Multivariate Ordination

Advanced Biostatistics

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Lecture 8

EEOB 590C

Objectives

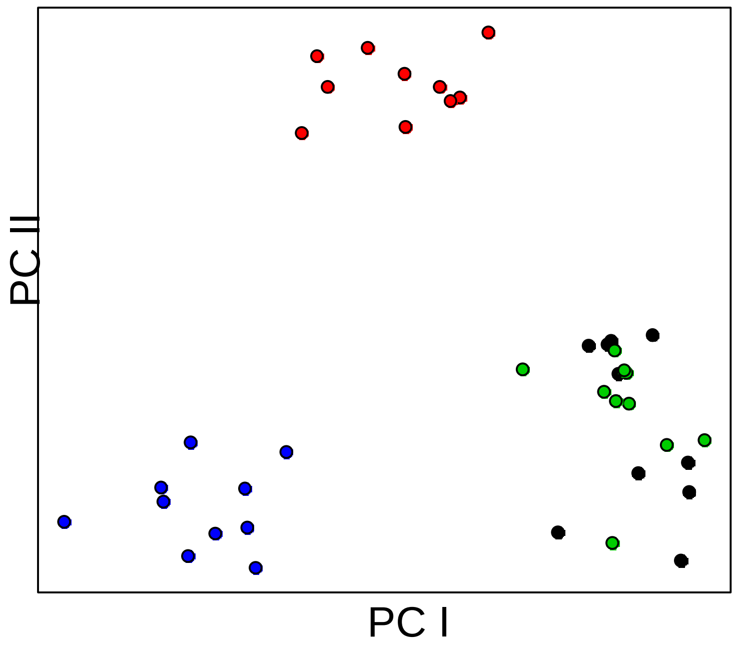
-Data are points in multivariate space

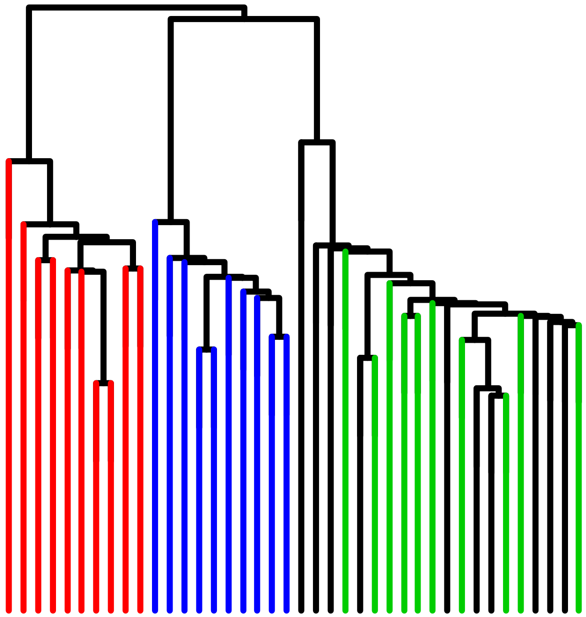
-Human perception does not work very well in >3 dimensions

-Look for patterns in high-dimensional spaces

-Generate summary plots of dataspace (ordination)

-Look for relationships of points (clustering)





Obtain summarized visualization of points in high-dimensional data space

Describe variation with a new set of variables (typically orthogonal)

These are produced under the criterion of finding what varies the most

They are ordered to represent more -> less variation

Plot first dimensions to summarize data

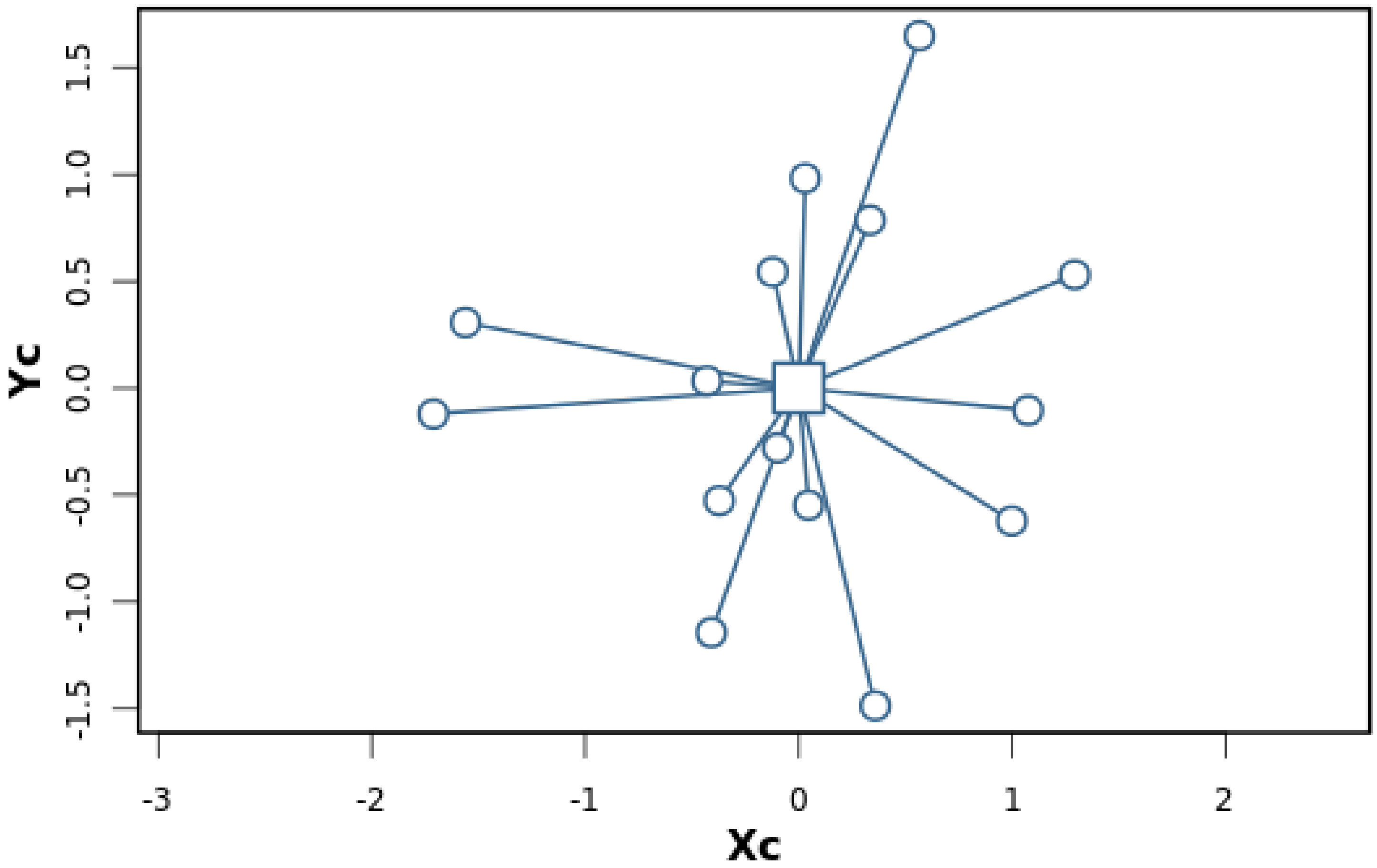
Look at association with original variables to know what varies

Some Ordination Approaches

- → Underlying matrices: covariance vs. correlation (vs. distances)
- → Rigid Rotation: Principal Components Analysis (PCA) Workhorse of ordination
- → Decomposing a distance matrix: Principal Coordinates Analysis (PCoA)
- → Iterative optimization: Non-Metric MultiDimensional Scaling (NMDS)
- → Non-continuous data: Correspondence Analysis (CA)

Ordination	Main Matrices	Slide 5
Create an *n* × 1 vector of 1s	$\mathbf{1}^{\mathbf{t}} = [1 \quad 1 \quad \dots \quad 1]$	
Find the variable means	$\bar{\mathbf{y}}^{\mathbf{t}} = (\mathbf{1}^{\mathbf{t}}\mathbf{1})^{-1}\mathbf{1Y}$	
Make a matrix of variable means	$\bar{\mathbf{Y}} = \mathbf{1}(\mathbf{1}^{\mathbf{t}}\mathbf{1})^{-1}\mathbf{1Y}$	
Mean-center the data matrix	$\mathbf{Y_c} = \mathbf{Y} - \bar{\mathbf{Y}}$	
Calculate the sums of squares and cross-products (SSCP)	$\mathbf{S} = \mathbf{Y_c}^{\mathbf{t}}\, \mathbf{Y_c}$	
Make the SSCP matrix into a covariance matrix	$\mathbf{C} = \dfrac{1}{(n-1)}\mathbf{S}$	
Make a diagonal matrix of just the variances	$\mathbf{V} = diag(\mathbf{C})$	
Calculate a correlation matrix	$\mathbf{R} = \mathbf{V}^{-1/2}\mathbf{C}\mathbf{V}^{-1/2}$	

Ordination	Variances and Distances	Slide 6
Mean-center the data matrix	$\mathbf{Y_c} = \mathbf{Y} - \bar{\mathbf{Y}}$	



Gower, J. C. 1966. Biometrika, 53, 325–338.

Principal Components Analysis

A **rigid rotation** of the multivariate data space

It preserves Euclidean Distances between objects (no distortion)

The rotation is done to find the direction of most variation

This is done using **Singular Value Decomposition (SVD)***

It produces a set of **new axes**, rank-ordered by % variation

These axes are linear combinations of initial variables

They are **orthogonal**, i.e. uncorrelated (mathematically!)

The data are **projected** on these new axes for visualization

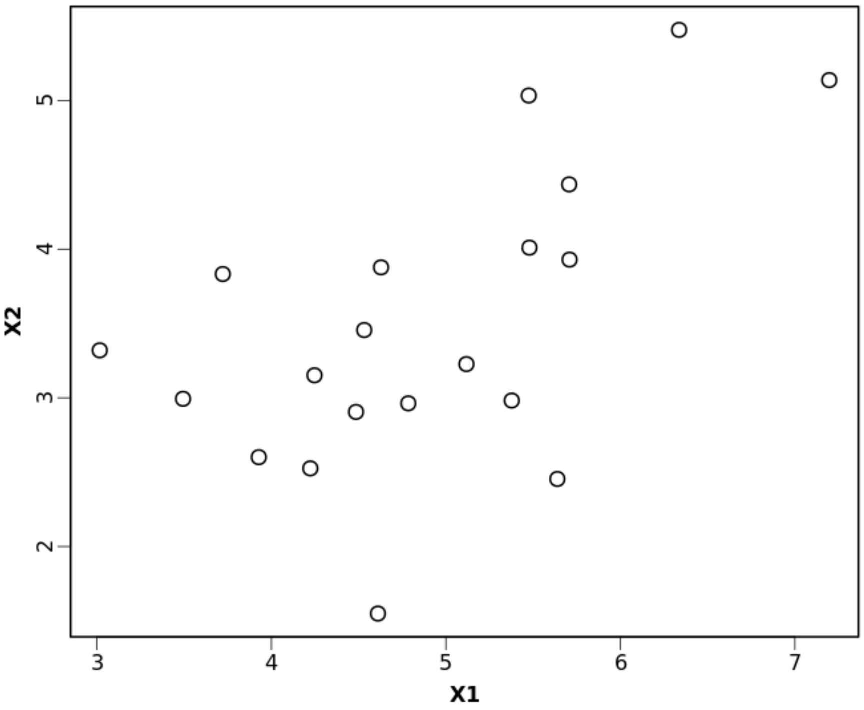
This is a separate step!
Have to project after making
the axes.

*Or eigenanalysis, which is a special case of SVD (see below)

PCA: Rigid Rotation of Data Space

Consider the simple case where k=2

X_1, X_2: two continuous variables that co-vary



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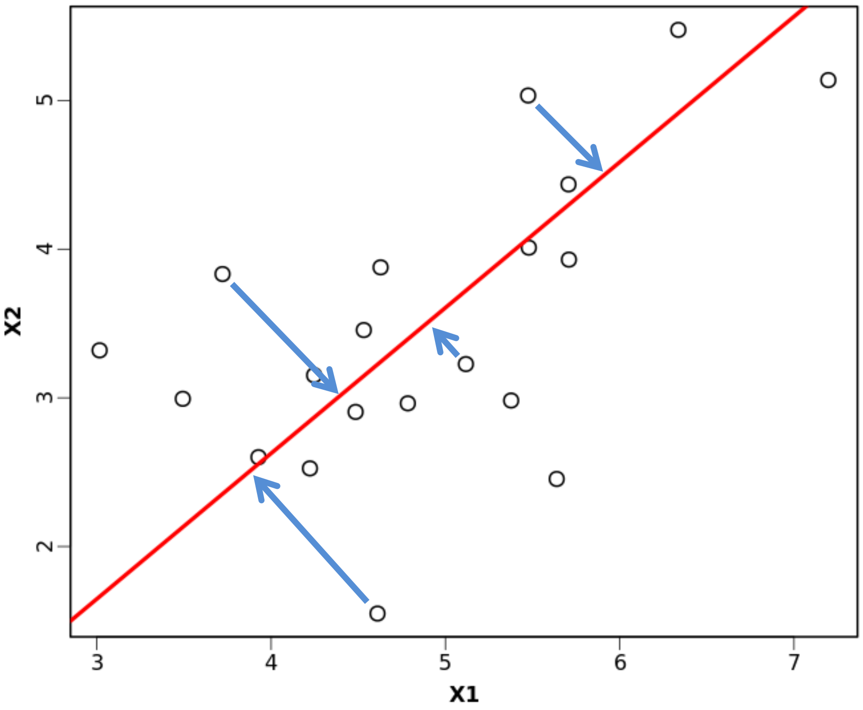

There is a line for which the distances of the points to it are minimized

This line summarizes the maximum covariation between X_1 and X_2

This is known as the major axis regression of X_2 on X_1

This is NOT the least squares regression line

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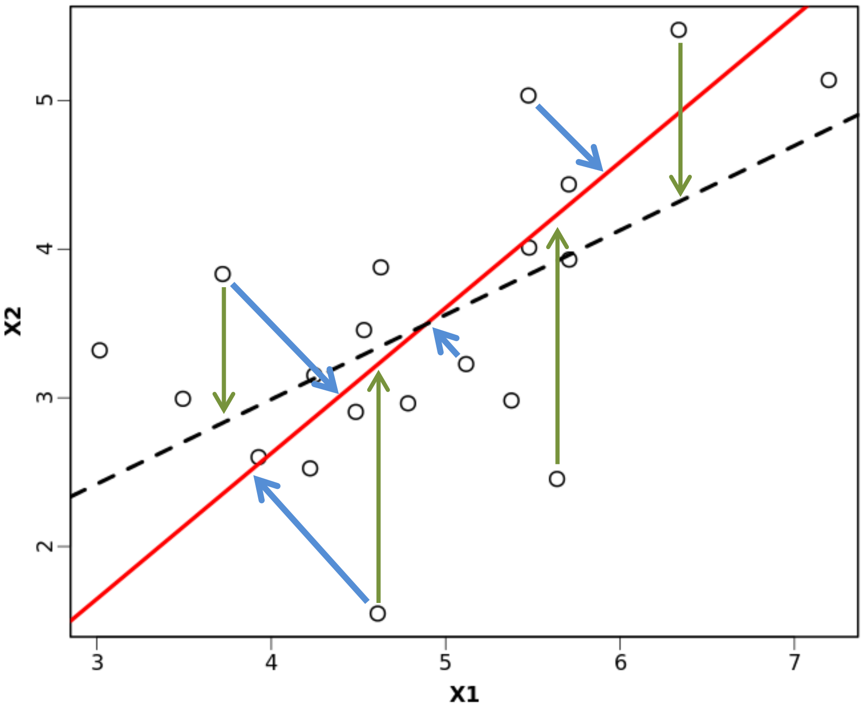

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This line summarizes the maximum covariation between X_1 and X_2

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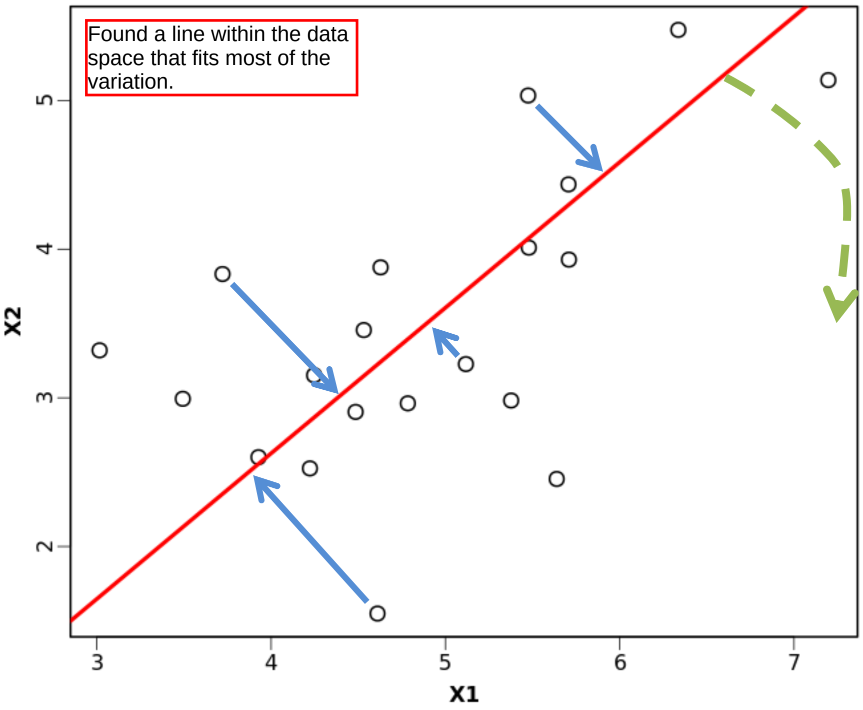
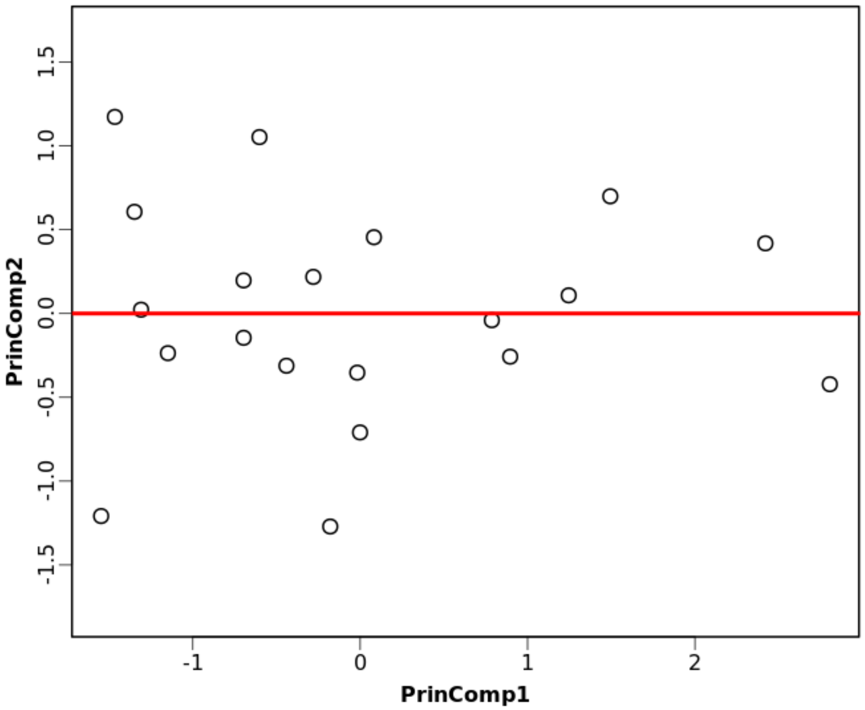
PCA: Rigid Rotation of Data Space

The orthogonal best fit line can be rotated to visualize the simplest covariation pattern

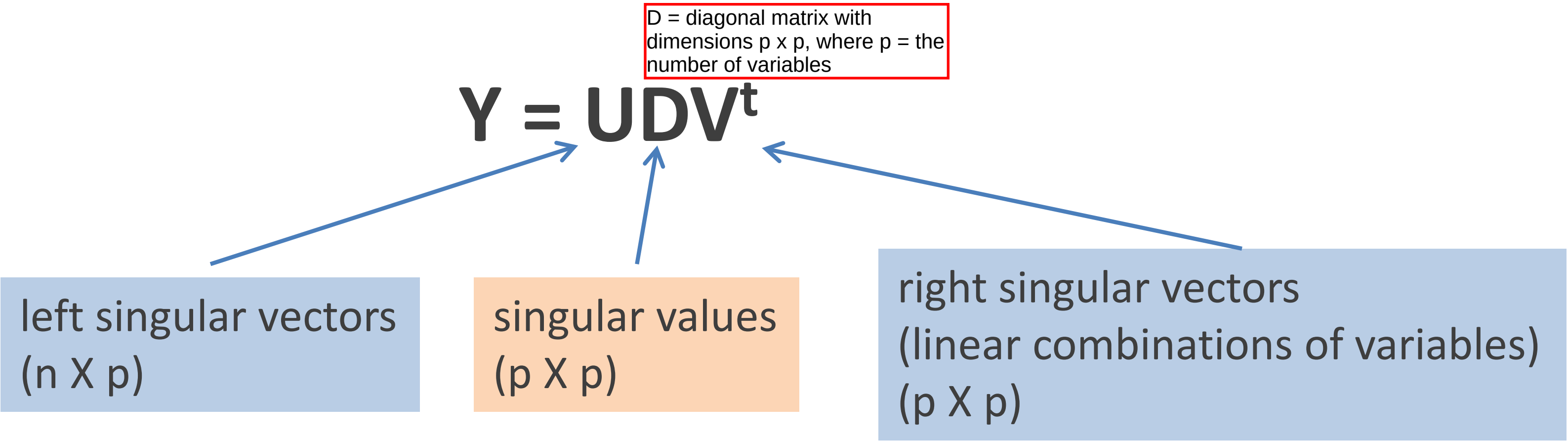

Found a line within the data space that fits most of the variation.



PCA: Procedure

Principal components (the direction of maximal variation) can be calculated for any number of dimensions

1. Start with the data matrix Y
2. Decompose this into singular vectors and singular values as



D = diagonal matrix with dimensions p x p, where p = the number of variables

$$Y = UDV^t$$

left singular vectors (n X p)

singular values (p X p)

right singular vectors (linear combinations of variables) (p X p)

D^2: expresses the % variance explained by each PC axis

UD: PC scores for each observation

n x n matrix

p x p matrix

NOTE: **U** also found from eigen-analysis of **YY**^{t}, and **V** found from eigen-analysis of **Y**^{t}**Y.**

The ‘classic’ implementation of PCA is using eigen-analysis: **S** = **E**Λ**E**^{t} , where **E** contains the eigenvectors and Λ the eigenvalues. PCA scores are found as: **P=YE**

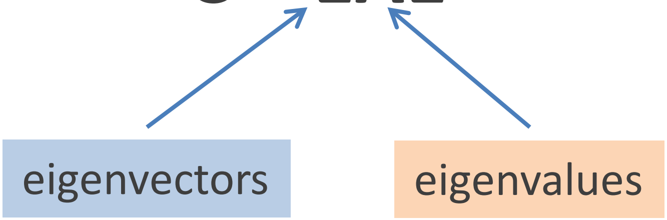
PCA Using Eigenanalysis

Usually we think of PCA using eigenanalysis

Eigenanalysis: a specific case of SVD for square symmetric matrices

1. Start with covariance (or correlation) matrix of Y
2. Decompose using eigenanalysis as

$$\mathbf{C} = \mathbf{E}\boldsymbol{\Lambda}\mathbf{E}^t$$



eigenvectors

eigenvalues

Λ: % variance explained

PC scores are found by projection of Y on the eigenvectors, as **P** = **YE**

PCA: Comments

PCA is a rigid rotation where new axes explain % variation

-Distances among objects preserved (so long as all dimensions containing variation are used)!

PC axes are loadings of each variable on PC axis (variables with values closer to -1 & +1 are more influential in that direction)

How well does a particular PC plot represent relationships among objects?

Assess by:

-% variation explained by PC axes

-Mantel correlation and Shepard diagram (plot of distances in reduced PC space vs. distances in full data space)

PCA: interpretation

To interpret a PCA we look at:

- % of variation explained
- structure of PCs (loadings of eigenvectors, or correlations with variables)
- ordination of observations in PC plot

-PCA does **nothing to the data**, it is just a rigid rotation

-It does **NOT find a particular dimension** (group differences, allometry, altitudinal gradient etc): it only finds the direction of highest variability in the data

-PC axes are orthogonal, so caution is needed in interpreting their biological meaning

-Some criteria exist for how many PC axes to interpret (broken stick model, log-ratio method, etc.: see Legendre & Legendre Num. Ecol.; also Mardia et al. 1979 Mult. Anal.; Jackson 1993, Ecol.)

PCA: Dimensionality

In some cases, some dimensions may have 0% variation ($\lambda<0$)

More rows than columns

When N > p, there are N-1 PC axes with variation*

When p > N, there are p PC axes

More columns than rows

Fewer PCs with variation may also occur when there is redundancy in the data

-compositional data: A+B+C=1,

-linear dependencies: $Y_1 = Y_2 + Y_3$

*This property holds for metric distances

PCA: Effect of Scale

If a variable varies much ***more*** than others, it dominates the PCA

(“more” is relative: 0 - 100cms is numerically “more” than 0 - 1m)

Particularly relevant when variables are measured in different units

Standardizing data often alleviates this problem

a) center and scale Y (subtract the mean and divide by standard deviation)

b) use the correlation matrix

a) and b) are equivalent, i.e. cov(Y.std) = cor(Y)

PCA: Example (Using Covariances)

```
> pca.bumpus <- prcomp(Y)

> summary(pca.bumpus)
Importance of components:
                           PC1     PC2     PC3     PC4     PC5     PC6
Standard deviation     0.06288 0.03574 0.02074 0.01577 0.01497 0.01485
Proportion of Variance 0.62225 0.20098 0.06766 0.03915 0.03527 0.03470
Cumulative Proportion  0.62225 0.82322 0.89088 0.93003 0.96530 1.00000
```

```
> pca.bumpus$rotation
          PC1         PC2         PC3         PC4         PC5        PC6
AE  0.2671219  0.02110608 -0.03036462  0.92618034 -0.16953793 -0.2018051
BHL 0.2591896 -0.08769478 -0.26991755 -0.17087231 -0.85357143  0.3073977
FL  0.4531669 -0.39808021  0.10060855  0.10052754  0.37487601  0.6895013
TTL 0.4603097 -0.51621150  0.35219851 -0.24850014 -0.07573124 -0.5745514
SW  0.2464018 -0.10723566 -0.87884180 -0.09611748  0.29570037 -0.2423767
SKL 0.6192799  0.74526673  0.14033437 -0.17859440  0.09469297 -0.0226606
```

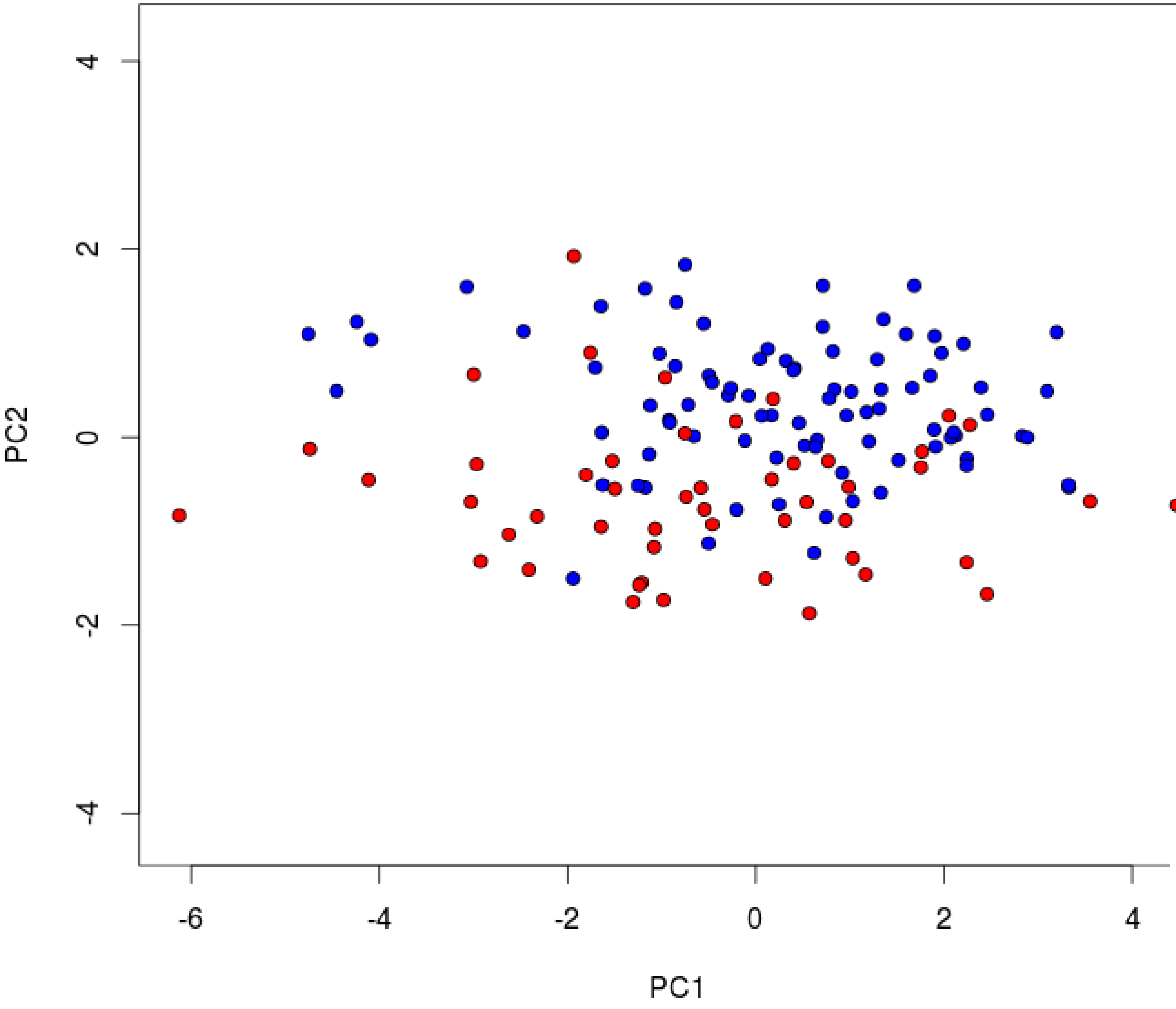
PCA: Example (Using Correlations)

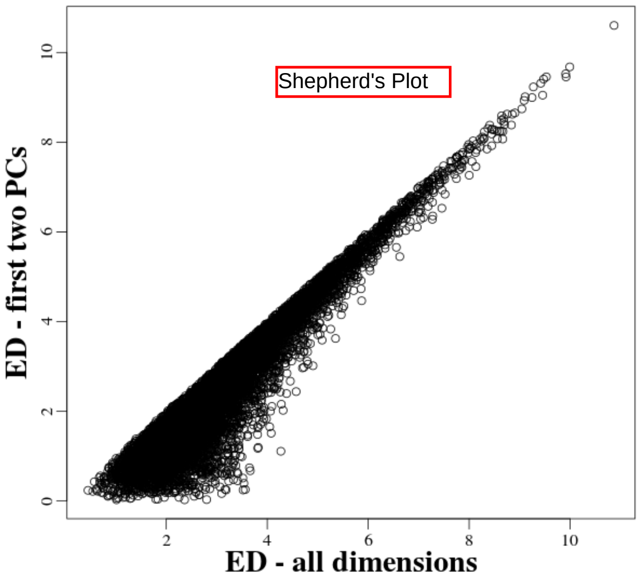
```
> pca.bumpus <- prcomp(Y, scale.=T)
> summary(pca.bumpus)
Importance of components:
                          PC1    PC2    PC3     PC4     PC5    PC6
Standard deviation     1.9110 0.8633 0.7838 0.66811 0.59933 0.4278
Proportion of Variance 0.6086 0.1242 0.1024 0.07439 0.05987 0.0305
Cumulative Proportion  0.6086 0.7329 0.8352 0.90963 0.96950 1.0000

> pca.bumpus$rotation
          PC1         PC2         PC3         PC4        PC5          PC6
AE  0.4061953  0.38791502 -0.25664018 -0.51015126 -0.5984397 -0.016694516
BHL 0.4196692 -0.07341605  0.21416001  0.73531917 -0.4815613  0.005262311
FL  0.4531108 -0.33702800 -0.26338163 -0.06552501  0.2371850  0.742416731
TTL 0.4303778 -0.43806789 -0.36689593 -0.03673575  0.2153367 -0.663731472
SW  0.3690596 -0.13025008  0.82639874 -0.37602393  0.1340963 -0.067225280
SKL 0.3635158  0.72227113 -0.03679566  0.22806658  0.5379220 -0.058756221
```

PC1: loadings similar and positive (size)

PC2: relative shape: SKL vs. TTL & FL

PCA: Scatter-Plot of PC Scores





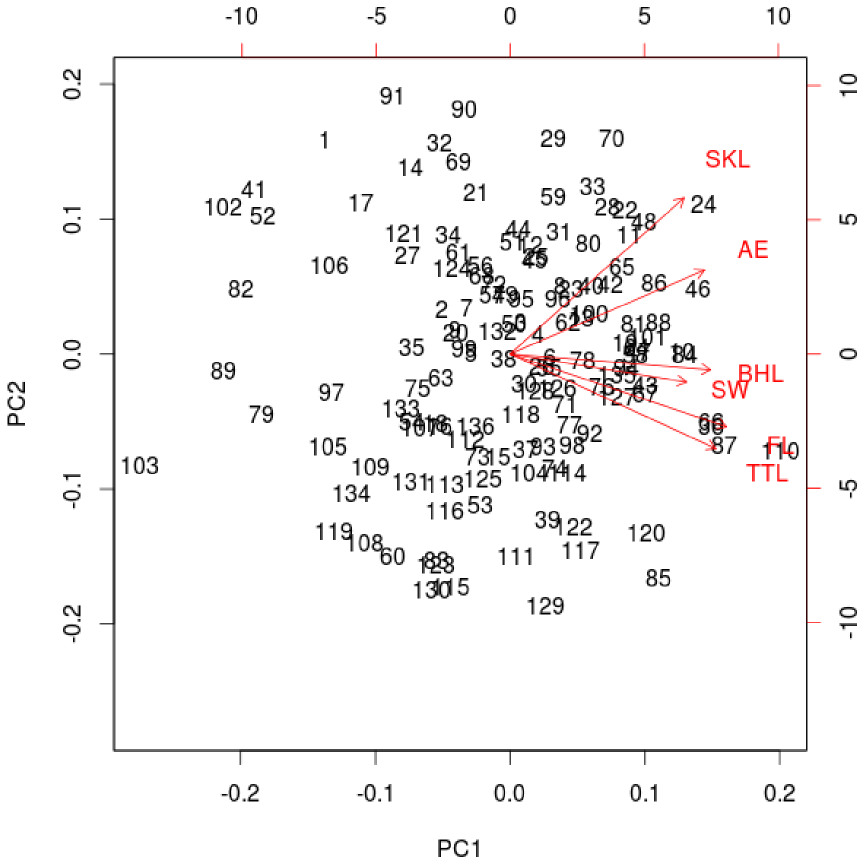
```
> pca.bumpus$rotation[,2]
        AE                BHL          FL               TTL              SW            SKL
0.38791502 -0.07341605 -0.33702800 -0.43806789 -0.13025008  0.72227113
```

PCA: Bi-Plot

-Ordination plot of objects (rows) and variables (columns)

-Look for sets of vectors with small angles, and clusters of points

-Can use to identify variables with high association with objects



Numbers are "n"s
Letter vectors are "p"s

PCA is helpful for exploring multivariate space and it may **suggest** patterns

It is exploratory! It is nothing more than a rigid rotation of the multivariate data space

No hypotheses are tested

Using the correlation vs. covariance matrix has important implications

It preserves the Euclidean distances among objects in the original multivariate data space because rigid rotation!

Very useful for summarizing the results of hypothesis-testing approaches applied to multivariate data(e.g. MANOVA)

Principal Coordinates Analysis (PCoA)*

Ordination from distance matrix among objects (Q-mode)

-Preserves object distances from *any* distance measure (D_E, Jaccard, etc.)

Projects data into Euclidean space

If D_{euclid} used, PCoA identical to PCA (Gower, 1966)

Sometimes called metric multidimensional scaling (MDS) because it preserves relationships among objects

*** *aka* Classical Multidimensional Scaling**

PCoA: Procedure

Start with **D**, the matrix of pairwise distances among objects (d_{ij}^2)

Transform as $$\mathbf{A} = -\frac{1}{2}\mathbf{D}^2$$

Double-center $$\mathbf{G} = \left(\mathbf{I} - \frac{1}{n}\mathbf{1}\mathbf{1}^{\mathrm{t}}\right)\mathbf{A}\left(\mathbf{I} - \frac{1}{n}\mathbf{1}\mathbf{1}^{\mathrm{t}}\right)$$

I = *n* × *n* identity matrix
1 = *n* × 1 matrix of 1s
G = double-centered matrix
(named after Gower, 1966)

This positions the centroid of the scatter at the origin by subtracting row and column means from each element, and adding the grand mean

Decompose using eigenanalysis $$\mathbf{G} = \mathbf{E}\mathbf{\Lambda}\mathbf{E}^{\mathrm{t}}$$

Eigenvectors are the *COORDINATES* of objects in Euclidean space

PCoA: Thoughts

PCoA embeds a set of objects into a Euclidean space

-Identical to PCA for continuous multivariate data

Strength: Enables ordination for *any* data types expressed as distances (genetic distance, Hamming distance, geographic distance, etc.)

Can yield negative eigenvalues (if distances are semi-metric or nonmetric)

Properties of Distances

Bray-Curtiss distances don't follow all of these

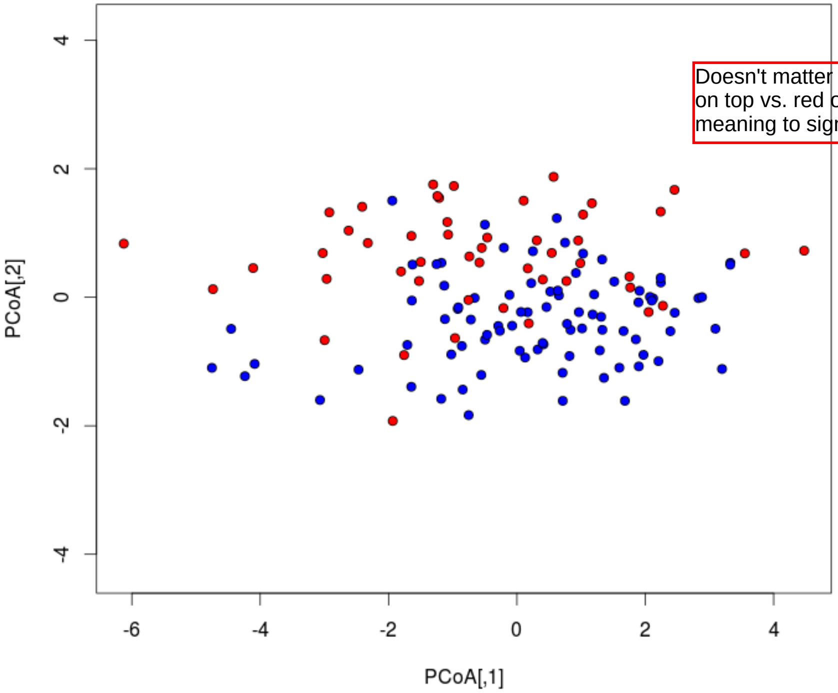
Metric (Euclidean): (1) $d_{11} = 0$, (2) $d_{12} = d_{21}$, (3) triangle inequality

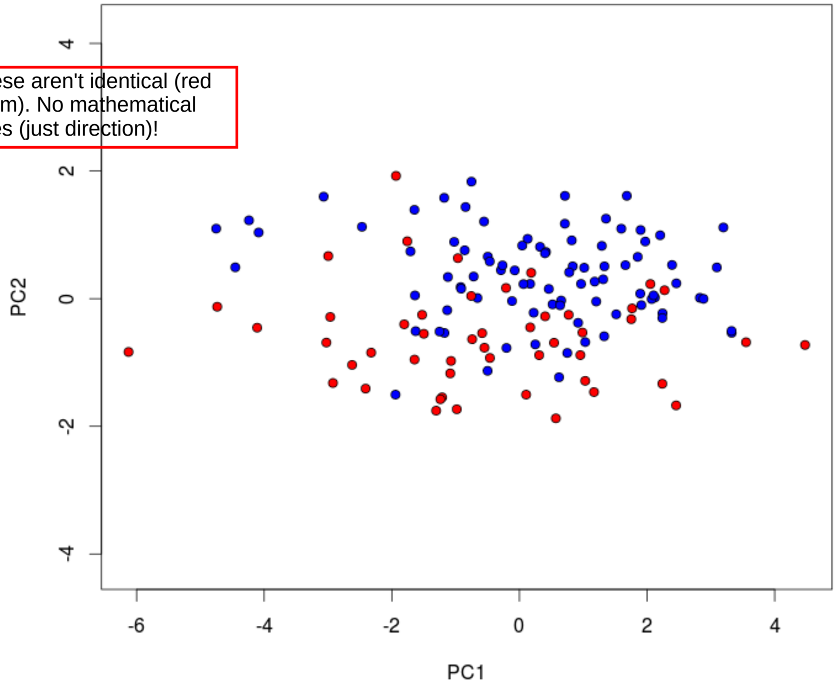
Semimetric (pseudometrics): no triangle inequality

Non-Metric: d_{12} may be negative

PCoA: Example

```
> Y <- scale(Y, center = T, scale = T)
> bumpus.dist <- dist(Y)
> PCoA <- cmdscale(bumpus.dist)
```





Doesn't matter that these aren't identical (red on top vs. red on bottom). No mathematical meaning to sign of axes (just direction)!

Non-Metric Multidimensional Scaling (NMDS)

PCA and PCoA preserve distances among objects

Non-metric MDS generates ordination where similar objects are close together and dissimilar objects are far apart

Preserves the relative **order** of the objects, but not the distances themselves

Objective is to find a low dimensional representation of the data space

-Start with distance matrix

-Specify number of dimensions for MDS ordination *a priori*

-Construct initial configuration of objects (a ‘guess’).

This step is **crucial**, as no distances are optimized, but rather the fit is tested and iterated until stable (the result from PCoA is often used)

Can use the MDS ordination as "guess"

-Calculate fitted distances in NMDS space and compare to true distances

-Calculate predicted distances, and goodness of fit (stress)

$$Stress1 = \sqrt{\sum \left(d_{fitted} - \hat{d}_{fitted}\right)^2 / \sum d_{fitted}{}^2}$$

-Move objects in NMDS plot and repeat (GOAL: Monotonic fit of D_{obs} vs. D_{fit})

-Iterate until Δ stress is below threshold (i.e., convergence)

Note: other Stress equations exist

NMDS: Comments

-NMDS seems arbitrary, but works rather well

Positives:

-Generally yields fewer dimensions than PCA,PCoA

-Does not require full distance matrix (missing values ok)

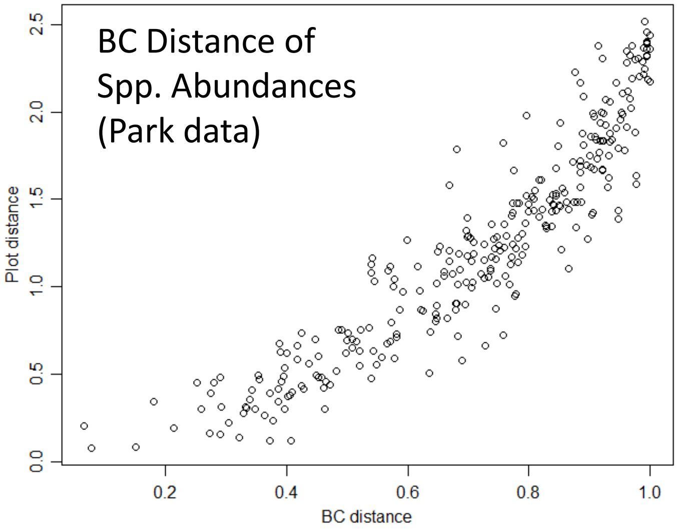
Negatives:

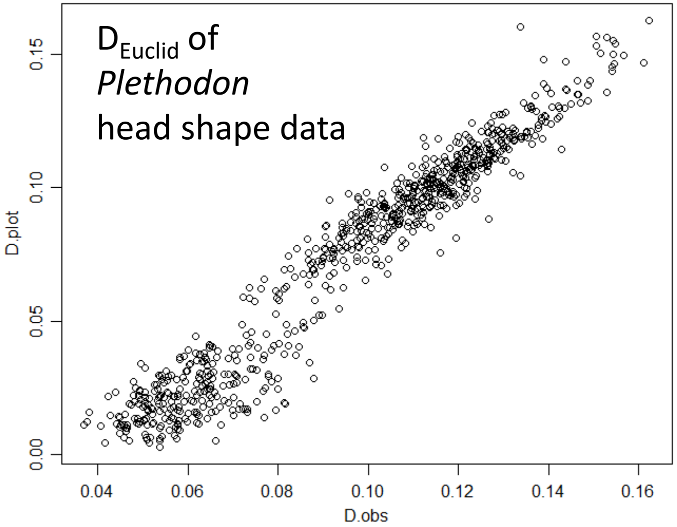
-Arbitrary optimization

-Results dependent on starting configuration ('guess')

-Does not preserve distances among objects (though that is not the objective)

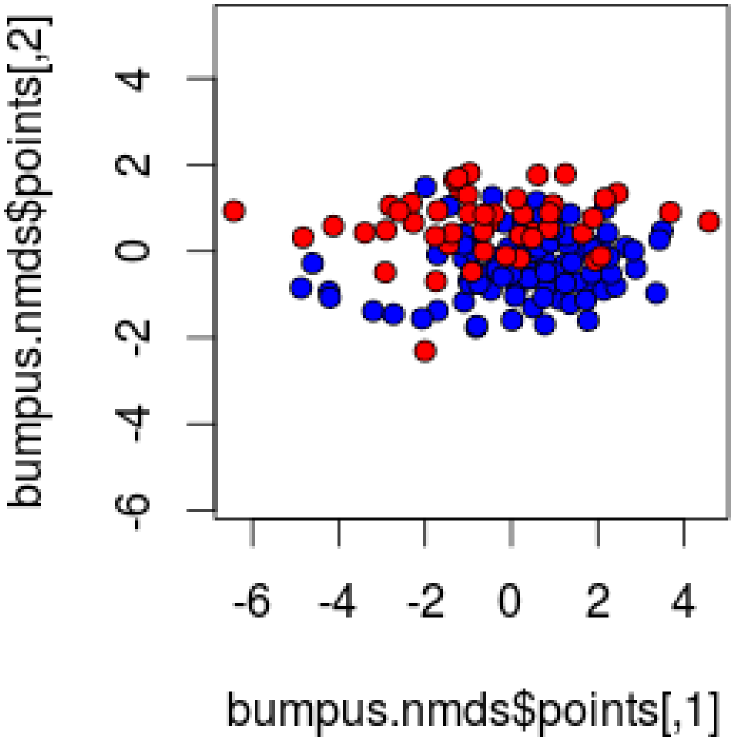
-Minimizes monotonic relationship of D_{fit} vs. D_{obs}

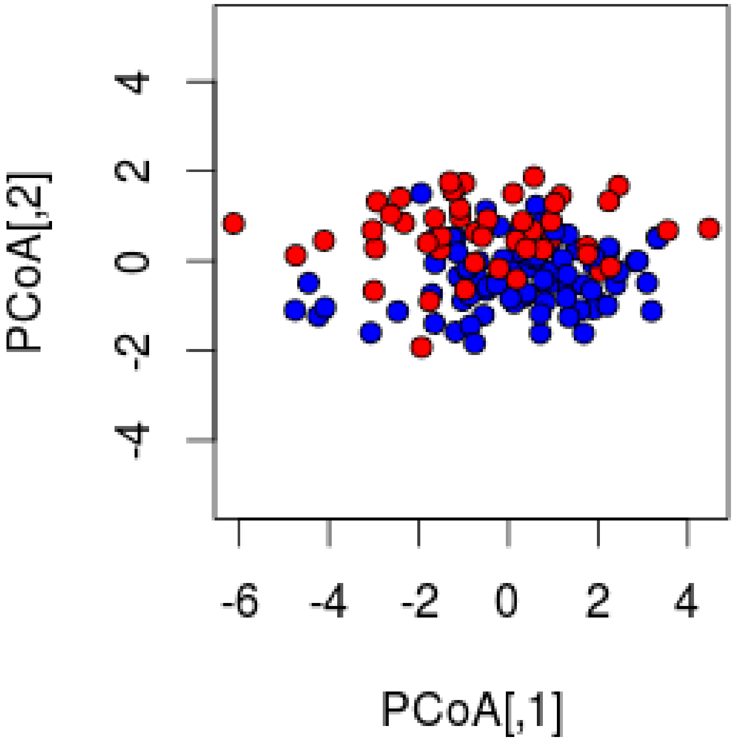




NMDS: example

NMDS DOES NOT PRESERVE DISTANCES;
SHOULD BE QUALITATIVELY INTERPRETED
WITH EXTREME CAUTION





Correspondence Analysis (CA)

Ordination for count and frequency data

Very frequently used in ecology (community data, species presence etc)

Preserves the χ^2 distance among objects (a weighted Euclidean distance of conditional probabilities)

$$D_{Euclid} = \sqrt{\sum_k \left(y_{ki} - y_{kj}\right)^2}$$

$$D_{X^2} = \sqrt{\sum_k \frac{1}{y_k}\left(\frac{y_{ki}}{y_i} - \frac{y_{kj}}{y_j}\right)^2}$$

where $y_k = \sum y_{+k} \Big/ \sum y_{++}$

and $y_i = \sum y_{i+}$

Provides a test of independence of rows and columns

CA also called reciprocal averaging

1. Calculate matrix of relative frequencies (Q) from contingency table

Elements of **Q**: $p_{ij} = f_{ij}/f_{total}$

2. Standardize Q by centering by rows and columns*

Elements of $\overline{\mathbf{Q}}$: $q_{ij} = \left[\dfrac{p_{ij} - p_{i+}p_{j+}}{\sqrt{p_{i+}p_{j+}}}\right]$

3. Decompose using SVD $SVD(\overline{\mathbf{Q}}) = \hat{\mathbf{U}}\mathbf{W}\mathbf{U}'$

- Û: eigenvectors for rows
- U´: eigenvectors for columns
- W: singular values
- Obtain ordination plot from scaled and projected row and column factors

* Hence CA is aka *reciprocal averaging*

CA: Comments

-Similarity of objects from frequency data can be viewed using ordination

-Test of independence between objects and variables (significance implies some objects have higher frequencies on particular variables)

-Ordination can be as a biplot (simultaneous plot of rows and columns: objects and variables)

-Interesting Note: Eigenanalysis of $\overline{\mathbf{Q}}\overline{\mathbf{Q}}'$ yields$\hat{\mathbf{U}}$, and

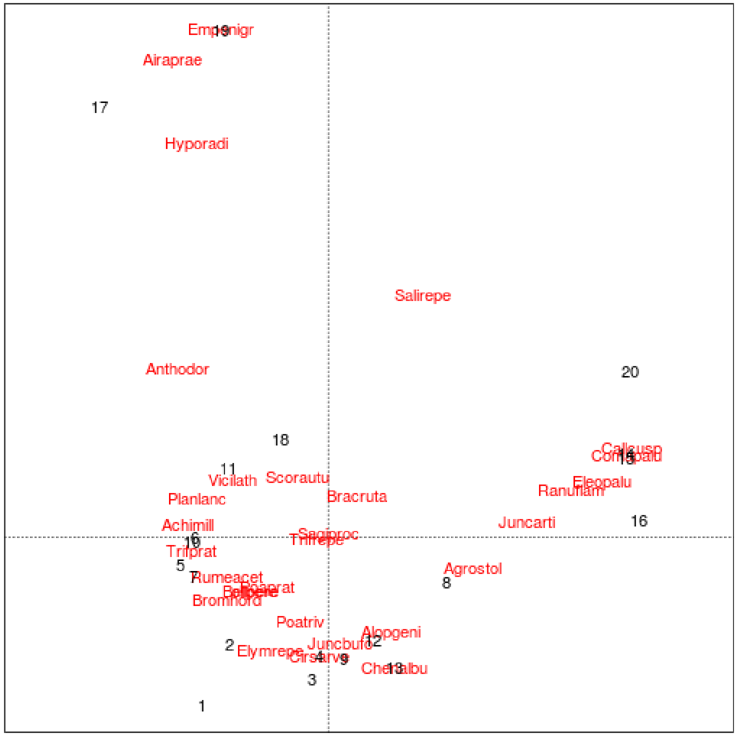
eigenanalysis of $\overline{\mathbf{Q}}'\overline{\mathbf{Q}}$ yields $\mathbf{U}'$

-Advantage of SVD is that it decomposes rows AND columns simultaneously

CA: example

data(dune) from vegan: dune meadow vegetation data from the Dutch island of Terschelling

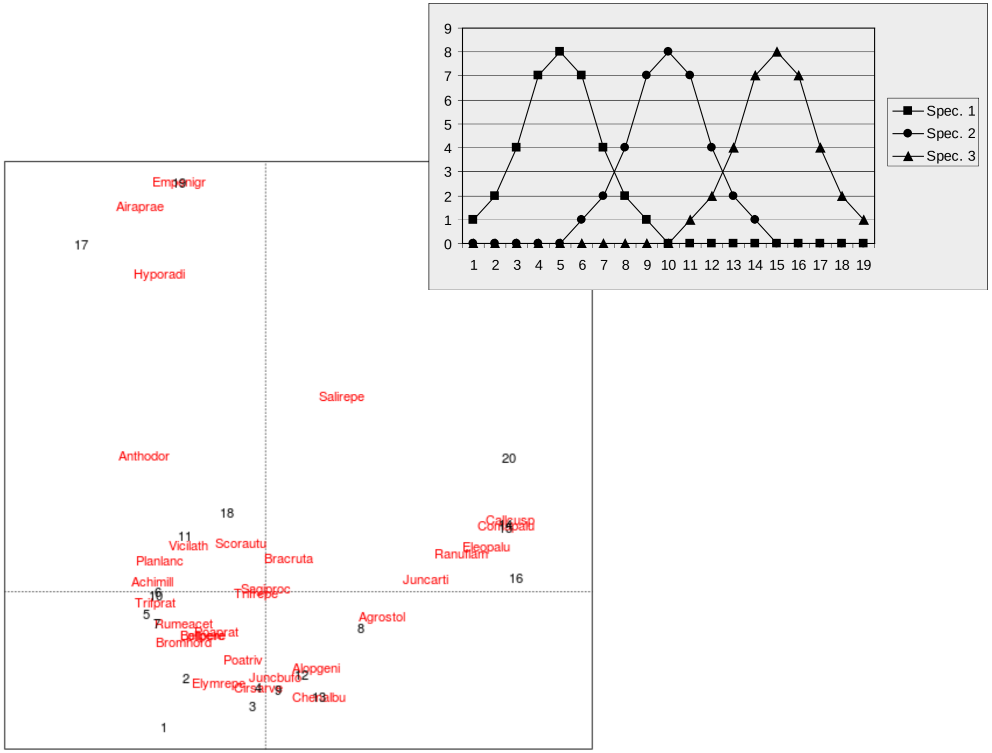
- rows: 20 2x2m randomly selected plots sampled in the island in 1982 for a dune conservation project
- columns: plant species
- values: cover class (standardized estimate of density)



Although most ordination methods construct **mathematically** orthogonal axes, real life is not constrained to be orthogonal!

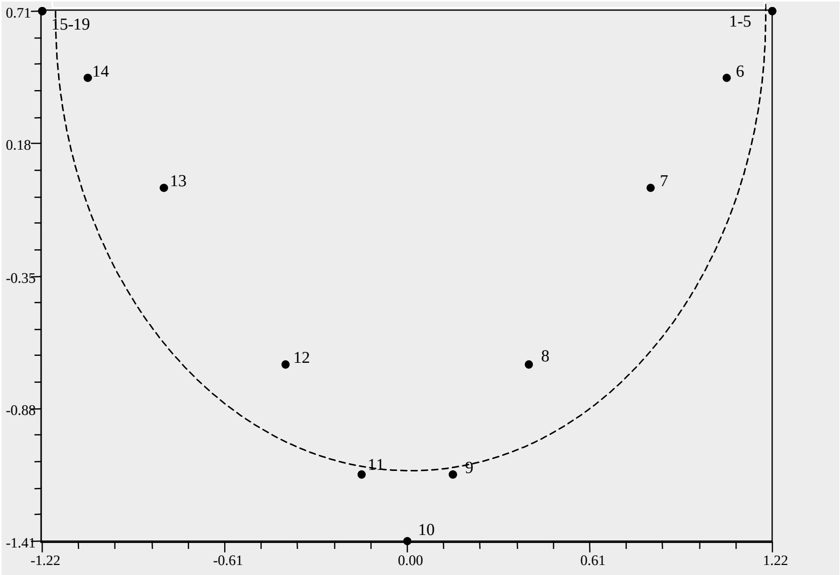
This may create problems, e.g. the “arch effect” (*aka* the “horseshoe” effect)

The 2nd axis is an arched function of the 1st axis (common in ecological data, e.g. species counts in sites along an environmental gradient)



Hypothetical abundance data of 3 species for sites along an ecological gradient

Ordination (CA) of sites from above data



Detrended Correspondence Analysis (DCA)

‘Correct’ for arch effect in CA plots to represent underlying gradient in ordination

Two common approaches:

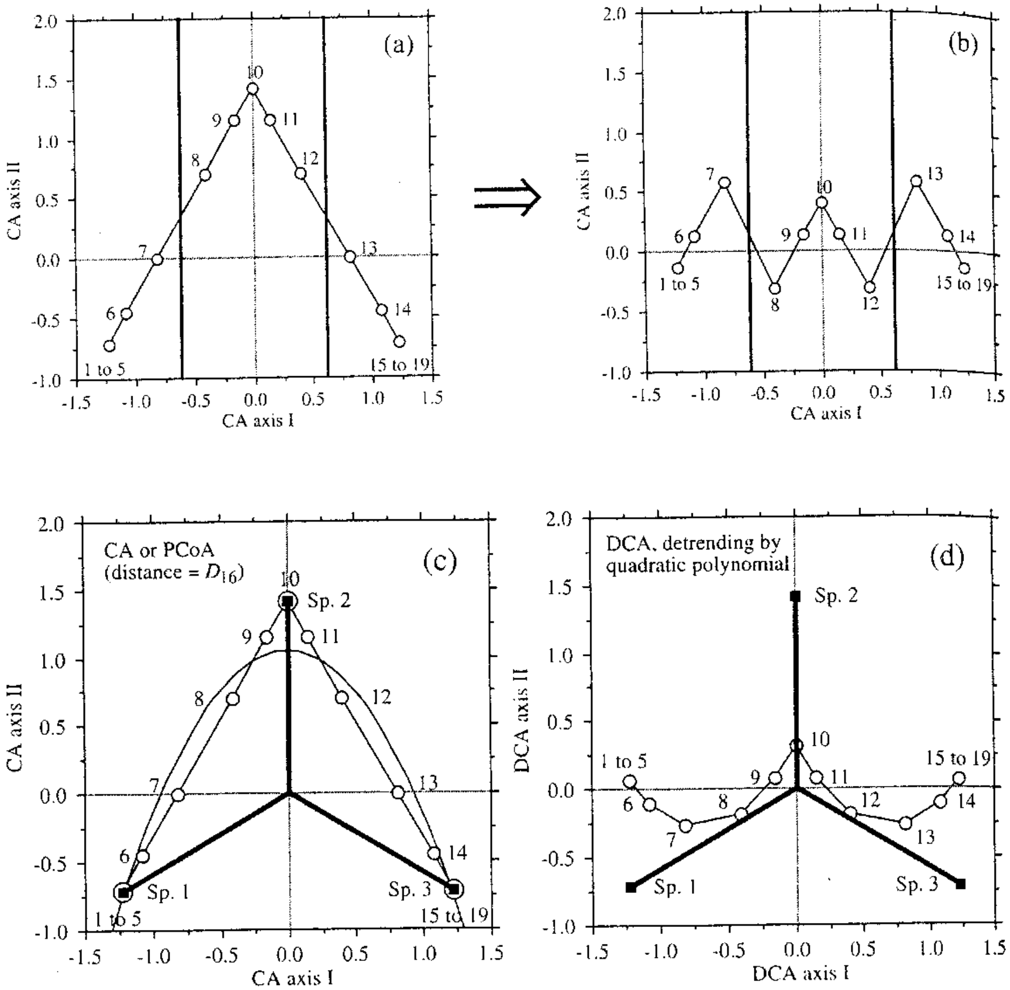
DCA by segments

- Calculate CA ordination and divide DC1 into segments
- Calculate DC2 (for each segment, calculate mean for DC2, and subtract from DC2 scores)

DCA by polynomials

- Additional constraint imposed in CA algorithm, such that DC2 is orthogonal to DC1 (linear), and also polynomials of DC1 (square, cubic, quartic, etc.)

DCA: approaches



DCA by segments

DCA by polynomials

From Legendre and Legendre (1998). *Numerical Ecology*.

DCA: Thoughts

Approaches are arbitrary (how to choose segments? What order polynomial to use?)

DC2 now completely **meaningless** (proximities of objects on DC2 cannot be interpreted)

DCA plot **distorted**, no longer represents distances among objects

Detrending eliminates (hides) arch effect, that may in itself be useful information (i.e., the arch *IS* the pattern in the data!)

Detrending should absolutely be avoided!

For detailed critique see Wartenberg, Ferson, and Rohlf, 1987. *Am. Nat.* 129:434-448.