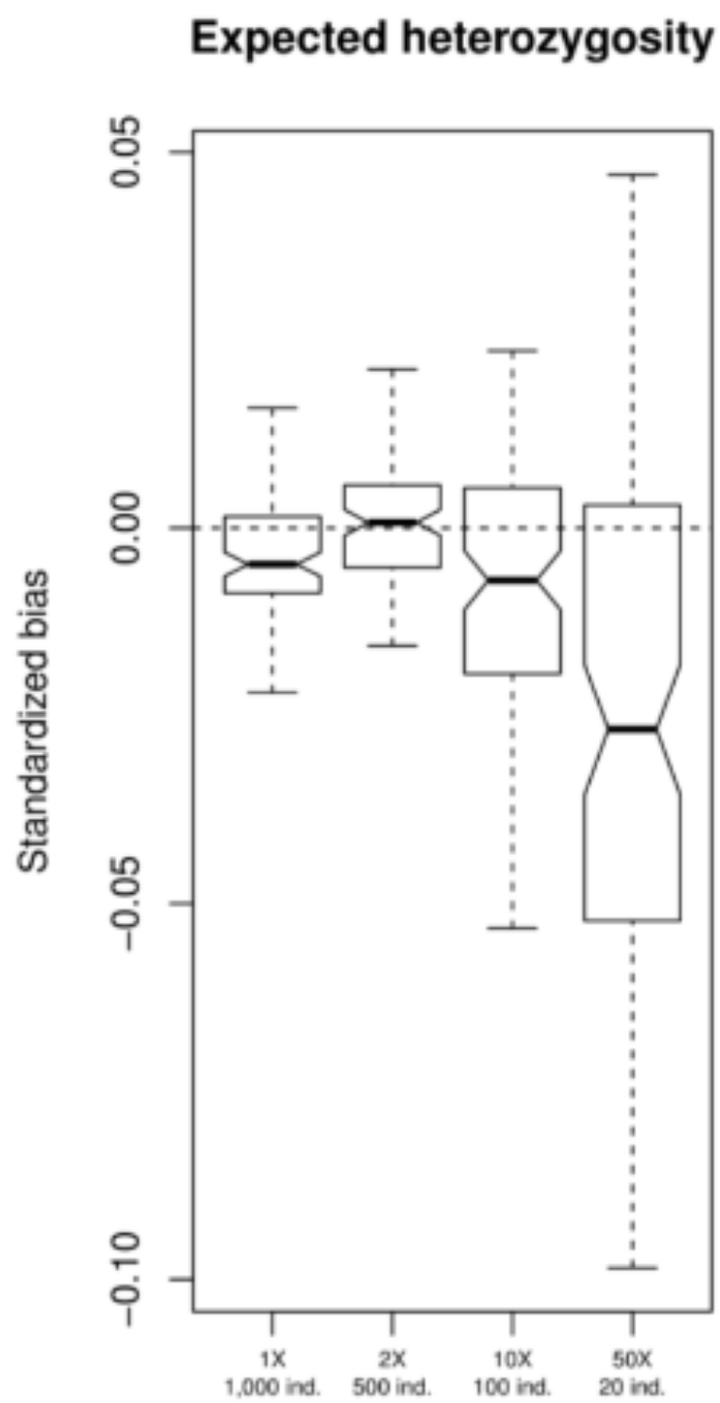

Fumagalli 2013

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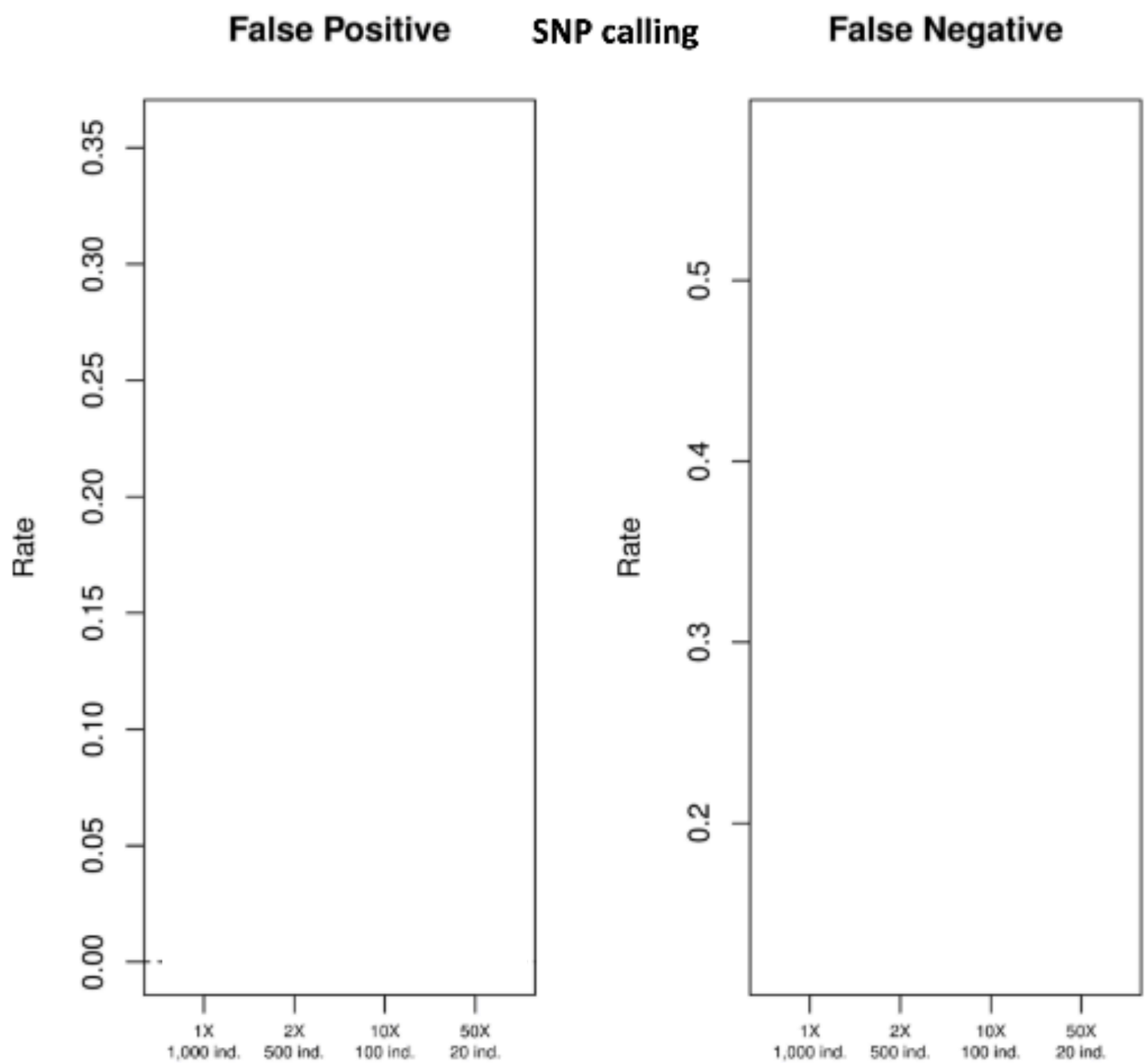

Fumagalli 2013

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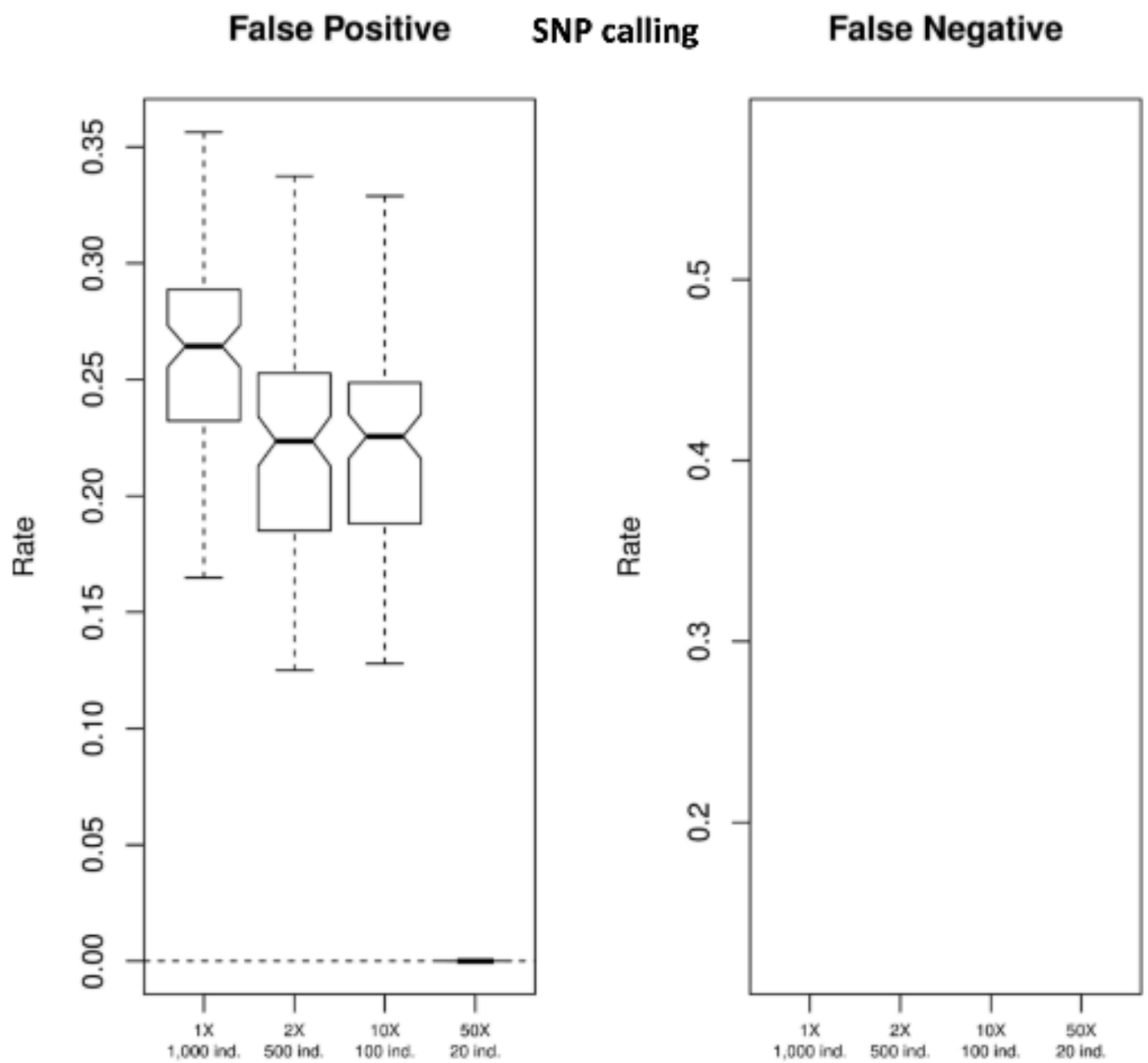

Fumagalli 2013

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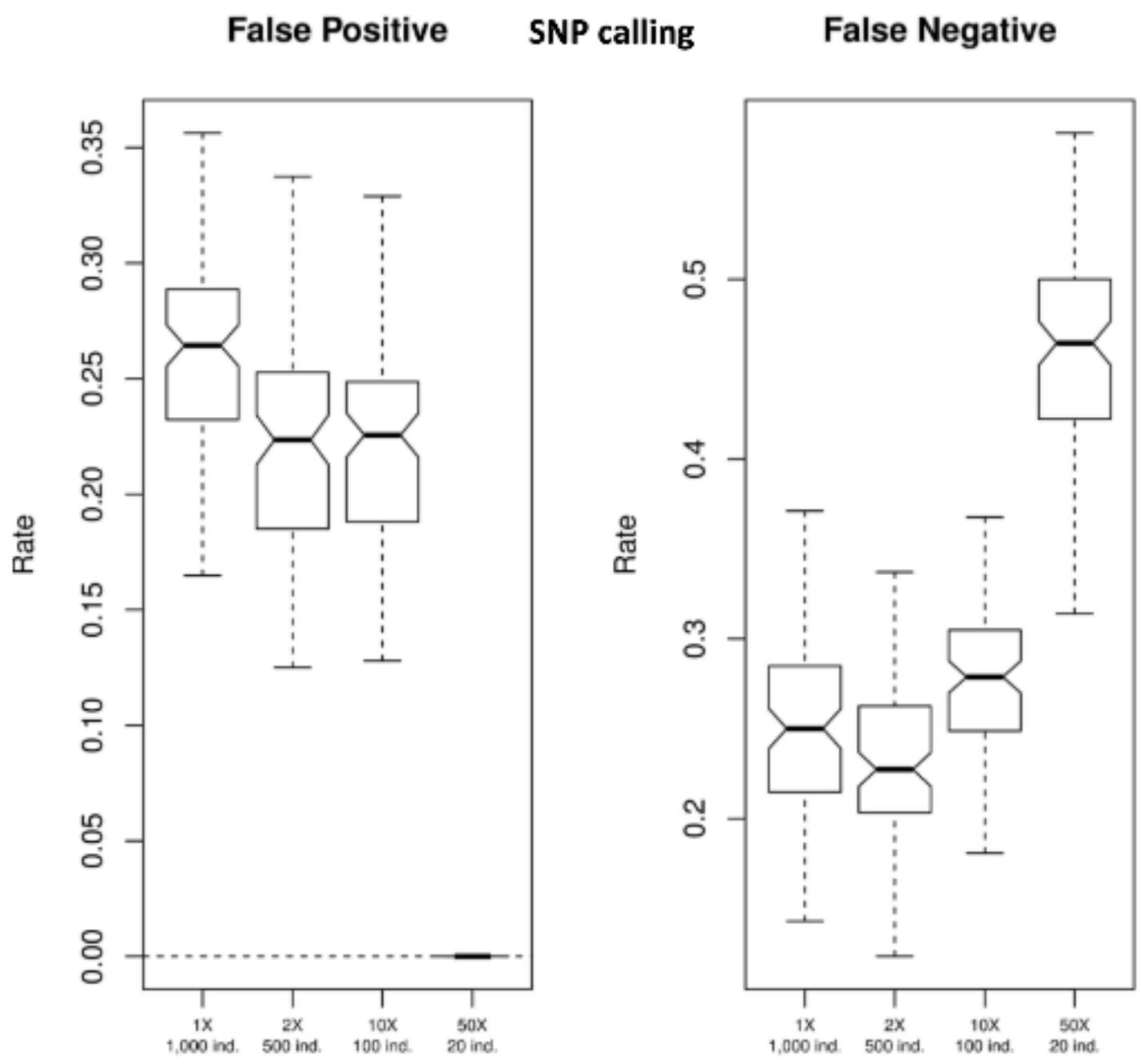

Fumagalli 2013

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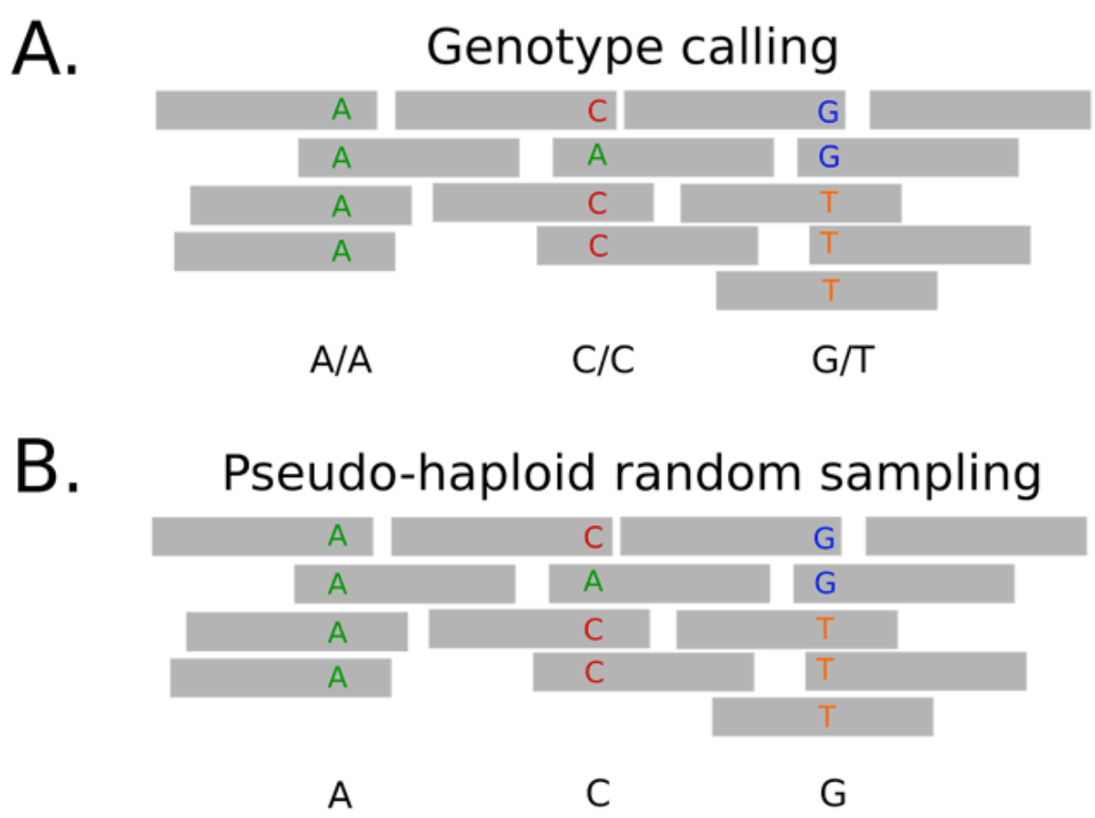
Alternative ways to deal with population genomic data



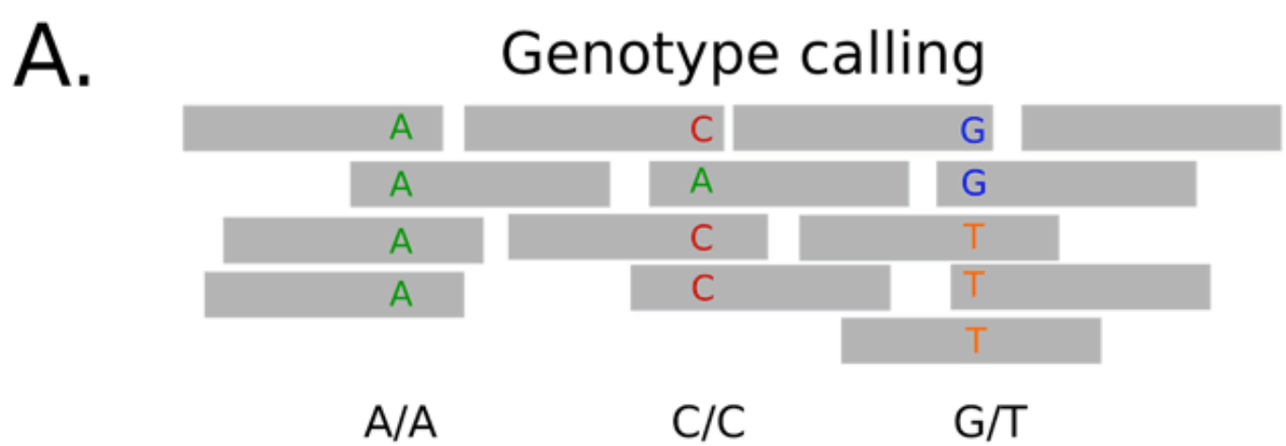
C.

Genotype likelihoods

Genotype	Likelihood	Genotype	Likelihood	Genotype	Likelihood
A/A	0.87	C/C	0.68	G/T	0.89
A/T	0.01	A/C	0.21	G/G	0.03
A/G	0.01	A/A	0.06	T/T	0.04
...		...		...	

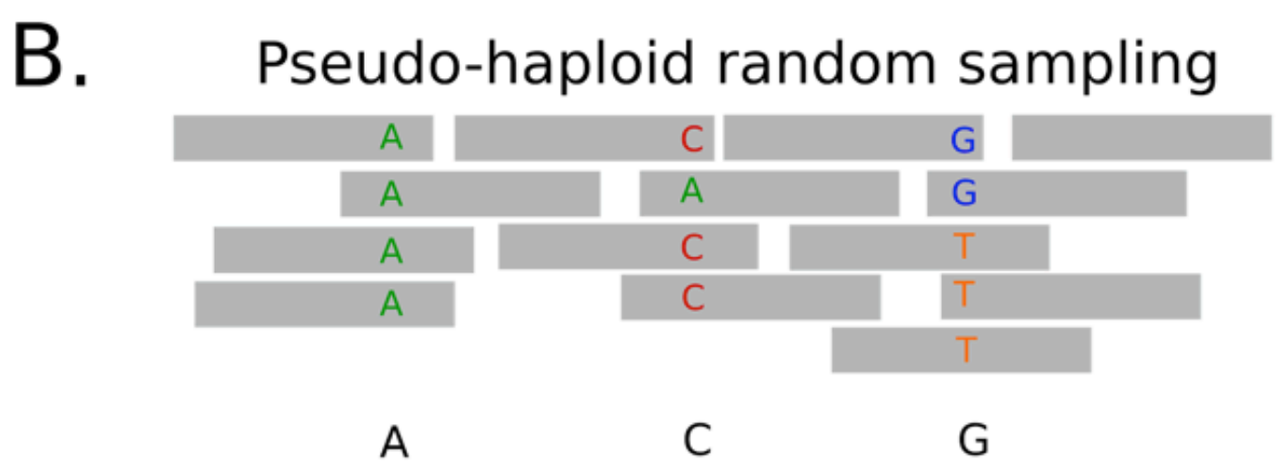
Genotype calling

- Generally requires high coverage data (> ~15X)
- Can lead to biases in comparisons with differences in coverage (more likely to over-call homozygous states on low-coverage data)
- Necessary for certain commonly used programs: PSMC, MSMC, etc.



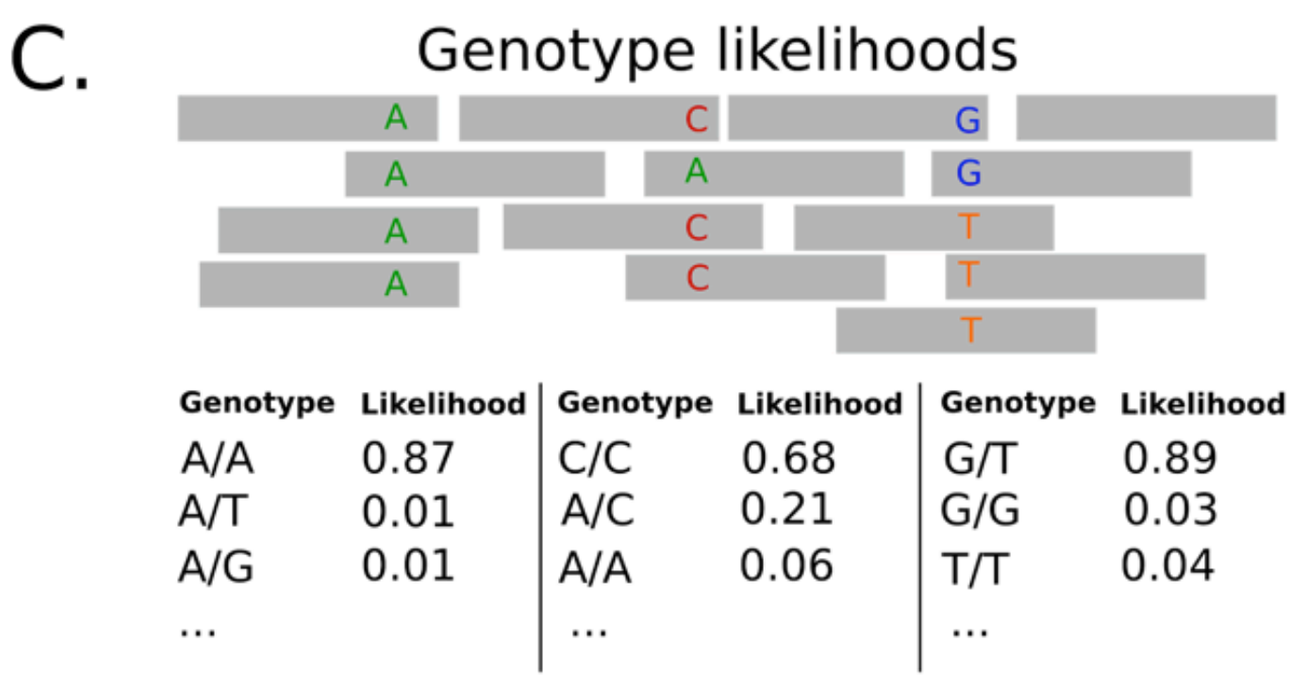
Pseudo-haploid sampling

- Unbiased with respect to differences in coverage
- Easy to produce and manipulate: we treat every diploid genome as haploid
- We lose information: we ignore all other reads that we do not sample!



Genotype likelihoods

- Uses the maximum amount of information
- Need a program to precompute likelihoods: ANGSD
- Need programs that can deal with genotype likelihoods: ngsAdmix, ngsTools, etc.
- Best for detecting selection (many individuals, low coverage data) -> good population allele frequency representation



Genotype likelihoods

- Genotype likelihood = P[data | a particular genotype]. The “log-likelihood” is the logarithm of the likelihood (easier to combine multiple probabilities: sums instead of products)

- 10 possible (unphased) genotypes: AA, AC, AG, AT, CC, CG, CT, GG, GT, TT

- Therefore, 10 log-likelihood values at each site, e.g. -10, -6.7, -8.3, -2.3, -3.5, -2.2, etc.

- Assuming we have M reads at a particular site:

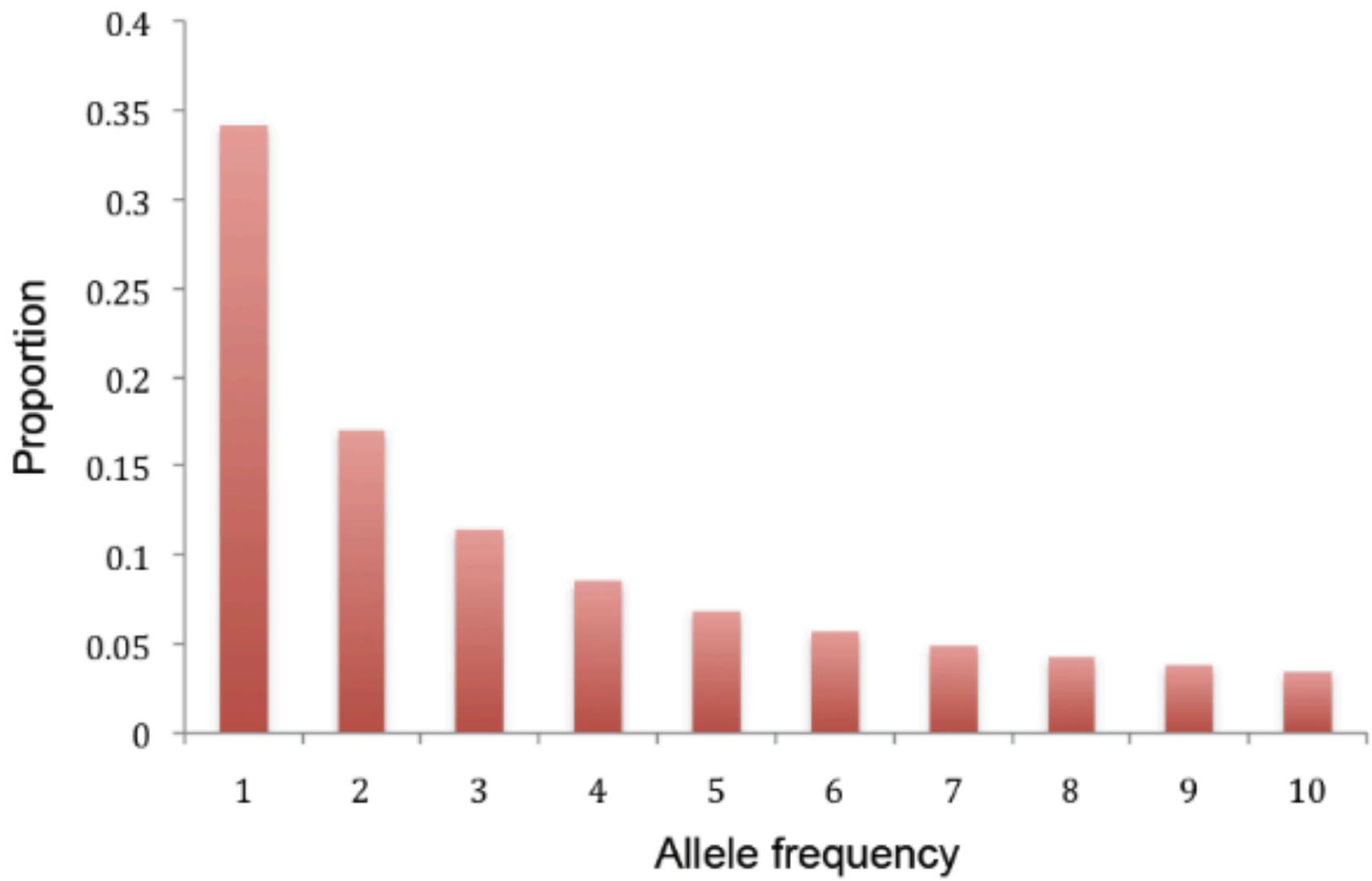
$$Pr\left(D|G=\{A_1,A_2\}\right)=\prod_{i=1}^{M}Pr\left(b_i|G=\{A_1,A_2\}\right)$$

$$=\prod_{i=1}^{M}\left(\frac{1}{2}p\left(b_i|A_1\right)+\frac{1}{2}p\left(b_i|A_2\right)\right),$$

$$p\left(b|A\right)=\begin{cases}\frac{e}{3} & b\neq A\\ 1-e & b=A.\end{cases}$$

Nielsen et al. 2012
Korneliussen et al. 2014

SFS likelihoods

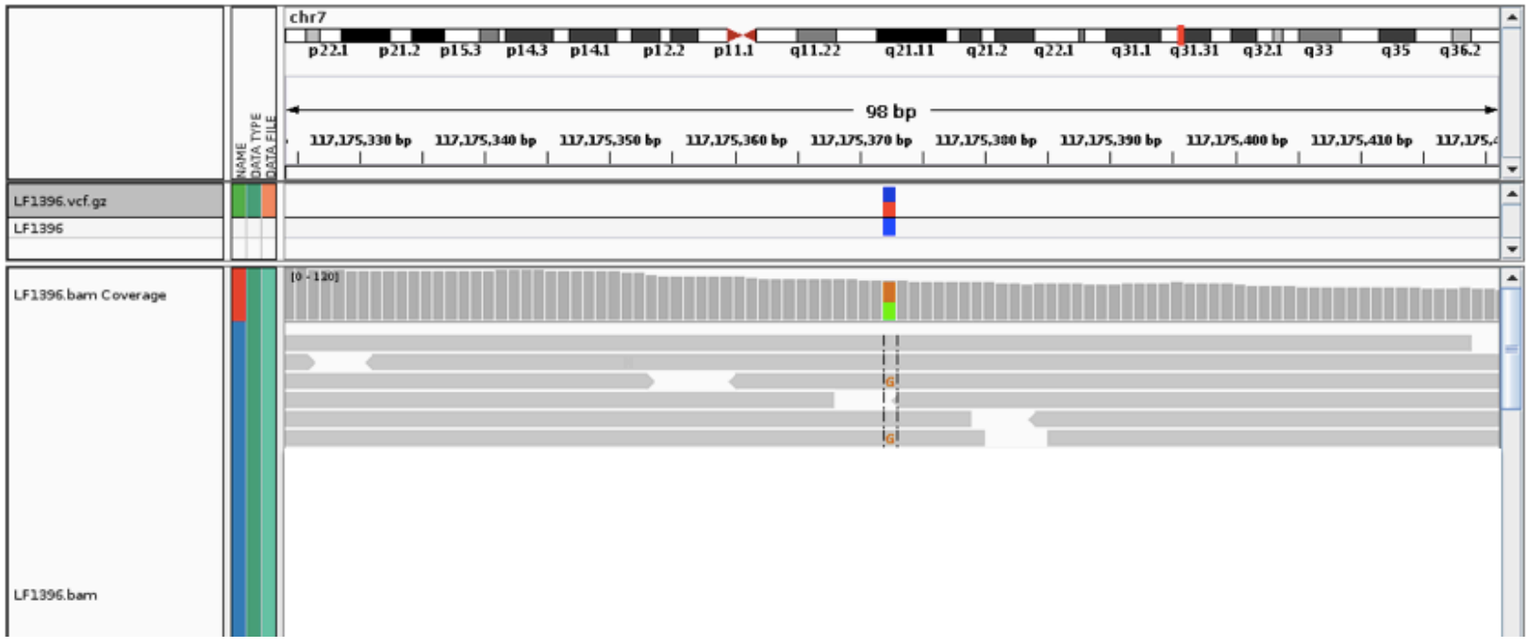


SFS likelihoods

- With low-coverage data, we don't have genotypes, so we cannot simply add up derived alleles to compute the SFS

- We can instead compute a likelihood **for each bin in the site-frequency spectrum**, given a set of reads from multiple individuals in a panel

- This approach is implemented in ANGSD and ngsTools



Fumagalli et al. 2014
Korneliussen et al. 2014

SFS likelihoods

- Let X be the sequencing data for our entire genome (all sites with ancestral and/or derived reads).
- X_s is the number of ancestral and derived reads at a particular site s.
- For 1 population, the SFS is a 1-dimensional vector $\vec{\gamma}$ with entries γ_i:
- $L(X|\gamma) = \prod_{s=1}^{N} L(X_s|\vec{\gamma}) = \prod_{s=1}^{N} \sum_{i=0}^{2n} \gamma_i P[X_s|D = i]$
- Then, we can use likelihood maximization algorithms to find a maximum likelihood estimate for each entry of the SFS (the values γ_i)

Nielsen et al. 2012
Korneliussen et al. 2014

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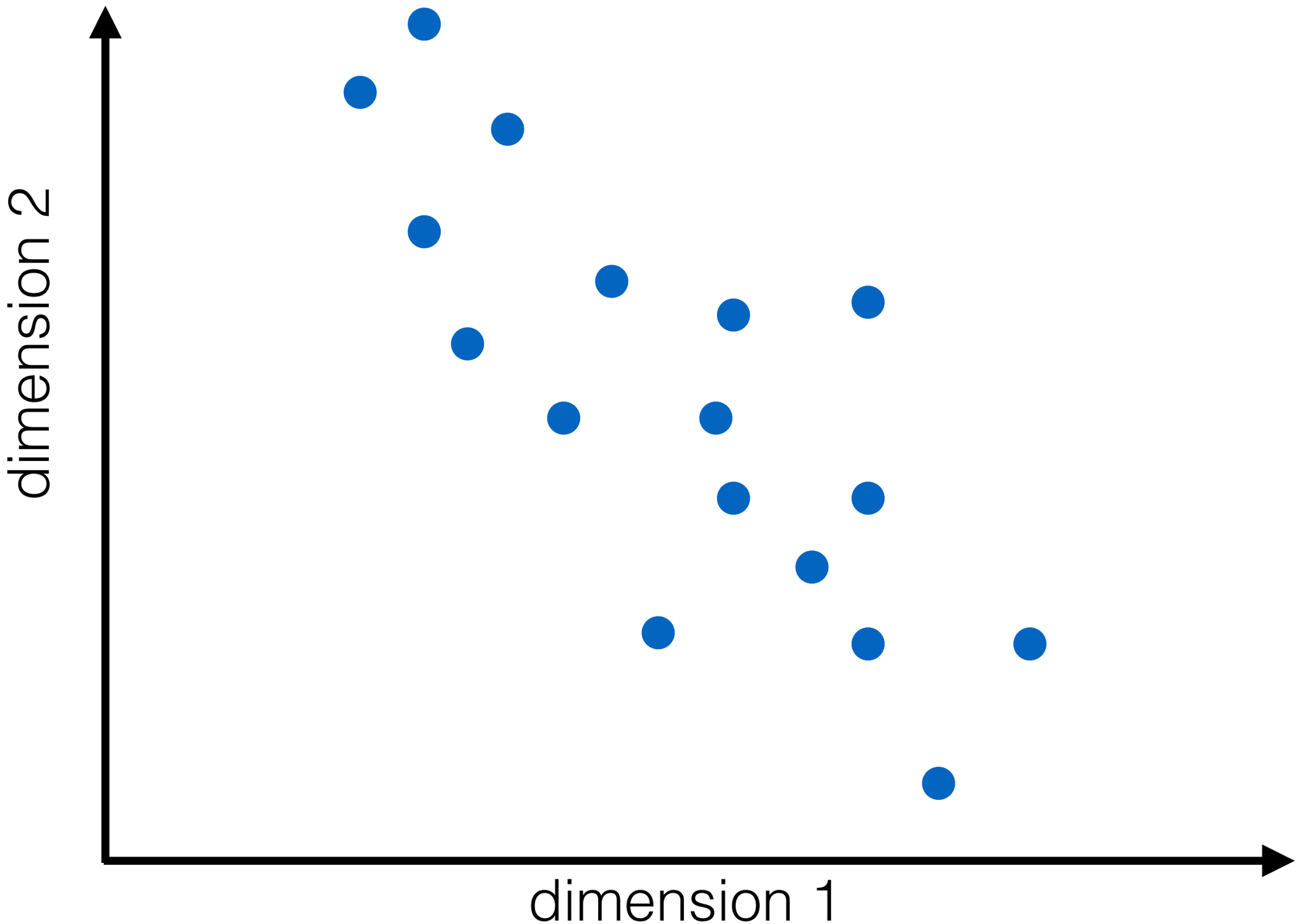
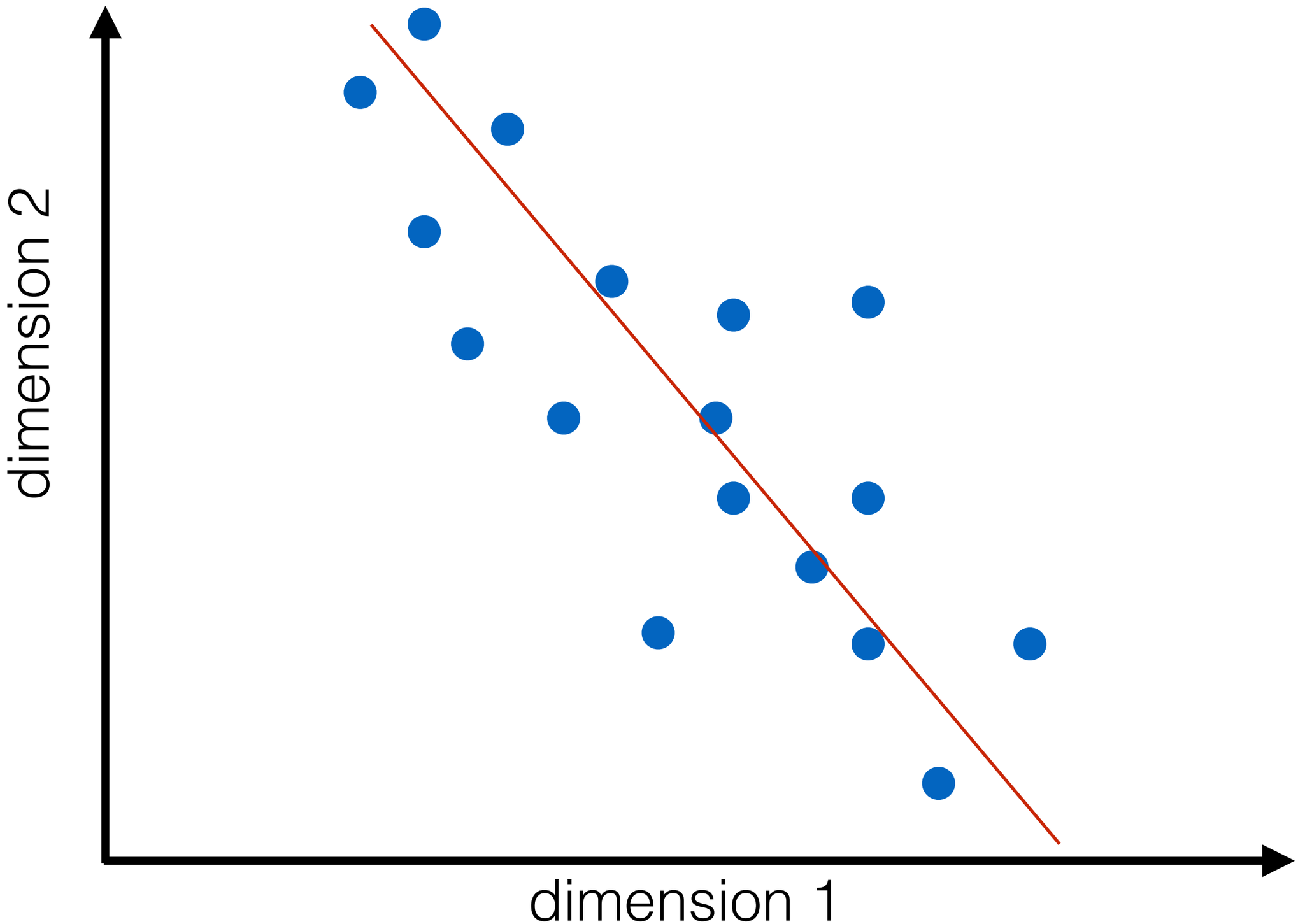
What is PCA?

- Principal Component Analysis: an orthogonal transformation of a set of observations of correlated variables into a set of values of linearly uncorrelated variables
- A technique for dimensionality reduction
- A technique for extracting the principal axes of variation in a dataset

Finding the best **orthogonal** axes of variation



Finding the best **orthogonal** axes of variation



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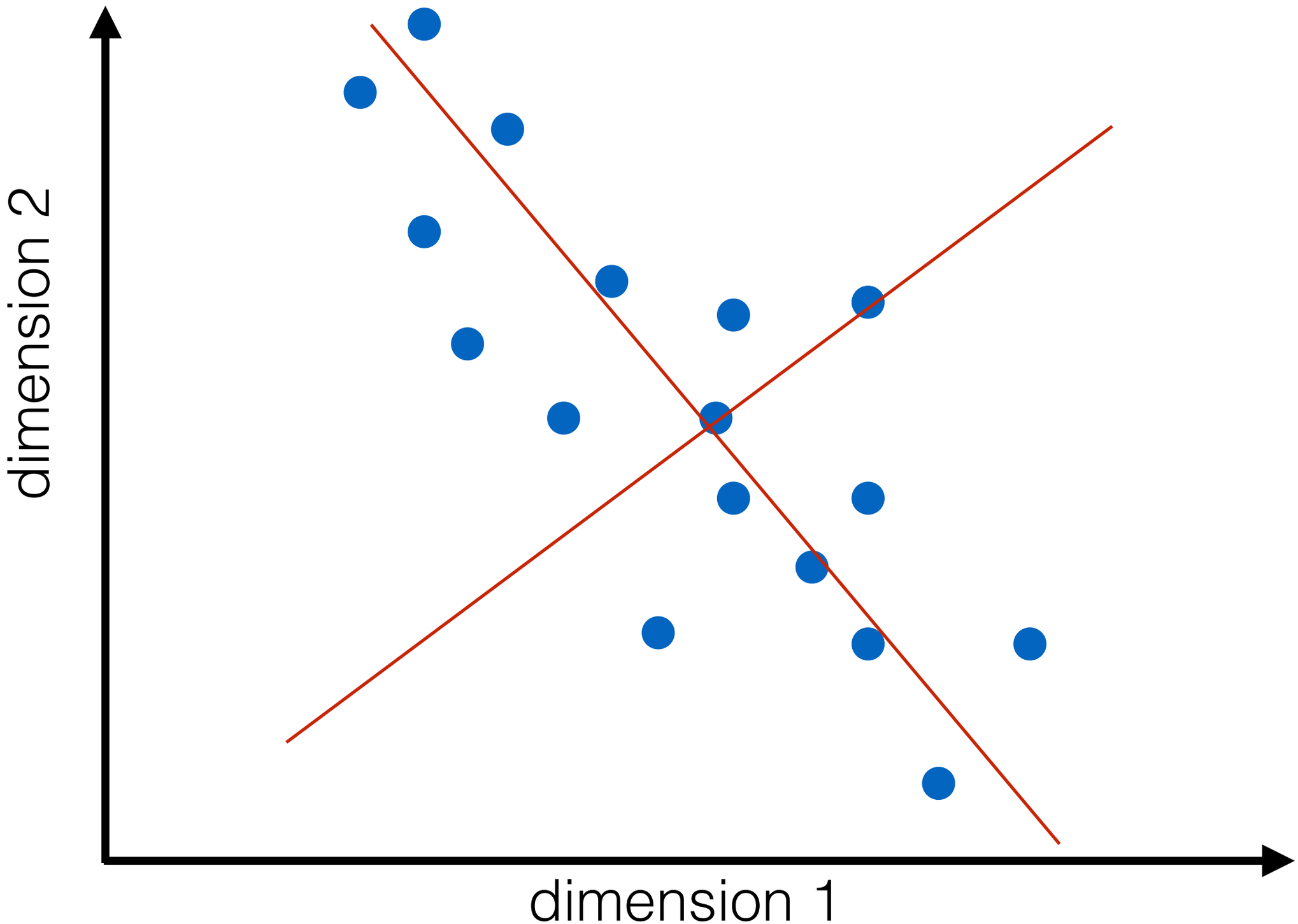
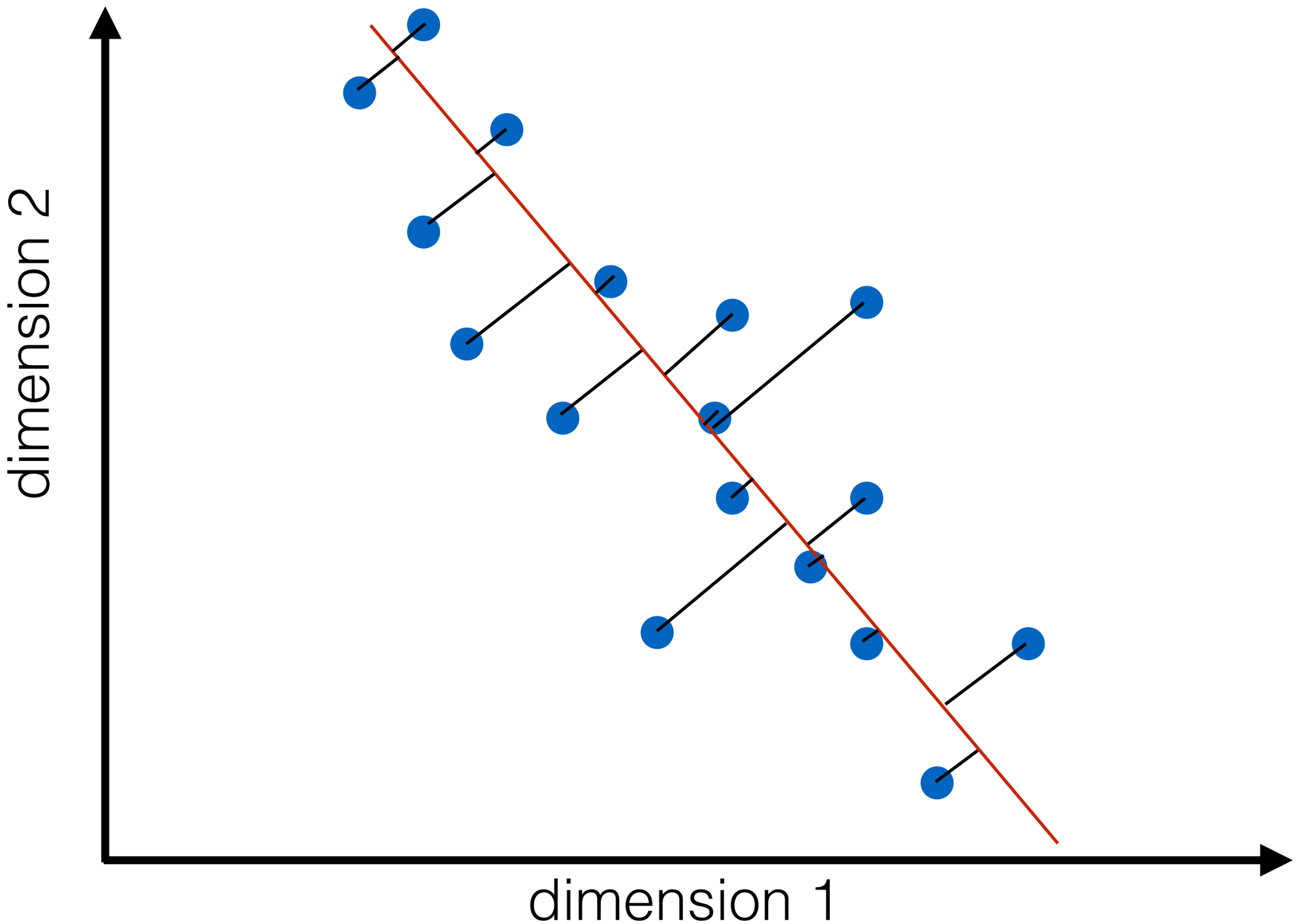


Projecting data onto **orthogonal** axes



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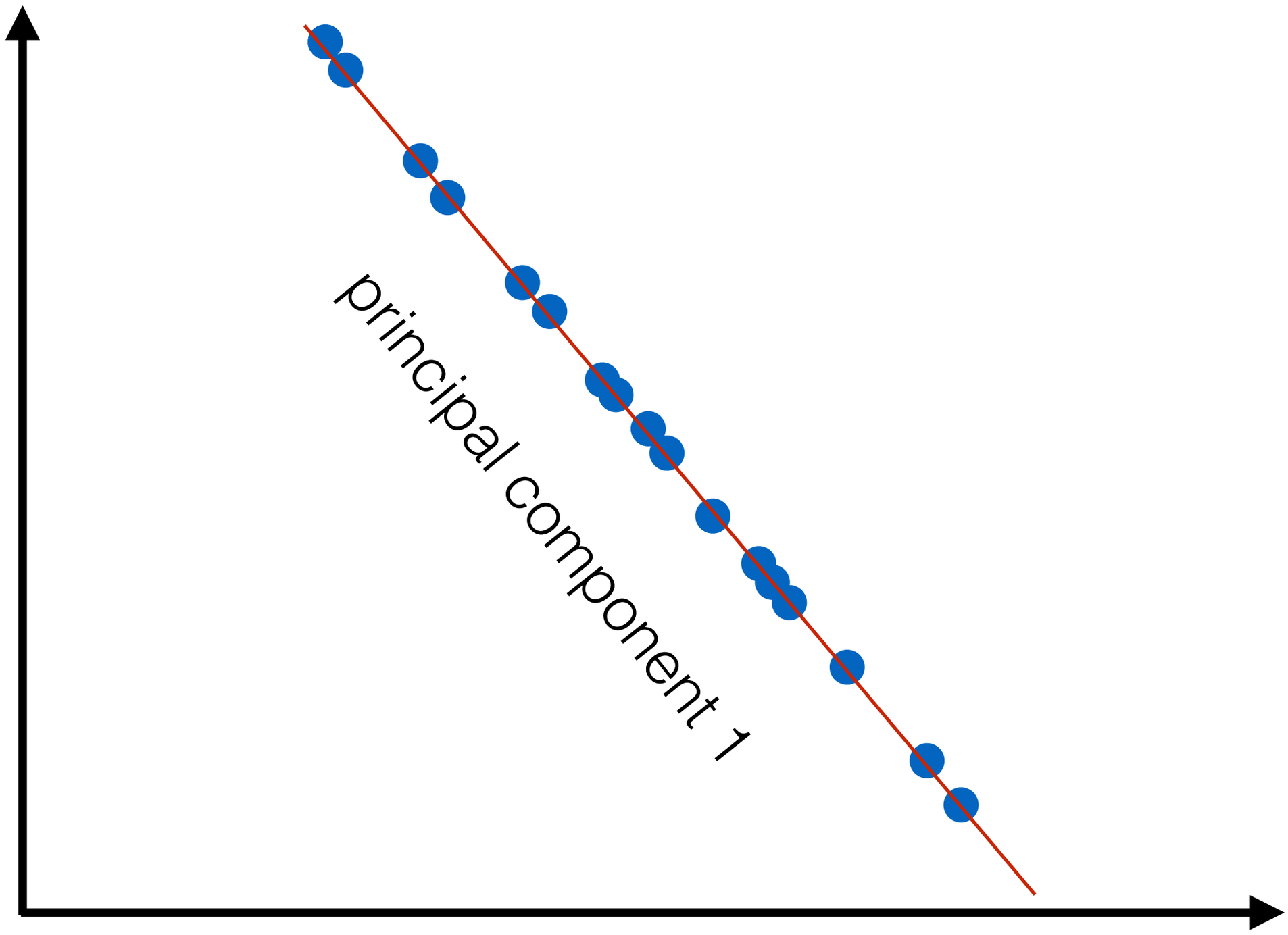

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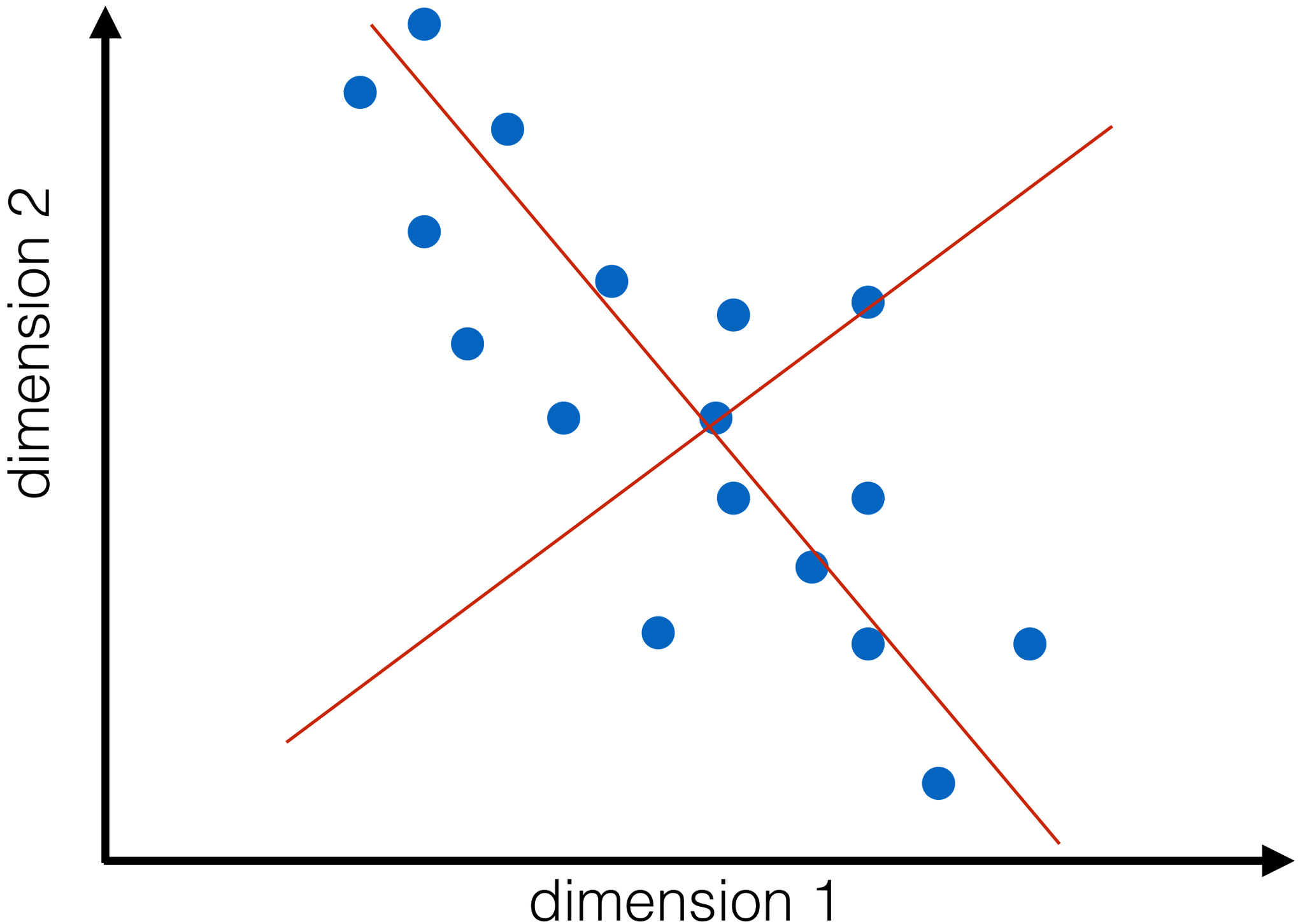

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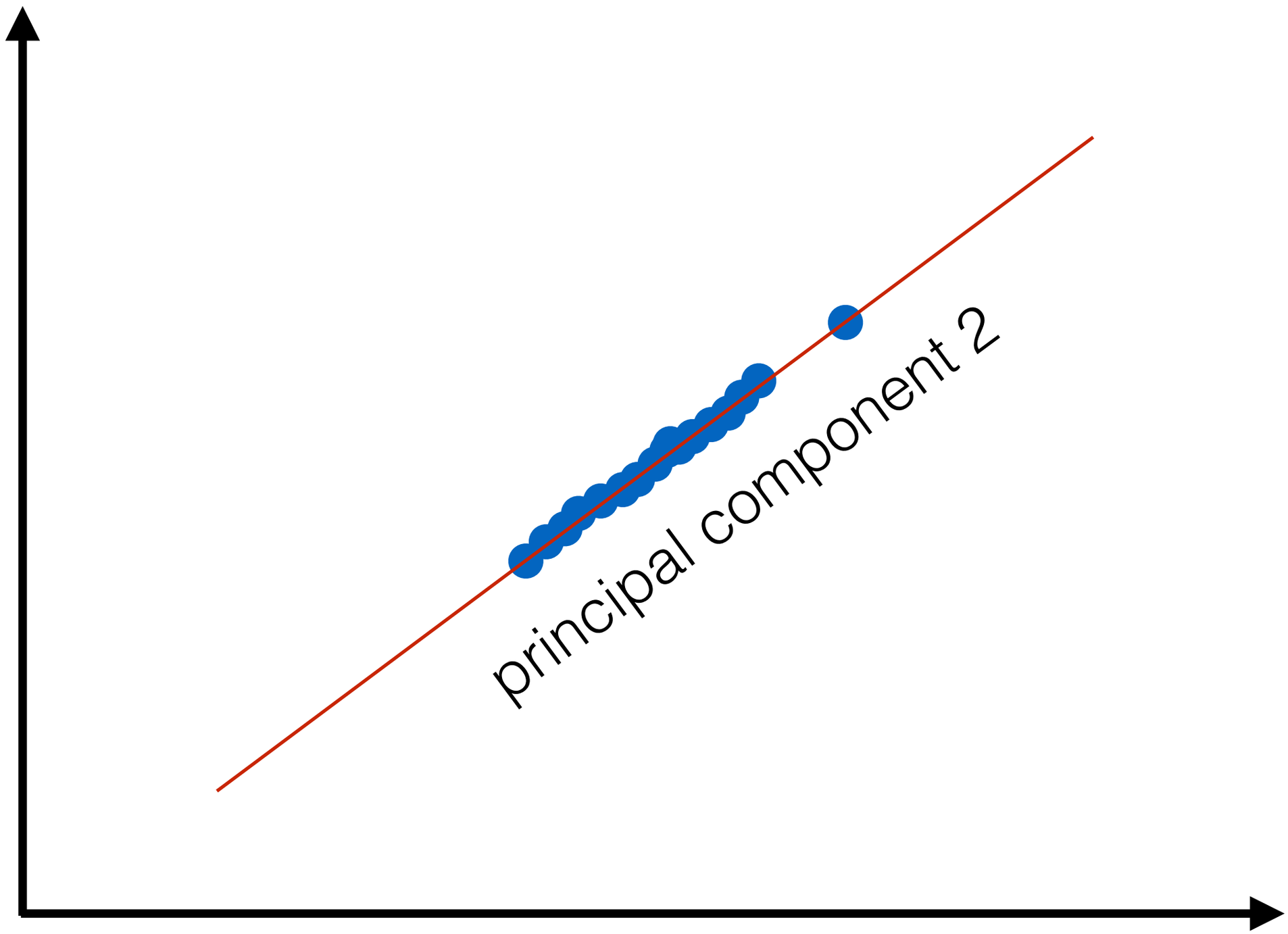

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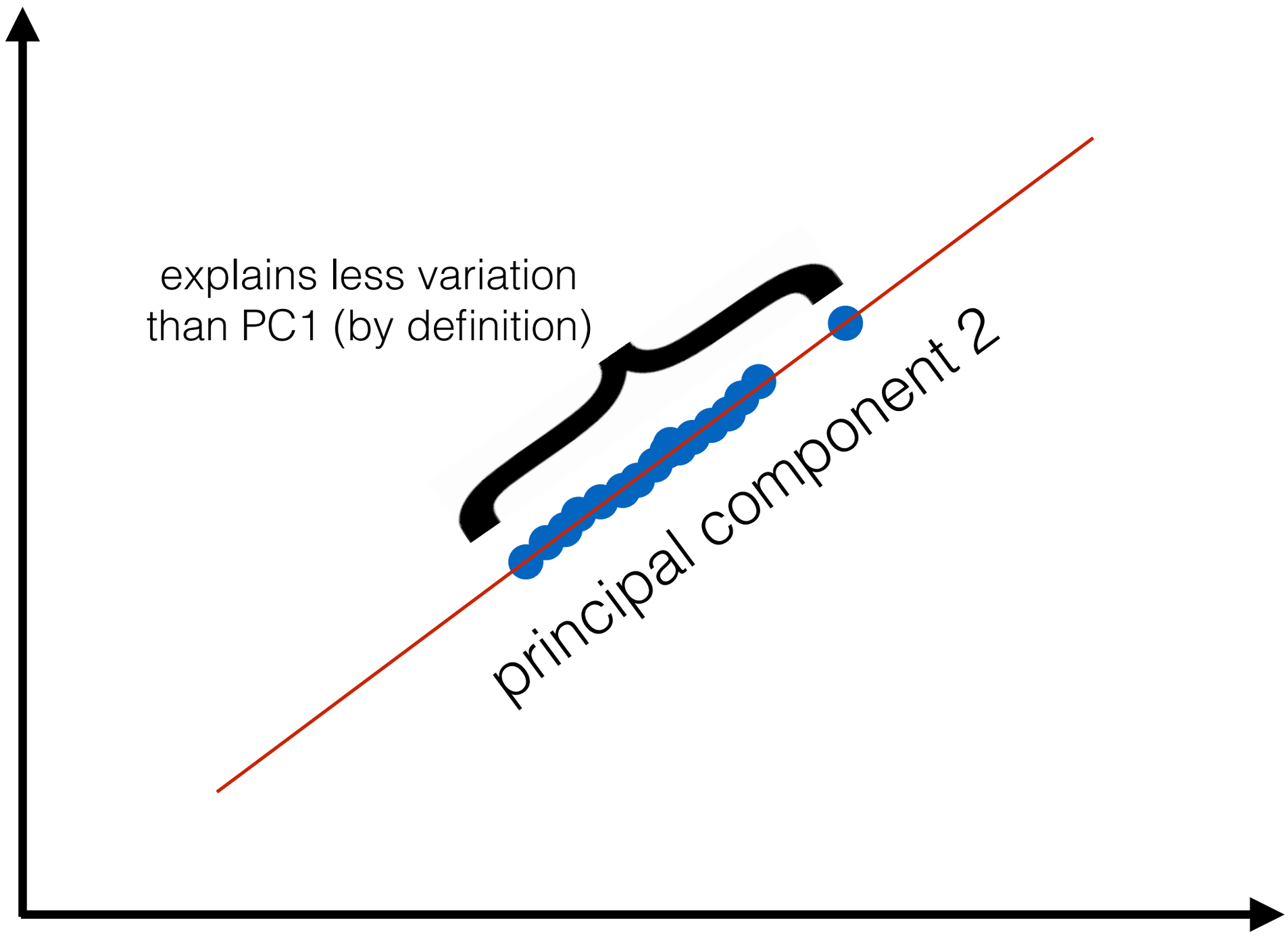

Genotype data are multi-dimensional

- Each SNP is a dimension!

M individuals

N SNPs

$$
\begin{bmatrix}
1 & 1 & 1 & 0 & 0 \\
0 & 1 & 2 & 1 & 2 \\
2 & 1 & 1 & 0 & 1 \\
0 & 0 & 1 & 2 & 2 \\
2 & 1 & 1 & 0 & 0 \\
0 & 0 & 1 & 1 & 1 \\
2 & 2 & 1 & 1 & 0
\end{bmatrix}
\xrightarrow[\text{each SNP}]{\text{Mean-center}}
\begin{bmatrix}
0.4 & 0.4 & 0.4 & -0.6 & -0.6 \\
-1.2 & -0.2 & 0.8 & -0.2 & 0.8 \\
1.0 & 0.0 & 0.0 & -1.0 & 0.0 \\
-1.0 & -1.0 & 0.0 & 1.0 & 1.0 \\
1.2 & 0.2 & 0.2 & -0.8 & -0.8 \\
-0.6 & -0.6 & 0.4 & 0.4 & 0.4 \\
0.8 & 0.8 & -0.2 & -0.2 & -1.2
\end{bmatrix}
= \mathbf{X}
$$

Solution: eigen-decomposition of covariance matrix

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1) Multiply **X** by itself: $\mathbf{X}^T\mathbf{X} = \mathbf{C} \longrightarrow$ covariance matrix (M x M)

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

$$\begin{bmatrix} V_a & C_{a,b} & C_{a,c} & C_{a,d} & C_{a,e} \\ C_{a,b} & V_b & C_{b,c} & C_{b,d} & C_{b,e} \\ C_{a,c} & C_{b,c} & V_c & C_{c,d} & C_{c,e} \\ C_{a,d} & C_{b,d} & C_{c,d} & V_d & C_{d,e} \\ C_{a,e} & C_{b,e} & C_{c,e} & C_{d,e} & V_e \end{bmatrix}$$

Solution: eigen-decomposition of covariance matrix

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2) Eigen-decompose **C**: $\mathbf{C} = \mathbf{V}\mathbf{D}\mathbf{V}^T$

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

2) Eigen-decompose **C**: $\mathbf{C = V D V^T}$

$$\mathbf{X} = \begin{matrix} 2 & -2 \\ 1 & -1 \\ 0 & 0 \\ -1 & 1 \\ -2 & 2 \end{matrix} \qquad \mathbf{C} = \begin{matrix} 10 & -10 \\ -10 & 10 \end{matrix} = \underbrace{\begin{bmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}} \underbrace{\begin{bmatrix} 20 & 0 \\ 0 & 0 \end{bmatrix}}_{\mathbf{D}} \underbrace{\begin{bmatrix} 1/\sqrt{2} & -1/\sqrt{2} \\ 1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V^T}}$$

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3) Columns of V are the eigenvectors of C (PCs): $\mathbf{C} * \mathbf{V_1} = D_{1,1} * \mathbf{V_1}$

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

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4) Diagonal entries of D are the eigenvalues of C

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

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$$\mathbf{X} = \begin{matrix} 2 & -2 \\ 1 & -1 \\ 0 & 0 \\ -1 & 1 \\ -2 & 2 \end{matrix} \qquad \mathbf{C} = \begin{matrix} 10 & -10 \\ -10 & 10 \end{matrix} = \underbrace{\begin{bmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}} \underbrace{\begin{bmatrix} 20 & 0 \\ 0 & 0 \end{bmatrix}}_{\mathbf{D}} \underbrace{\begin{bmatrix} 1/\sqrt{2} & -1/\sqrt{2} \\ 1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V^T}}$$

3) Columns of V are the eigenvectors of C (PCs): $\mathbf{C} * \mathbf{V_1} = D_{1,1} * \mathbf{V_1}$

4) Diagonal entries of D are the eigenvalues of C

5) Eigenvectors with largest eigenvalues are top PCs

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

2) Eigen-decompose **C**: $\mathbf{C = V D V^T}$

$$\mathbf{X} = \begin{matrix} 2 & -2 \\ 1 & -1 \\ 0 & 0 \\ -1 & 1 \\ -2 & 2 \end{matrix} \qquad \mathbf{C} = \begin{matrix} 10 & -10 \\ -10 & 10 \end{matrix} = \underbrace{\begin{bmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}} \underbrace{\begin{bmatrix} 20 & 0 \\ 0 & 0 \end{bmatrix}}_{\mathbf{D}} \underbrace{\begin{bmatrix} 1/\sqrt{2} & -1/\sqrt{2} \\ 1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V^T}}$$

PC1

3) Columns of V are the eigenvectors of C (PCs): $\mathbf{C} * \mathbf{V_1} = D_{1,1} * \mathbf{V_1}$

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5) Eigenvectors with largest eigenvalues are top PCs

Solution: eigen-decomposition of covariance matrix

1) Multiply **X** by itself: $\mathbf{X^T X = C} \longrightarrow$ covariance matrix (M x M)

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PC1 **PC2**

3) Columns of V are the eigenvectors of C (PCs): $\mathbf{C} * \mathbf{V_1} = D_{1,1} * \mathbf{V_1}$

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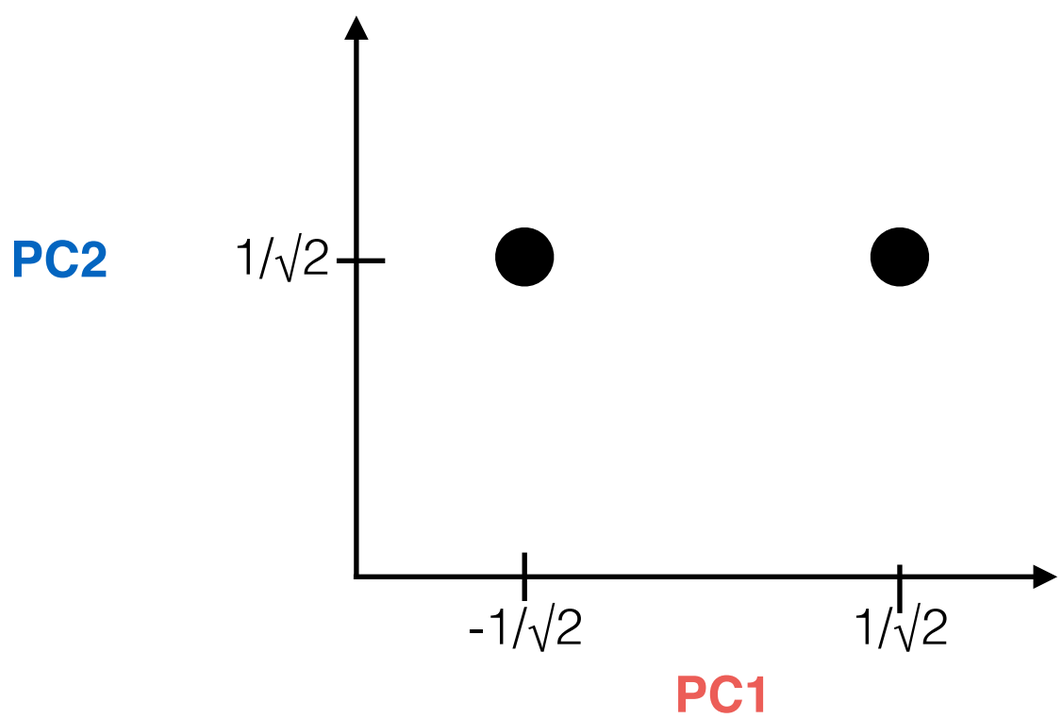
Solution: eigen-decomposition of covariance matrix

$$\mathbf{X} = \begin{matrix} 2 & -2 \\ 1 & -1 \\ 0 & 0 \\ -1 & 1 \\ -2 & 2 \end{matrix} \qquad \mathbf{C} = \begin{matrix} 10 & -10 \\ -10 & 10 \end{matrix} = \underbrace{\begin{bmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}} \underbrace{\begin{bmatrix} 20 & 0 \\ 0 & 0 \end{bmatrix}}_{\mathbf{D}} \underbrace{\begin{bmatrix} 1/\sqrt{2} & -1/\sqrt{2} \\ 1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}^{\mathbf{T}}}$$

PC1 **PC2**

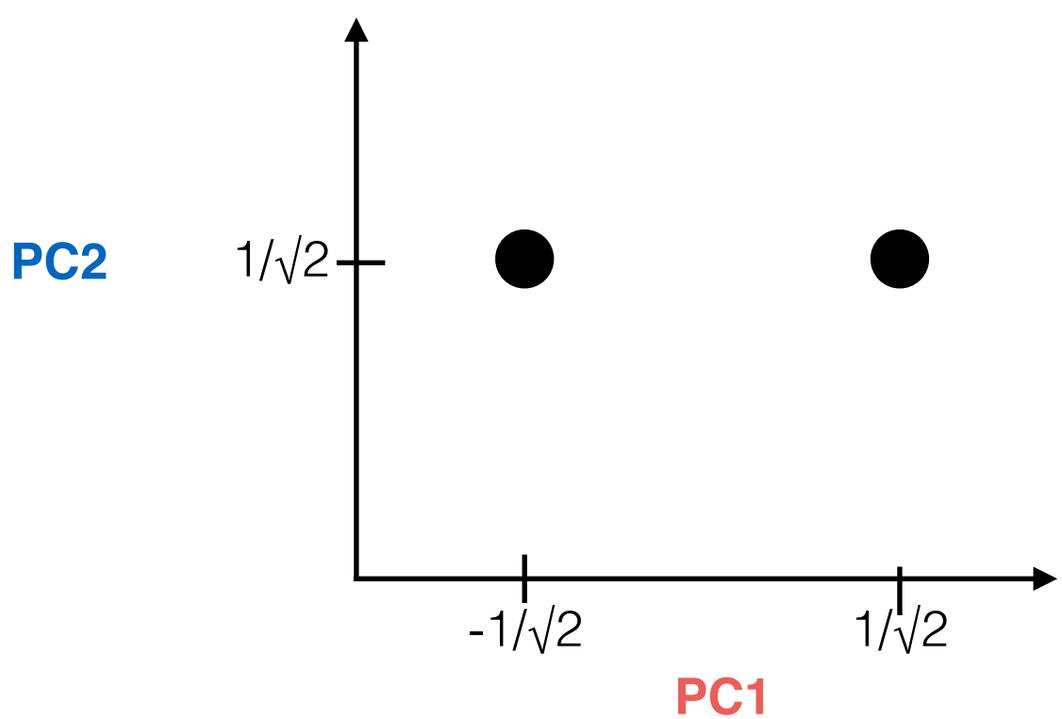


Solution: eigen-decomposition of covariance matrix

$$\mathbf{X} = \begin{matrix} 2 & -2 \\ 1 & -1 \\ 0 & 0 \\ -1 & 1 \\ -2 & 2 \end{matrix} \qquad \mathbf{C} = \begin{matrix} 10 & -10 \\ -10 & 10 \end{matrix} = \underbrace{\begin{bmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}} \underbrace{\begin{bmatrix} 20 & 0 \\ 0 & 0 \end{bmatrix}}_{\mathbf{D}} \underbrace{\begin{bmatrix} 1/\sqrt{2} & -1/\sqrt{2} \\ 1/\sqrt{2} & 1/\sqrt{2} \end{bmatrix}}_{\mathbf{V}^{\mathbf{T}}}$$

PC1 **PC2**

The **relative sizes** of the eigenvalues indicate the **proportion of total variance** each eigenvector explains



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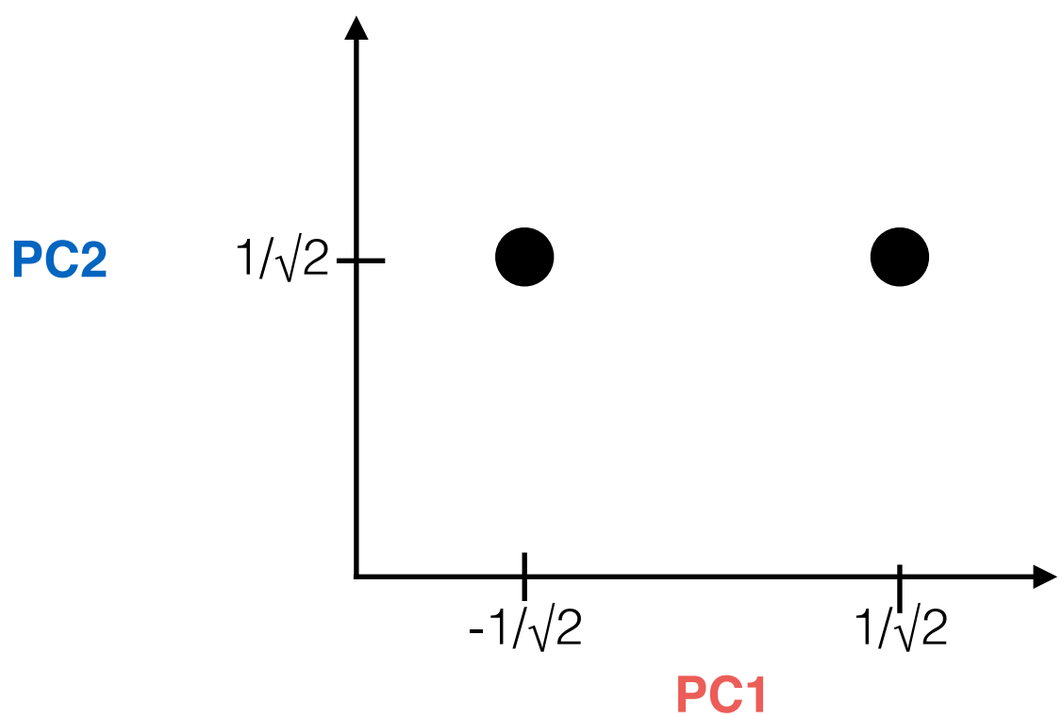
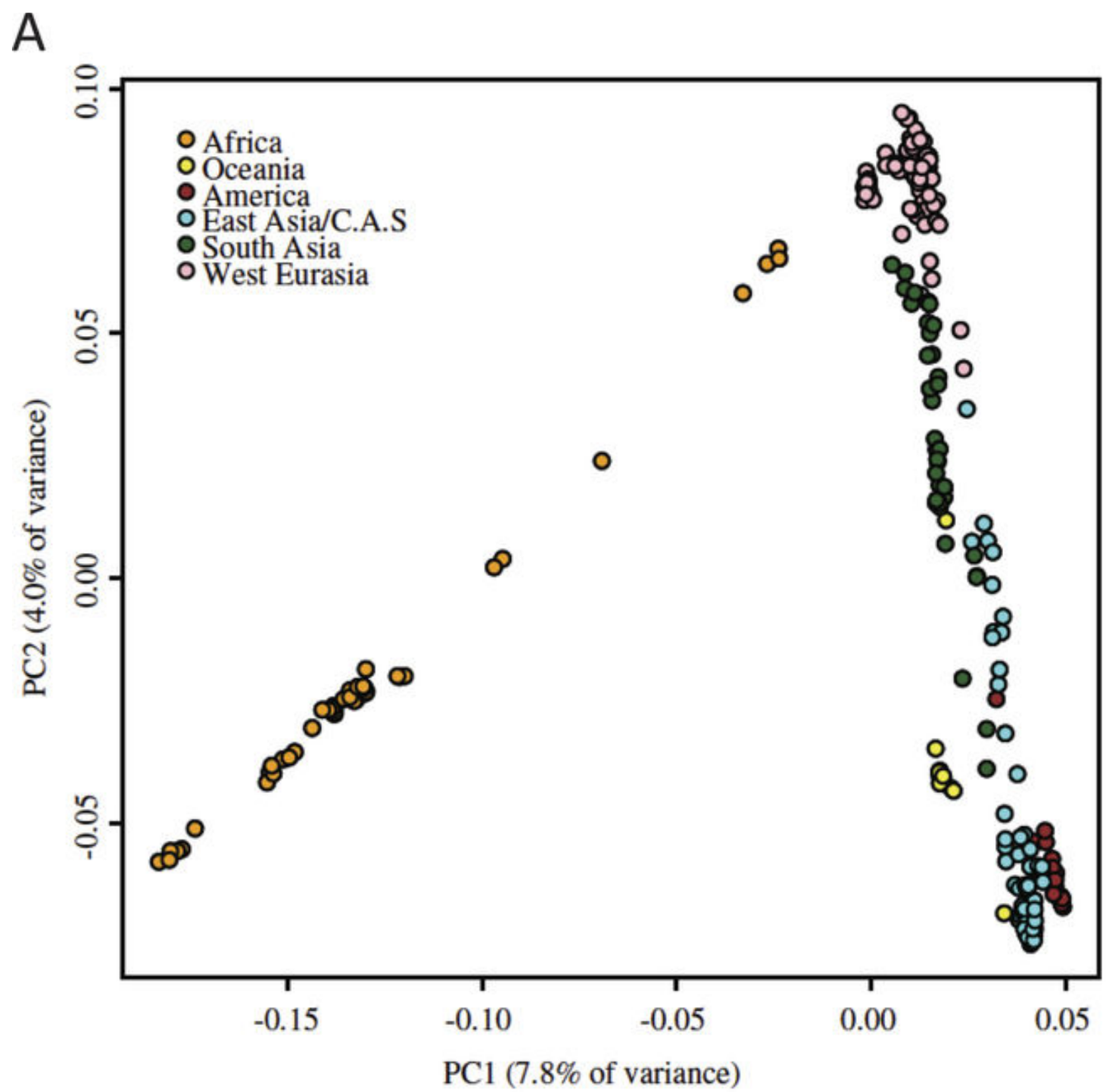
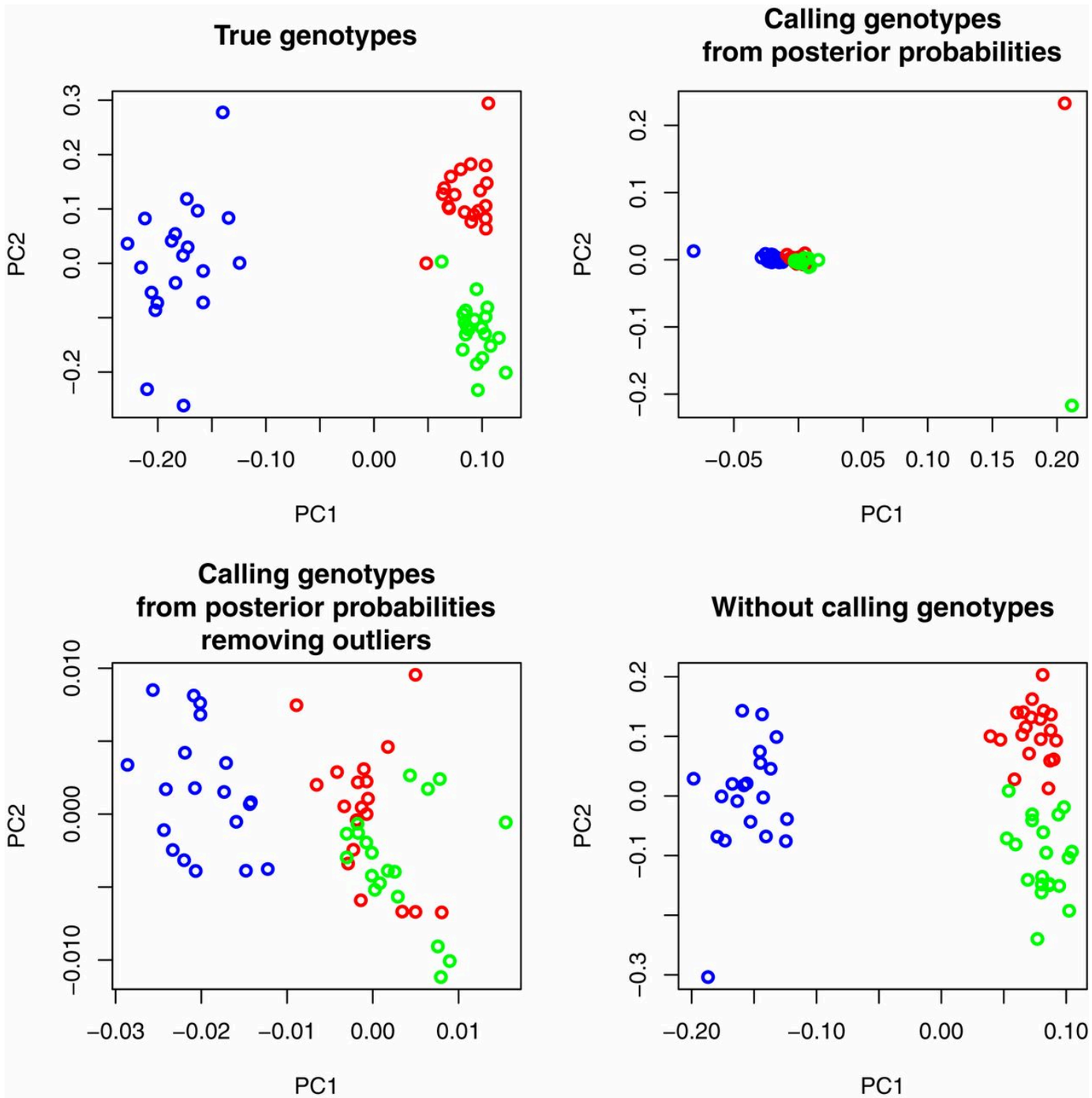


In this toy example, PC1 explains ALL of the variance

PCA of worldwide human genomes



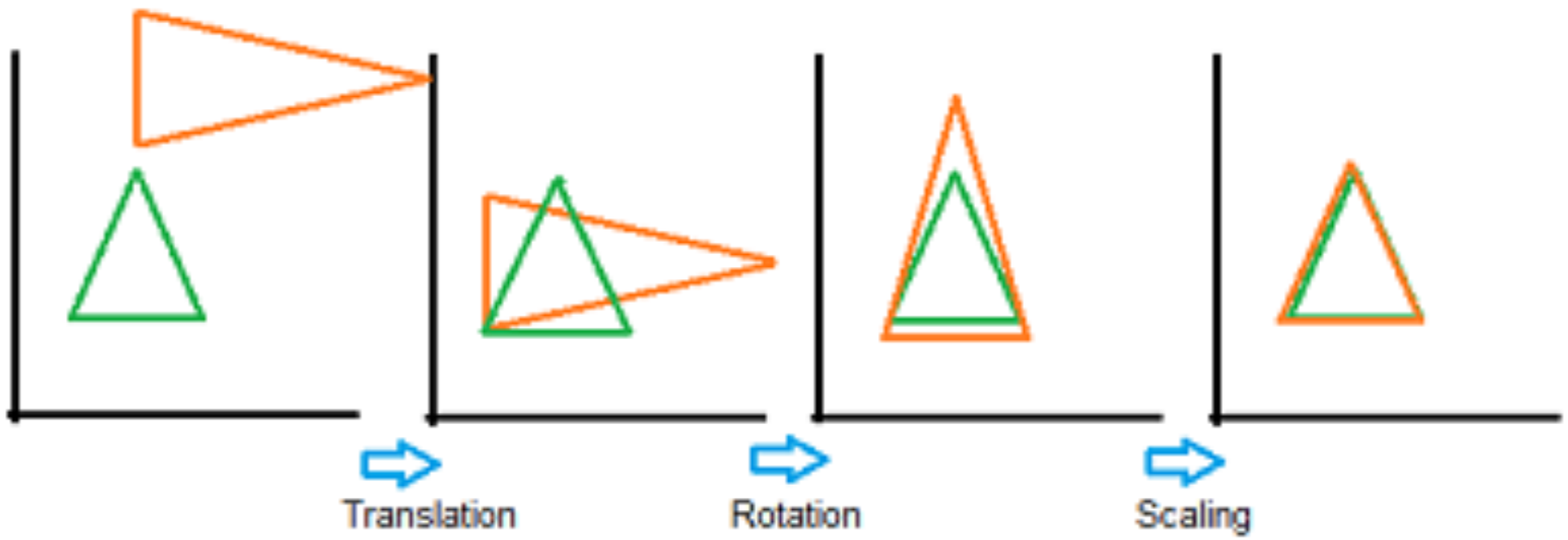
PCA from genotype likelihoods



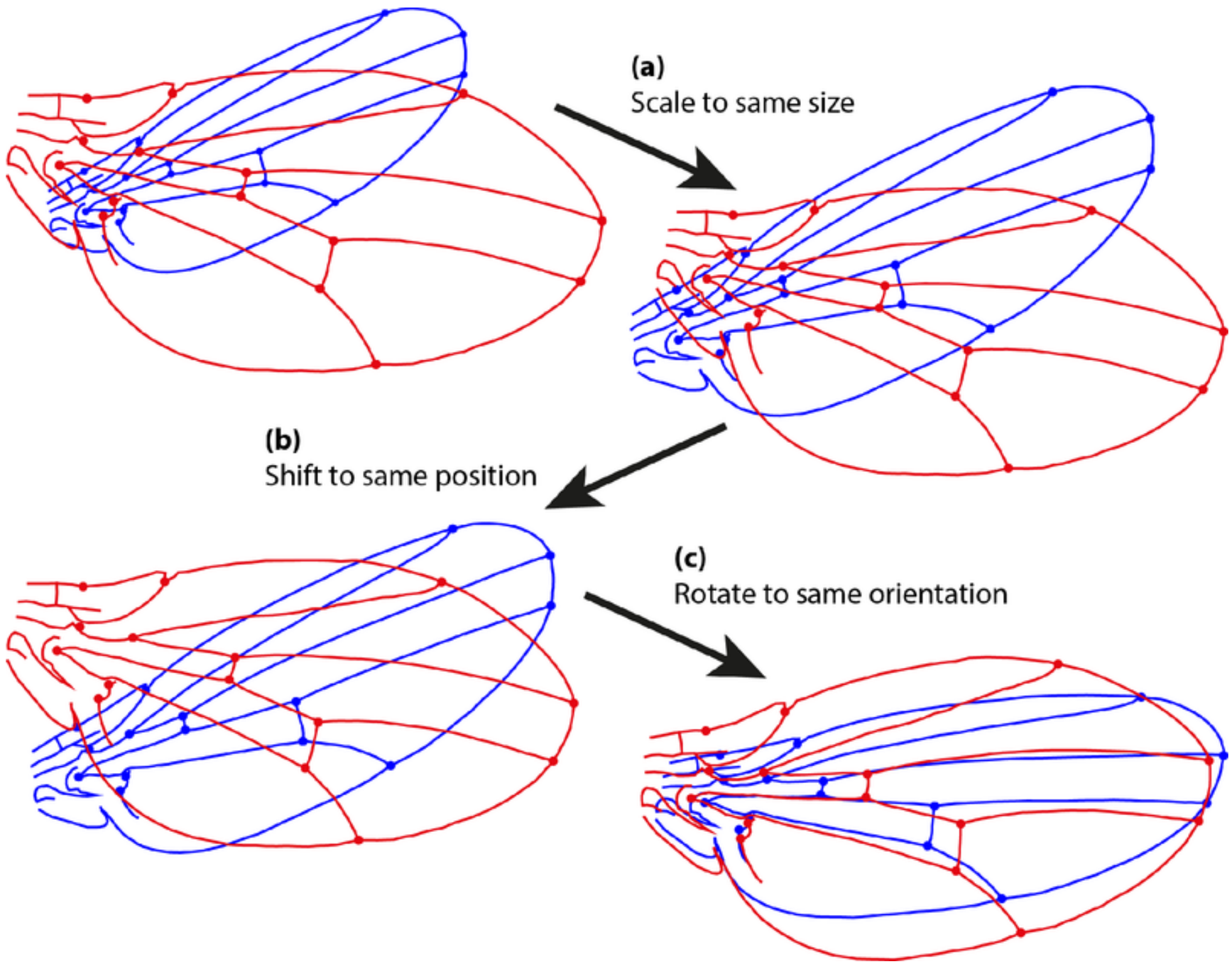
Dealing with missing data: Procrustes transformation

- SNPs in which at least 1 sample has missing data are unusable in a PCA
- Problem: low coverage genomes -> many sites with missing data
- Even bigger problem: combination of many low-coverage genomes -> very few sites with overlap in coverage across all of them
- Solution (Skoglund et al. 2012):
    - For each low-coverage genome, run 1 PCA (with many high-coverage genomes included)
    - Combine loadings from each individual PCA into an overall-PCA, using Procrustes transformation

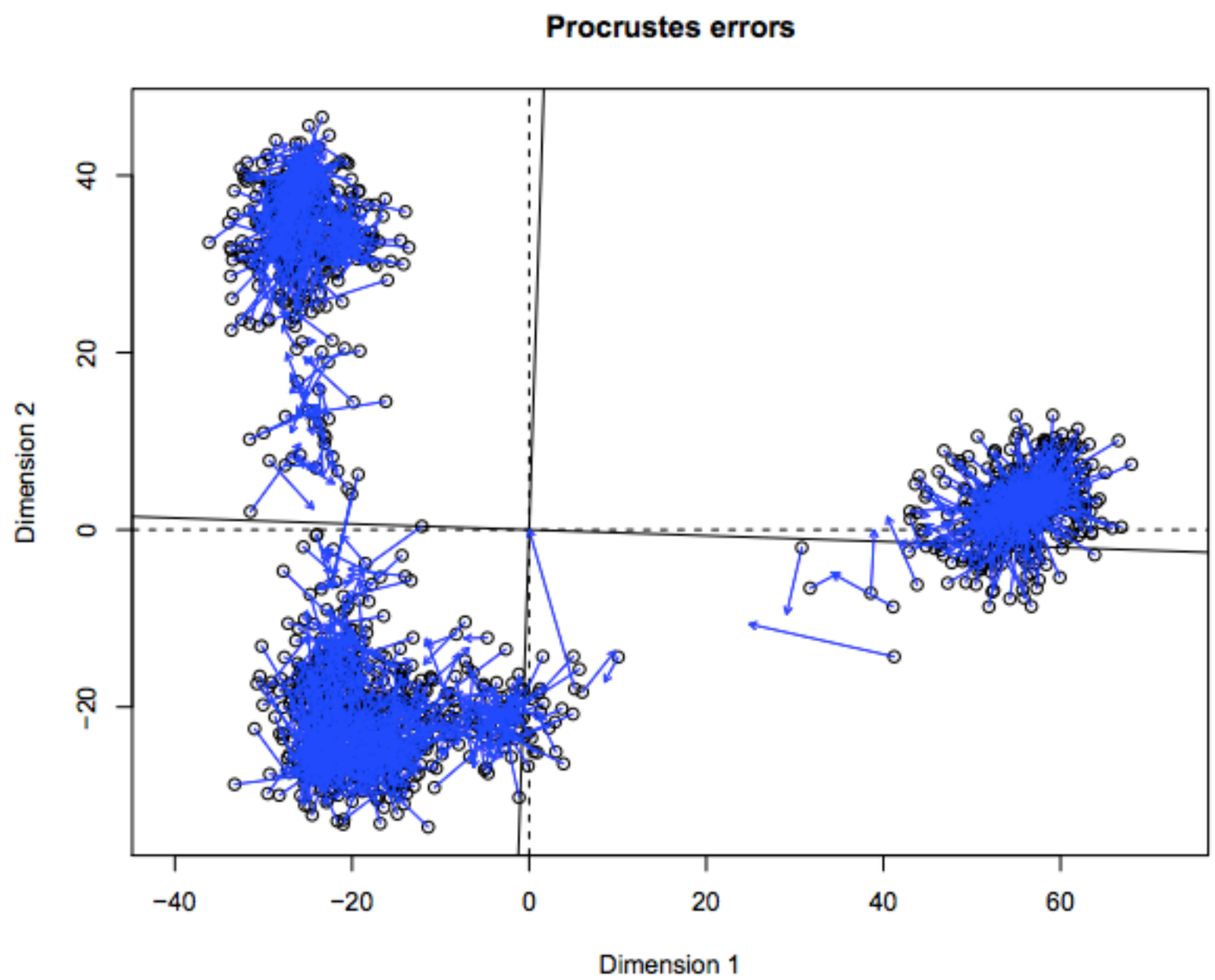
Shape-preserving Procrustes transformation



Shape-preserving Procrustes transformation

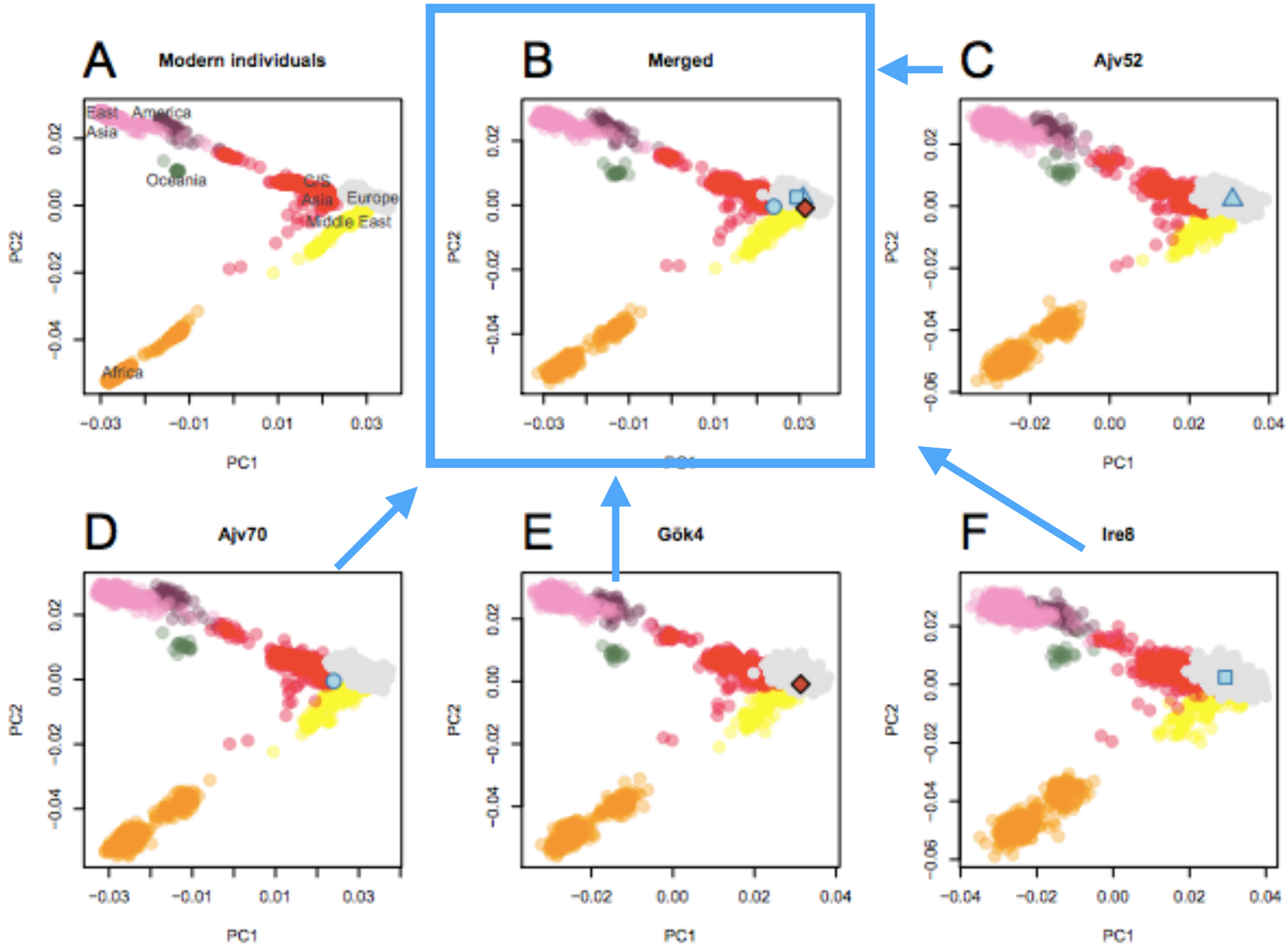


Use a Procrustes transformation using a **high-coverage reference PCA**



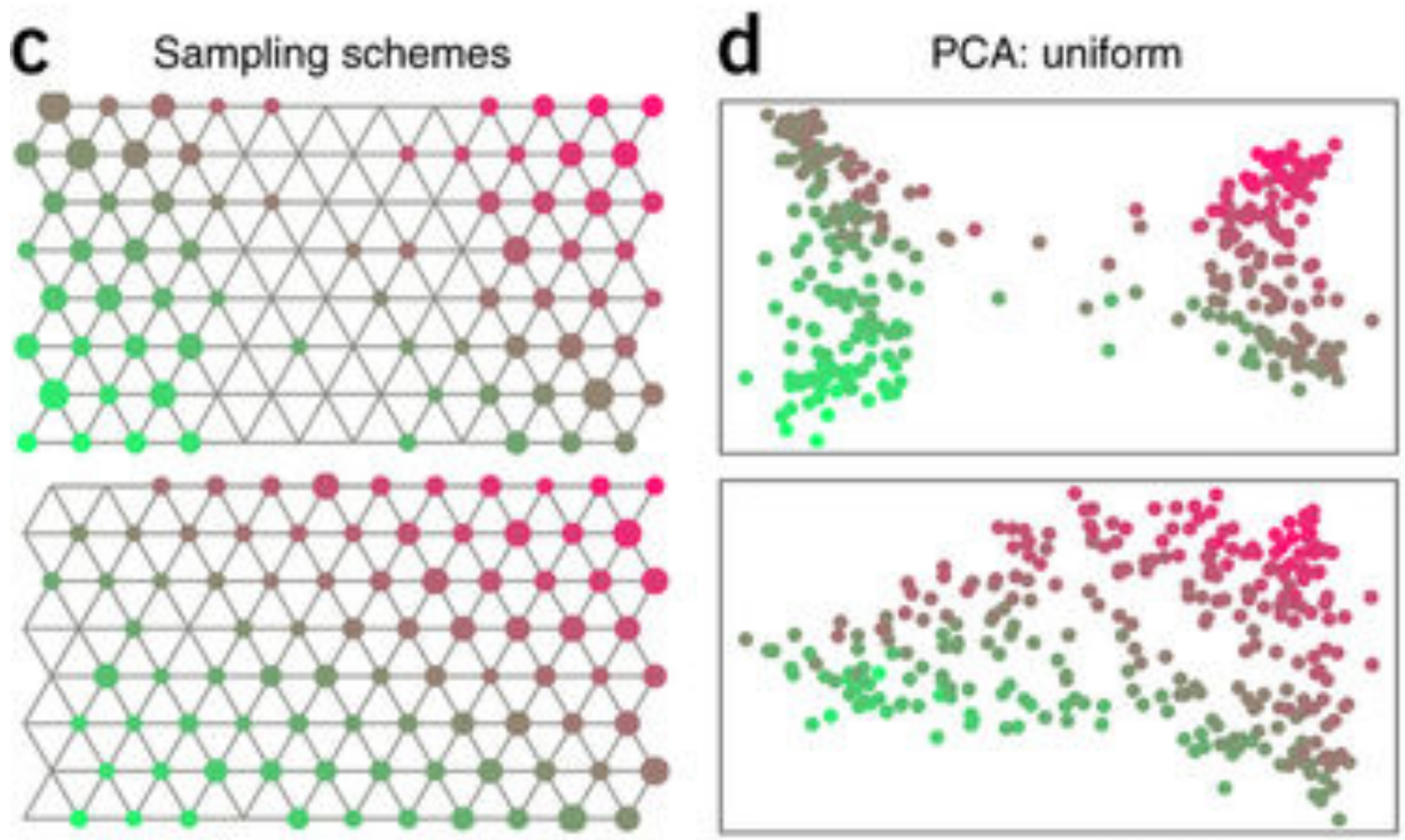
Skoglund et al. 2012

Procrustes transformation

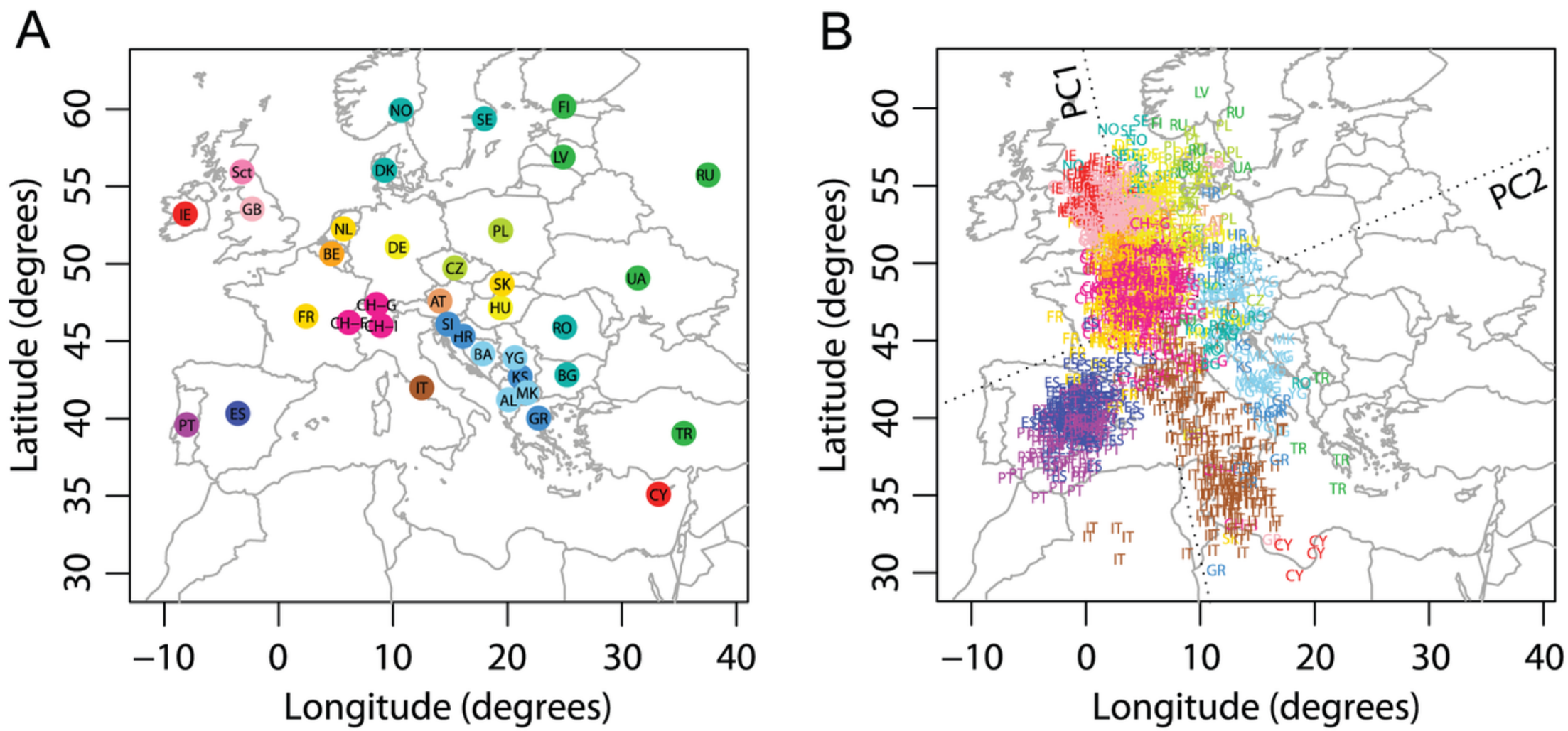


Skoglund et al. 2012

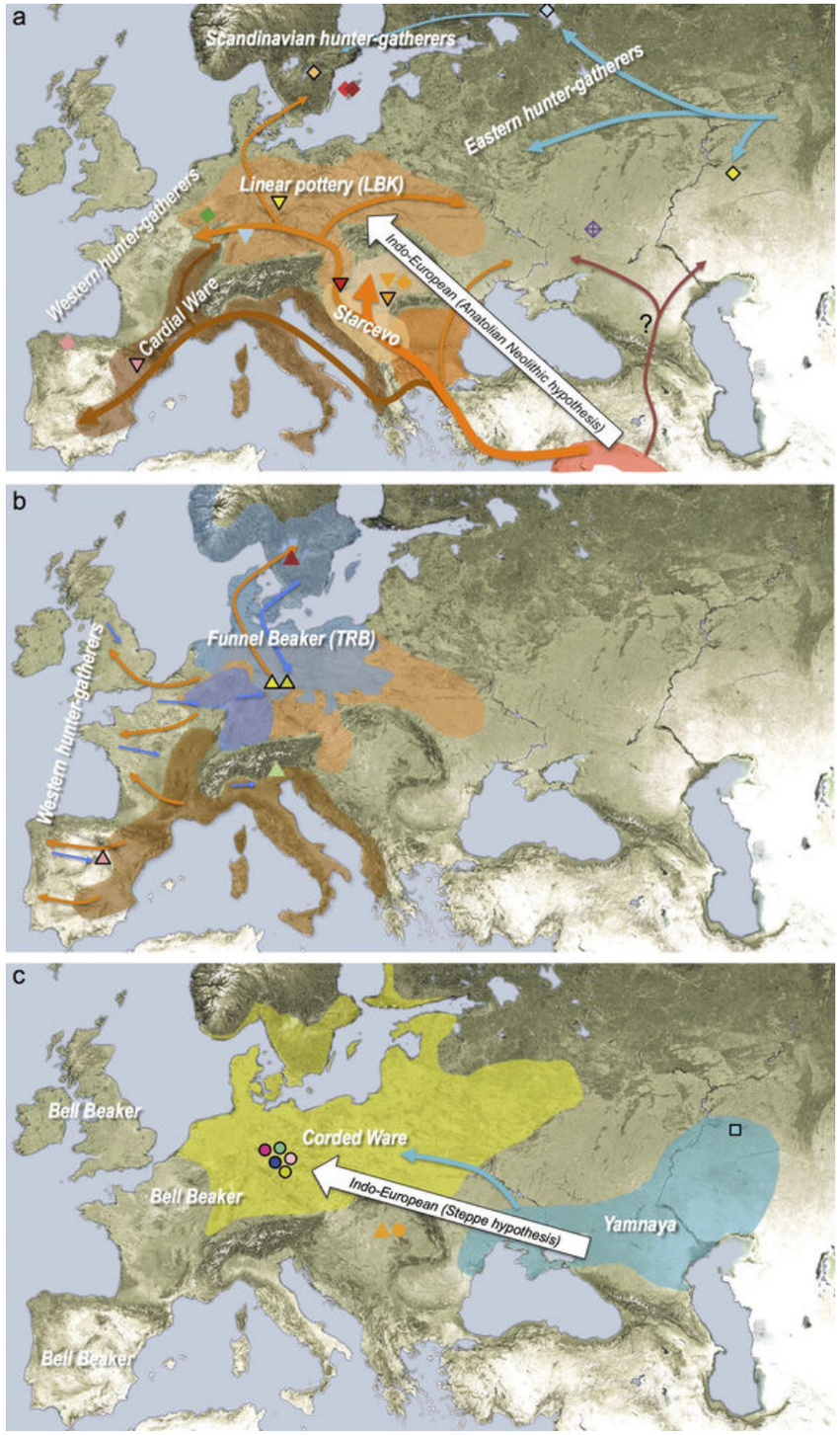
Sampling scheme can be misleading



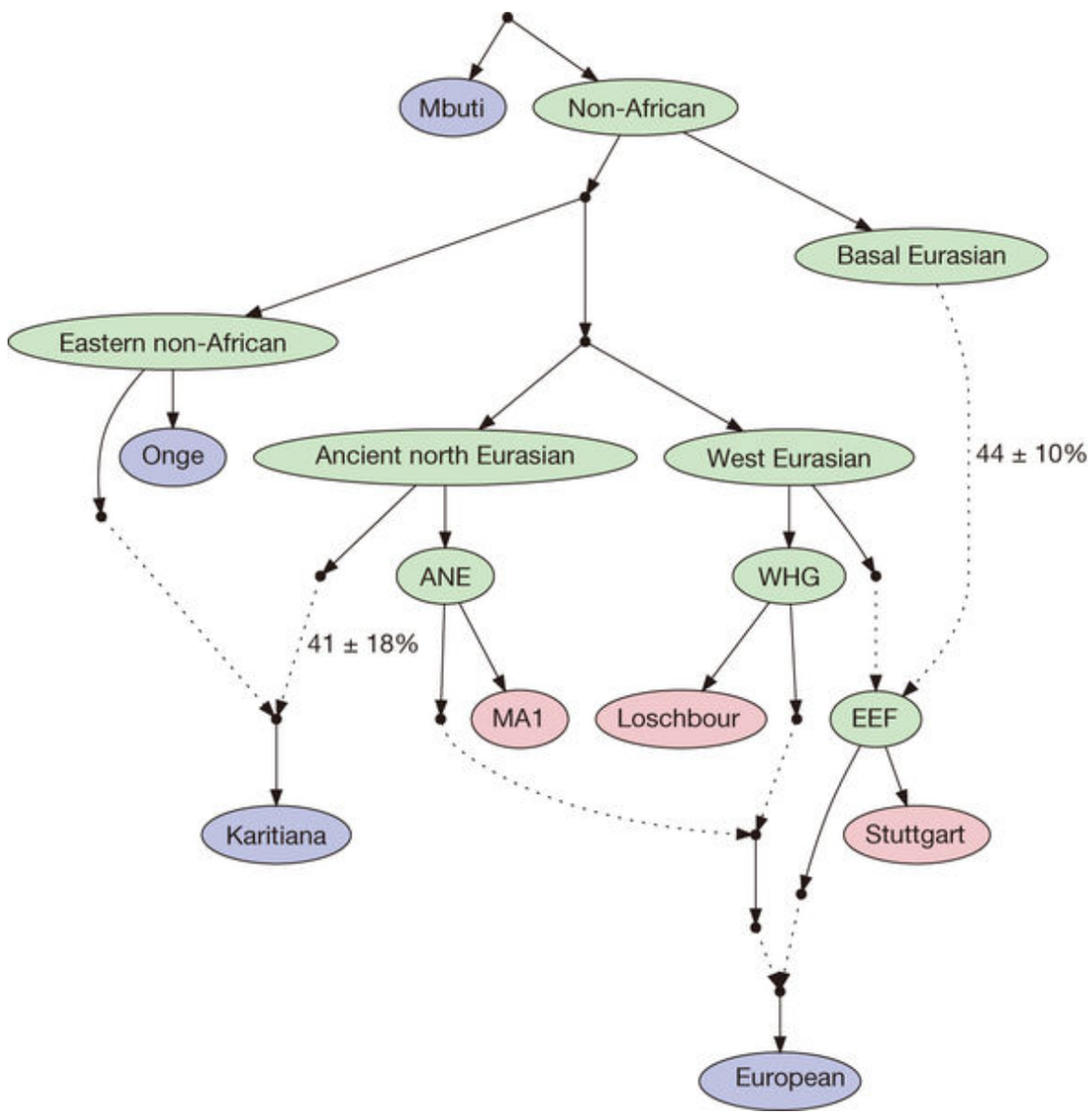
PCA can be misinterpreted!



PCA can be misinterpreted!



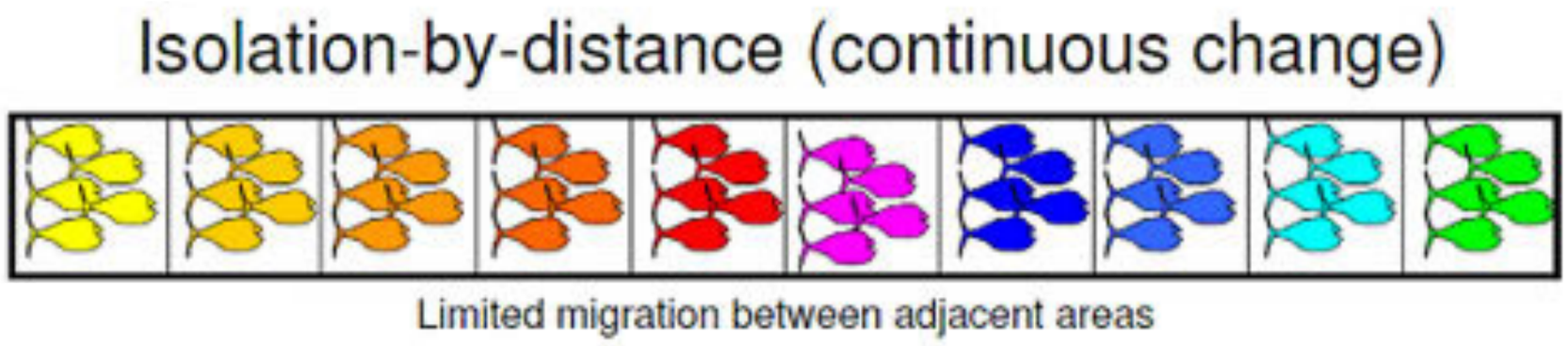
PCA can be misinterpreted!



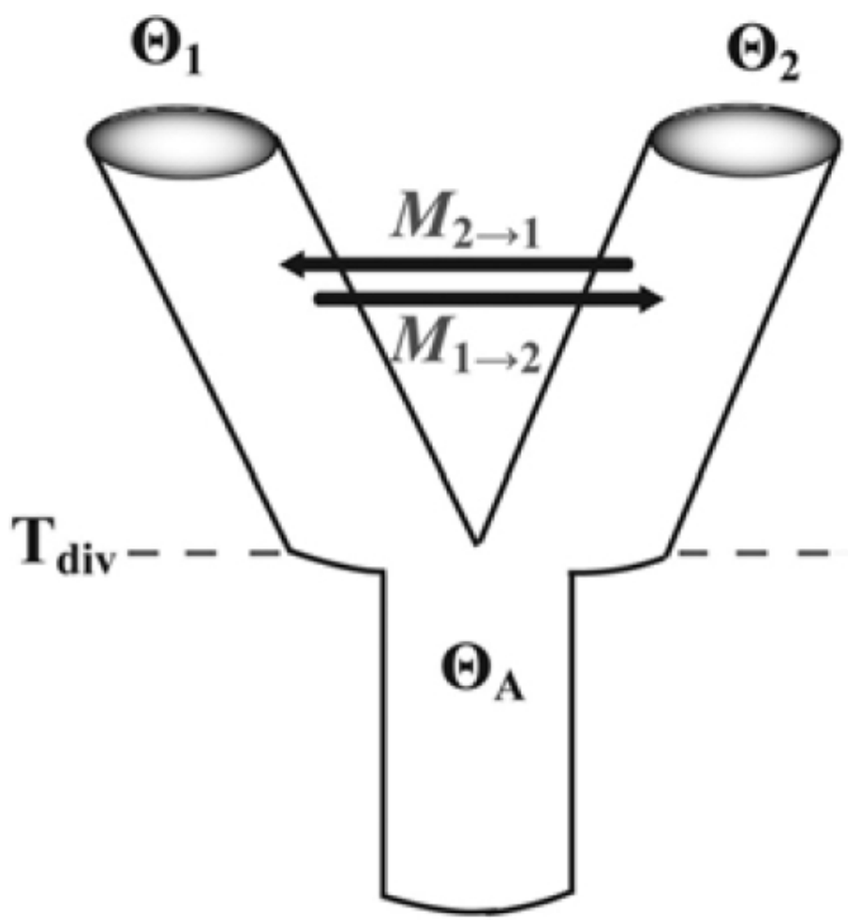
Today

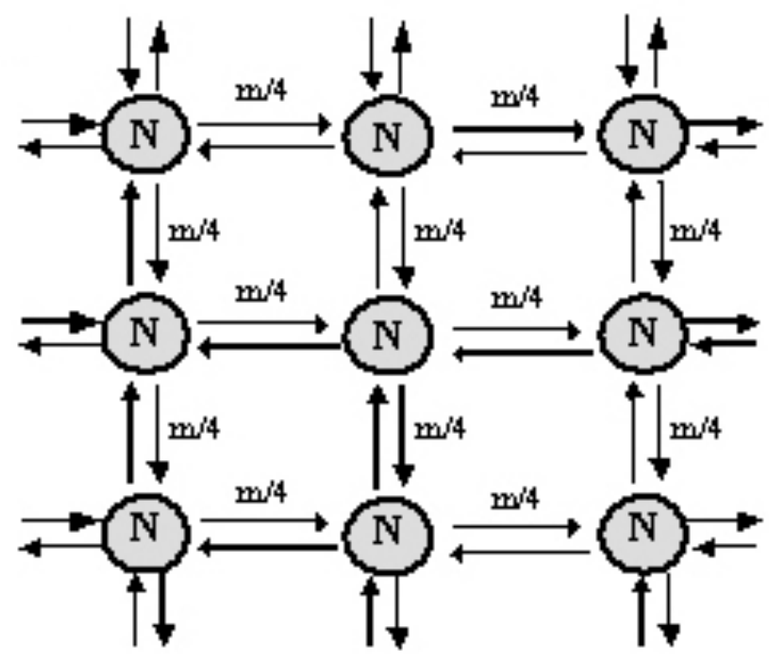
- Experimental design
- Data handling
- PCA
- **Spatial and isolation-by-distance methods**

Isolation-by-distance

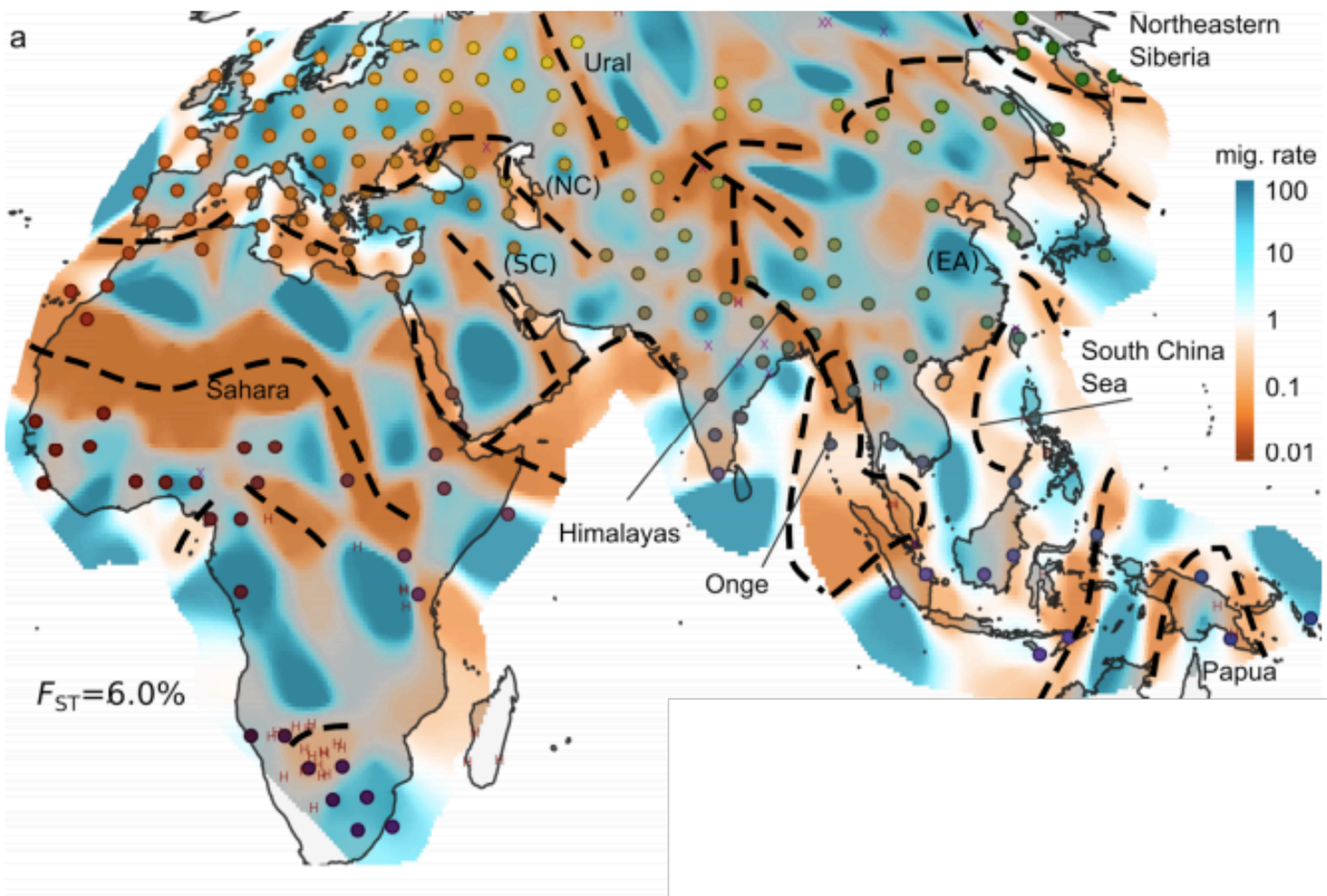


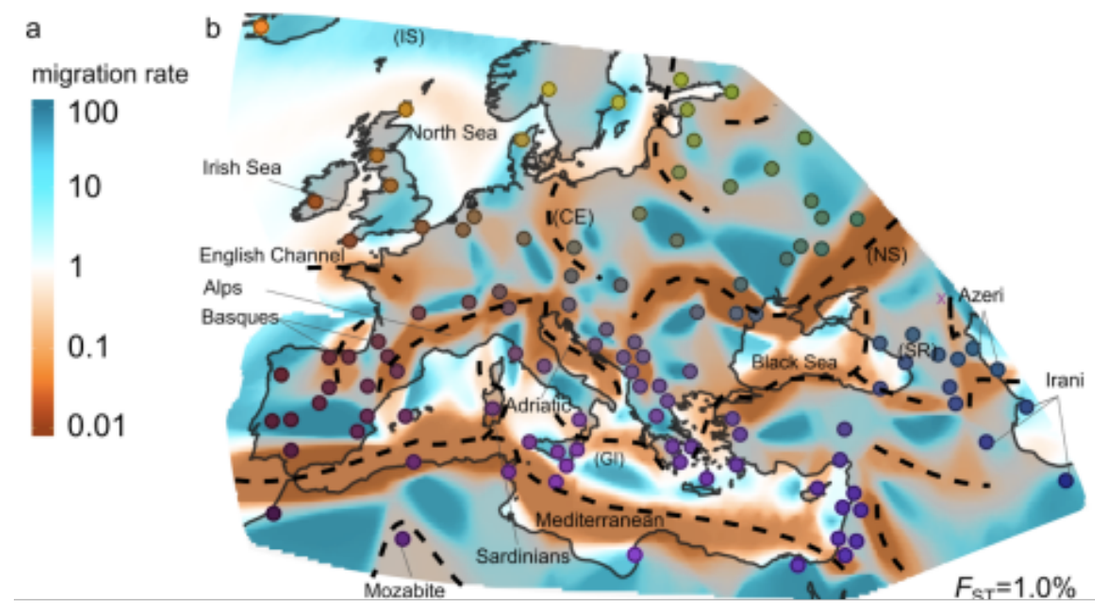
Long-range admixture vs. isolation-by-distance





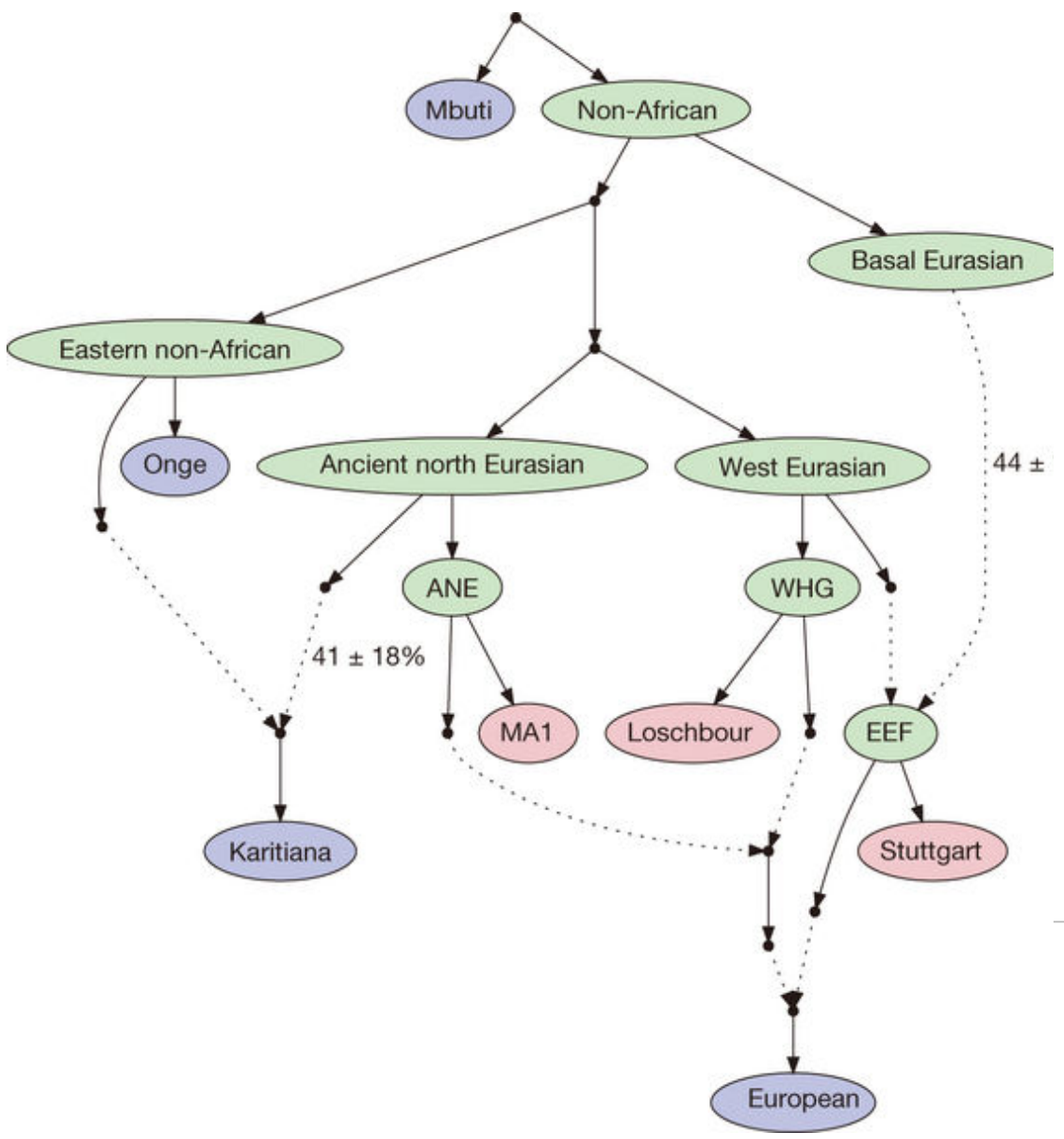
EEMS: a method to model isolation-by-distance

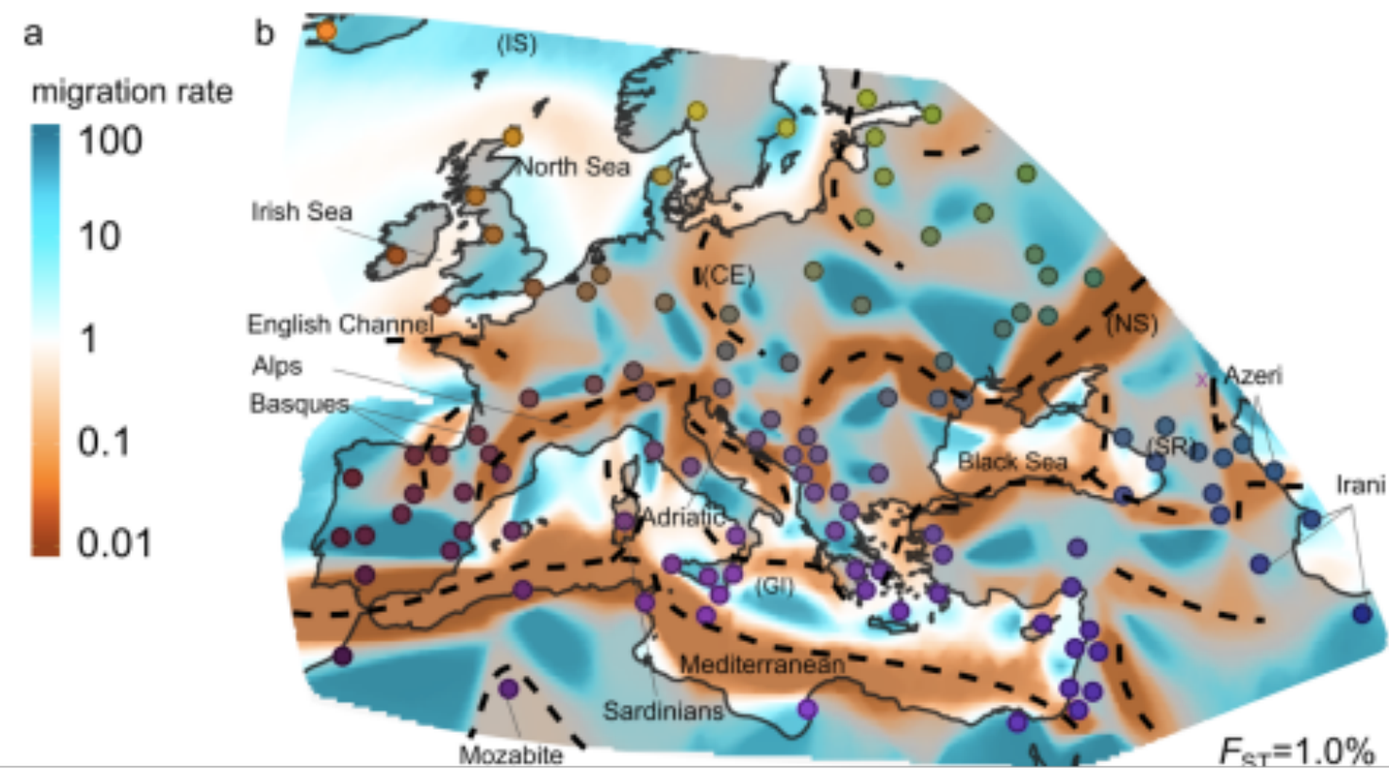




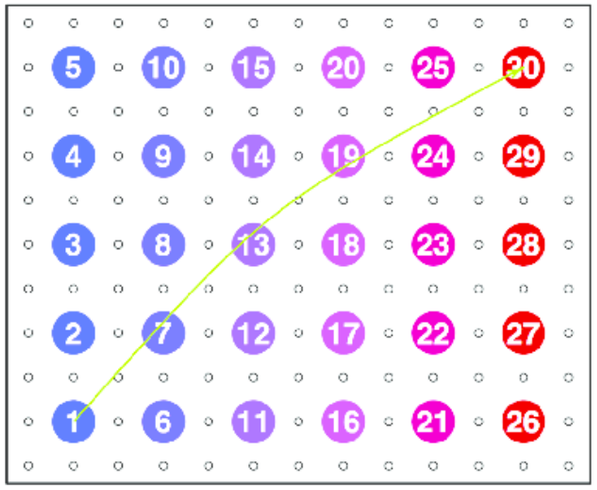
Petkova et al. (2016)
Peter et al. (2017)

Model assumptions are important

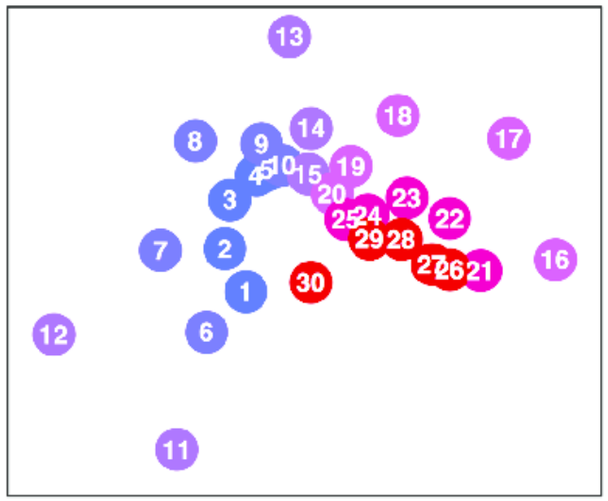




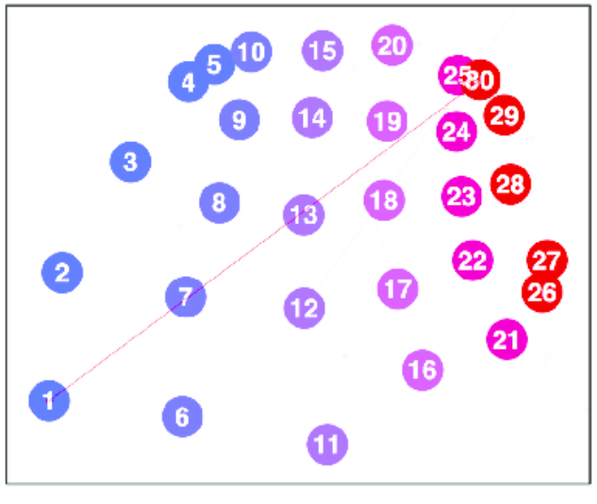
Long-range admixture + isolation-by-distance



(a) simulated lattice with admixture

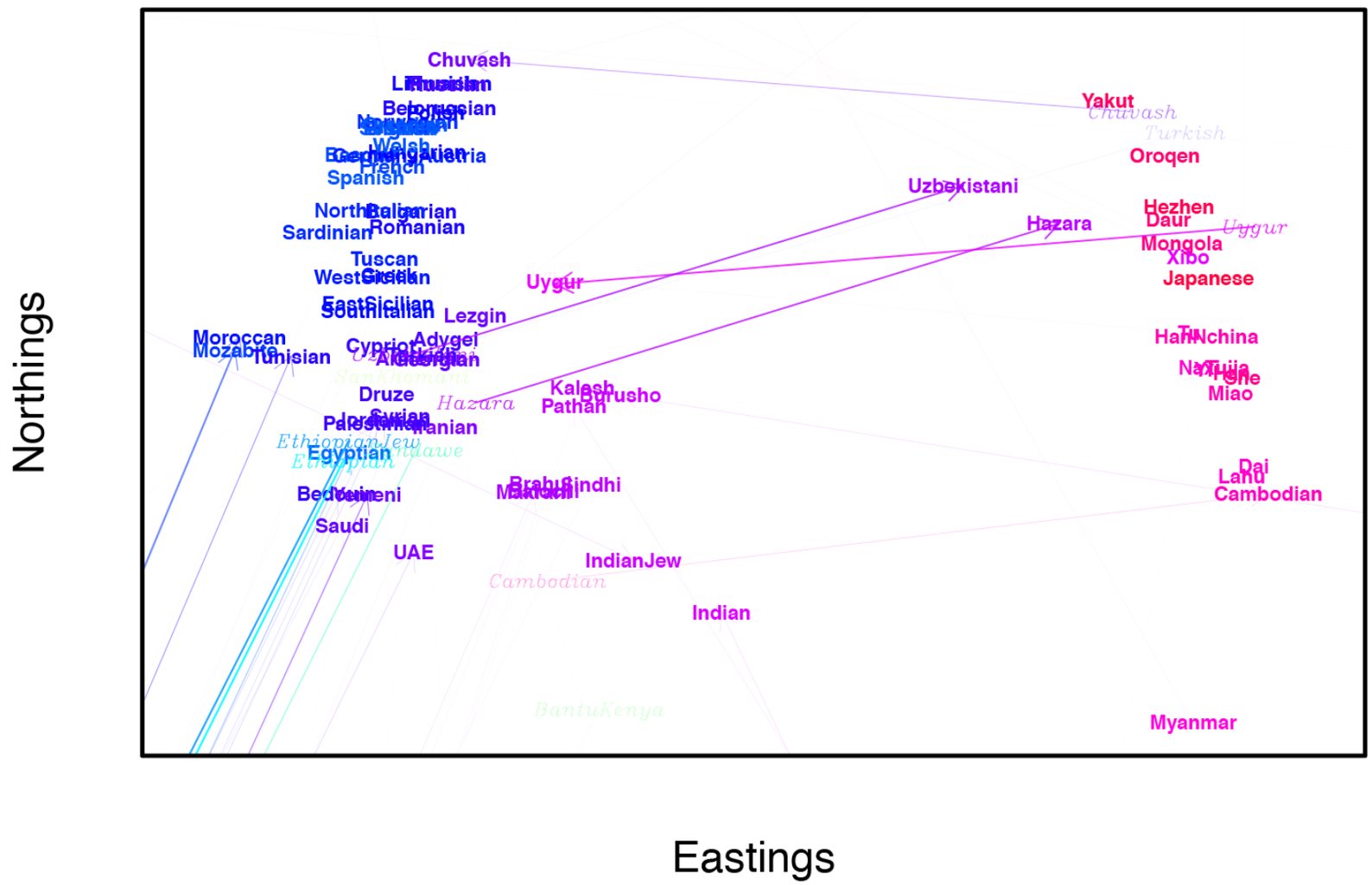


(b) geogenetic map without admixture inference



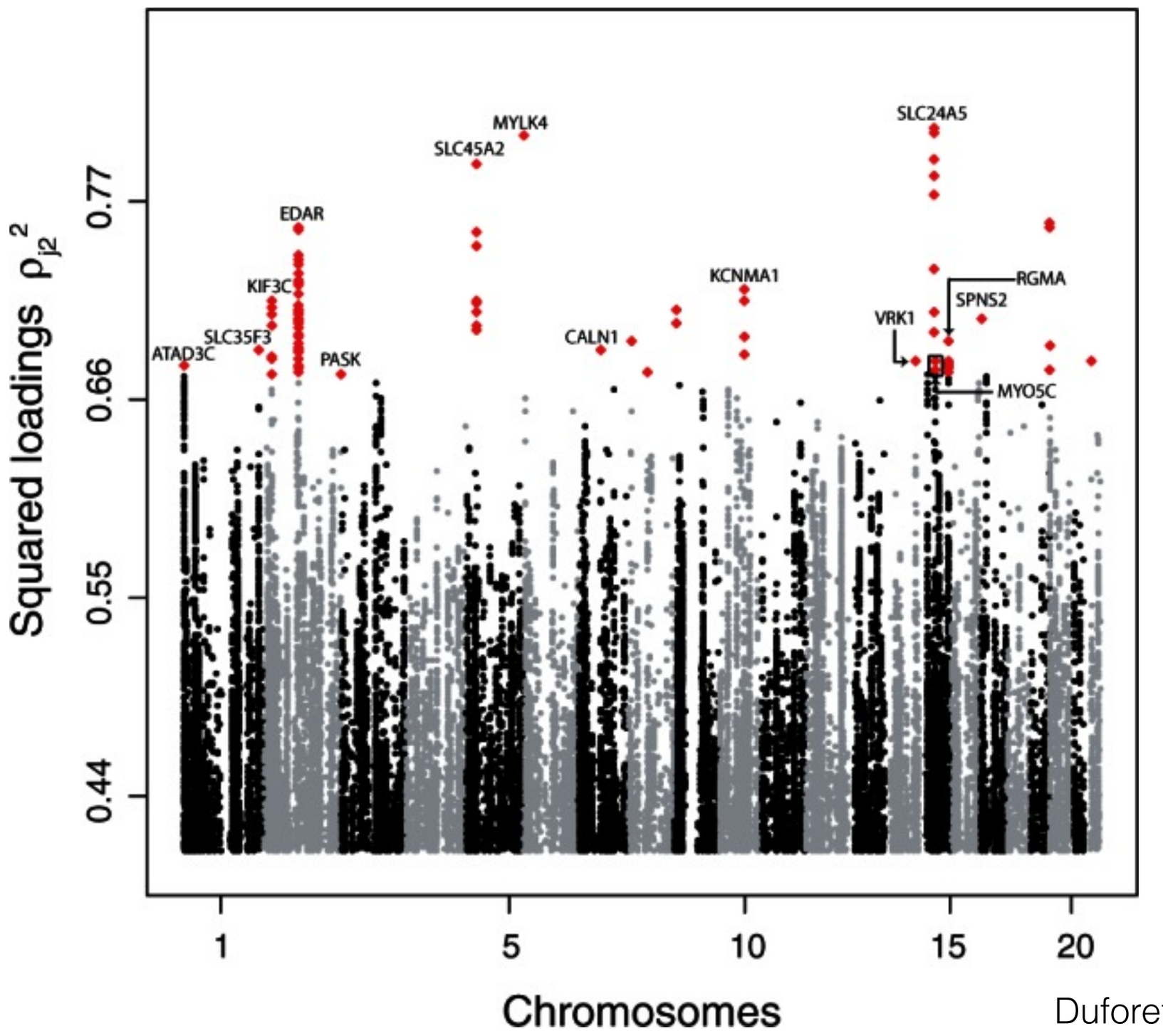
(c) geogenetic map with admixture inference

Long-range admixture + isolation-by-distance



(b) Close-up of Eurasian samples

Using PCA loadings to detect loci under selection



Duforet-Frebourg et al. (2016)