Lecture 5: Ecological distance metrics; Principal Coordinates Analysis

Univariate testing vs. community analysis

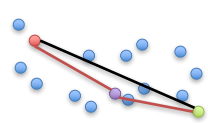
- Univariate testing deals with hypotheses concerning individual taxa
  - Is this taxon differentially present/abundant in different samples?
  - Is this taxon correlated with a given continuous variable?
- What if we would like to draw conclusions about the community as a whole?

Useful ideas from modern statistics

- Distances between anything (abundances, presence-absence, graphs, trees);
- Direct hypotheses based on distances;
- Decompositions through iterative structuration;
- Projections;
- Randomization tests, probabilistic simulations.

What is a distance metric?

- Scalar function d(.,.) of two arguments
- d(x, y) >= 0, always nonnegative;
- d(x, x) = 0, distance to self is 0;
- d(x, y) = d(y, x), distance is symmetric;
- d(x, y) <= d(x, z) + d(z, y), triangle inequality.



Microbiome analysis pipeline:

Specimens → Sequence → Abundance Matrix → Distances → Statistics* →Paper

	S-1	S-2	…	S-16
OTU-1				
OTU-2				
OTU-3				
OTU-4				
OTU-5				
….				
OTU-10,000				

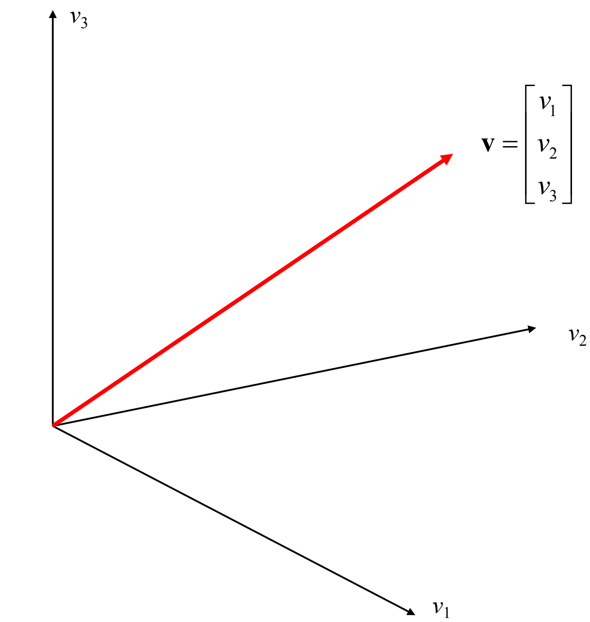
0.54															
0.54	0.34														
0.60	0.17	0.10													
0.01	0.75	0.17	0.72												
0.24	0.34	0.74	0.89	0.58											
0.08	0.43	0.82	0.91	0.69	0.64										
0.57	0.06	0.59	0.03	0.69	0.89	0.07									
0.88	0.68	0.59	0.49	0.19	0.64	0.54	0.26								
0.24	0.71	0.58	0.52	0.93	0.94	0.46	0.70	0.30							
0.92	0.46	0.17	0.21	0.17	0.02	0.81	0.22	0.45	0.59						
0.51	0.23	0.74	0.36	0.15	1.00	0.95	0.71	0.88	0.94	0.65					
0.29	0.30	0.75	0.28	0.04	0.45	0.15	0.44	0.59	0.89	0.07	0.19				
0.57	0.63	0.27	0.72	0.34	0.70	0.19	0.17	0.85	0.07	0.97	0.24	0.45			
0.95	0.53	0.86	0.01	0.10	0.63	0.40	0.10	0.51	1.00	0.20	0.41	0.11	0.14		
0.83	0.43	0.12	0.39	0.43	0.87	0.42	0.17	0.62	0.57	0.03	0.58	0.54	0.48	0.83	

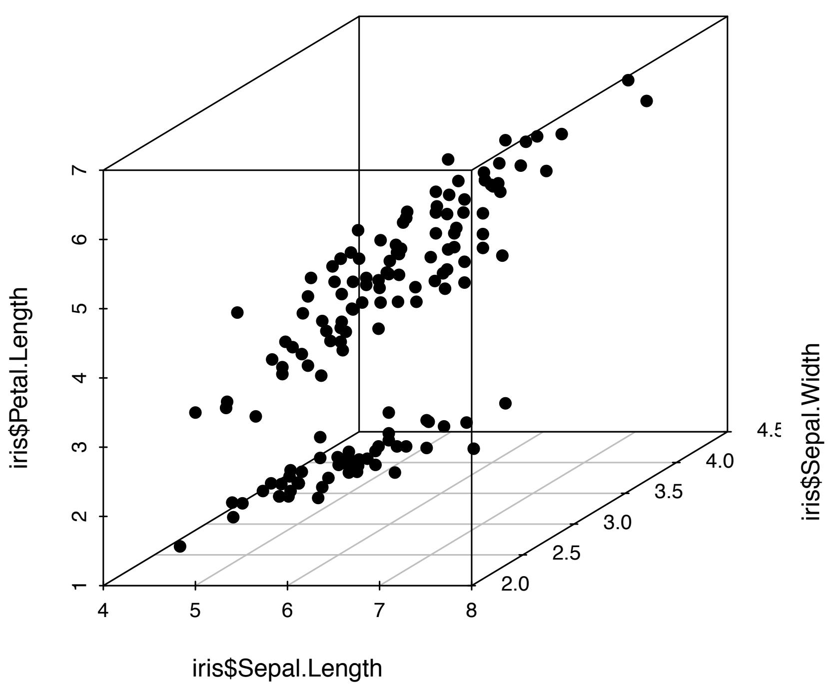
Visualization (Principal coordinates analysis)
Statistical hypothesis testing (PERMANOVA)

*This step can sometimes be omitted for lack of necessity.

PRINCIPAL COORDINATES ANALYSIS - MULTIDIMENSIONAL SCALING

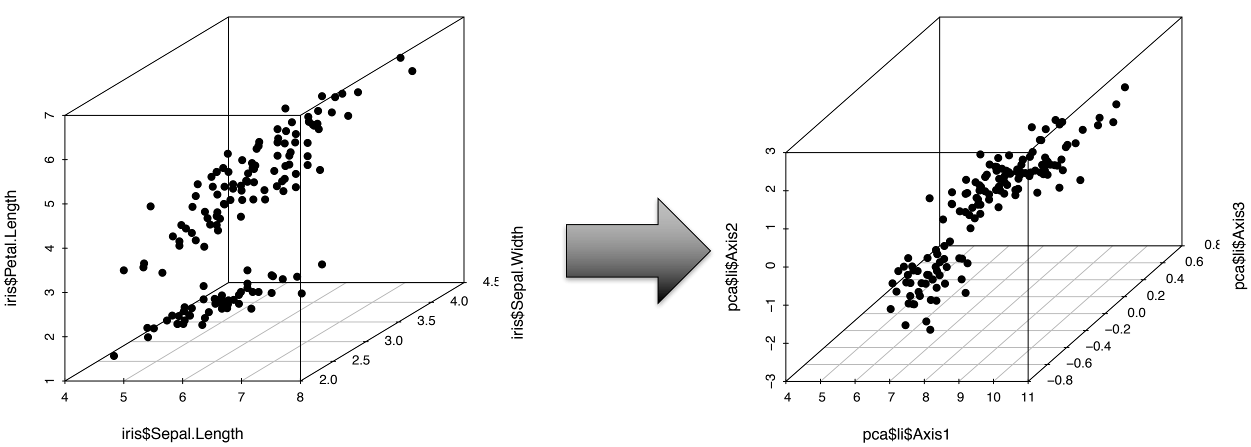
Every multivariate sample can be represented as a vector in some vector space



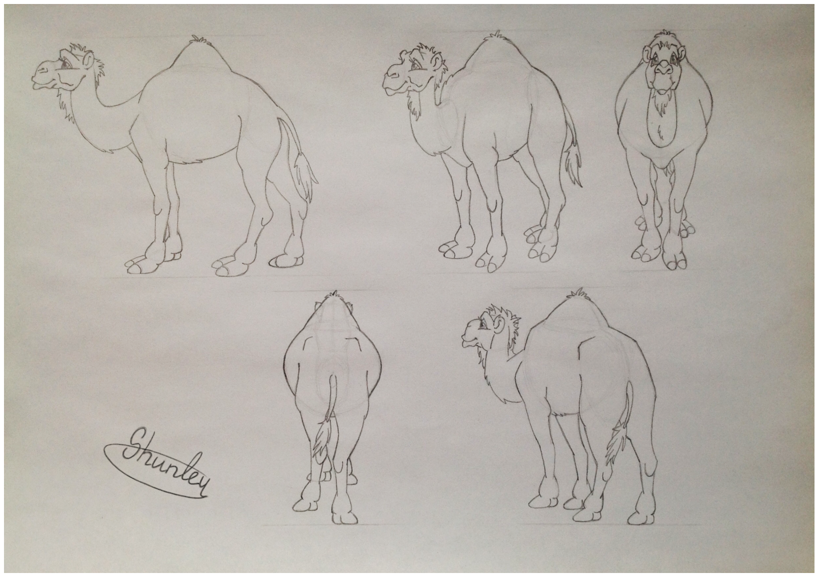


Vector Basis

- A basis is a set of linearly independent (dot product is zero) vectors that **span** the vector space.
- **Spanning** the vector space: Any vector in this vector space may be represented as a linear combination of the basis vectors.
- The vectors forming a basis are orthogonal to each other. If all the vectors are of length 1, then the basis is called orthonormal.



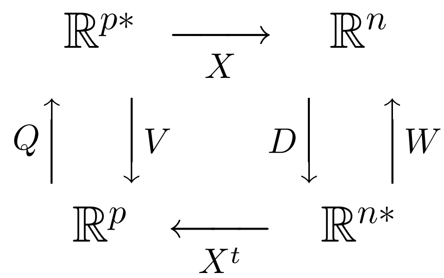
Idea: Change basis so that we can better discover patterns in the data.



Basic idea for analysis of multidimensional data

- Compute distances
- Reduce dimensions
- Embed in Euclidean space
- The general framework behind this process is called **Duality diagram**: ($\mathbf{X}_{nxp}$, $\mathbf{Q}_{pxp}$, $\mathbf{D}_{nxn}$)
  - $\mathbf{X}_{nxp}$ (centered) data matrix
  - $\mathbf{Q}_{pxp}$ column weights (weights on variables)
  - $\mathbf{D}_{nxn}$ row weights (weights on observations)

Duality diagram defines the geometry of multivariate analysis



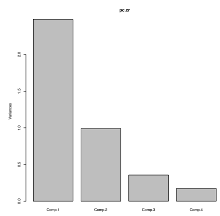
- $V = X^T D X$
- $W = X Q X^T$
- Duality:
  - The eigen decomposition of VQ leads to eigen-decomposition of WD
- *Inertia* is equal to trace (sum of the diagonal elements) of VQ or WD.

Principal Component Analysis (PCA)

- Let Q = I and D = 1/n I and let X be centered.
- $VQ = X^T DXQ = 1/n\ X^T X$.
- The inertia Tr(VQ) = sum of the variances.
- PCA decomposes the variance of X into independent components.
- To decompose the inertia means to find the eigen-system of VQ or equivalently WD matrices.
- Eigenvalues give the amount of inertia explained in corresponding dimension.
- Eigenvectors give the dimensions of variability.

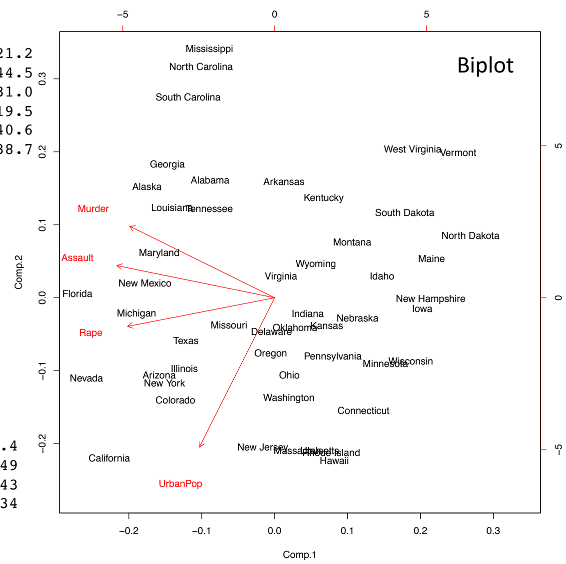
Example PCA

```
head(USArrests)
        Murder Assault UrbanPop Rape
Alabama      13.2     236       58 21.2
Alaska       10.0     263       48 44.5
Arizona       8.1     294       80 31.0
Arkansas      8.8     190       50 19.5
California    9.0     276       91 40.6
Colorado      7.9     204       78 38.7
```



Screeplot: plot of inertia

```
Loadings:
         Comp.1 Comp.2 Comp.3 Comp.4
Murder   -0.536  0.418 -0.341  0.649
Assault  -0.583  0.188 -0.268 -0.743
UrbanPop -0.278 -0.873 -0.378  0.134
Rape     -0.543 -0.167  0.818
```



Centering

- Let Y be not centered data matrix with n observations (rows) and p variables (columns)
- Let **H** = (**I** – 1/n **1**x**1'**)
- Then X = HY is centered

From Euclidean distances to PCA to PCoA

- Note that if **D** is a Euclidean distance, then
- **X X'** = 1/n **H** $\mathbf{D}^{(2)}$ **H**.
- PCoA is a generalization of PCA in that knowledge of **X** is not required, all you need to represent the points is **D**, the inter-point distance matrix.

Representation of (arbitrary) distances in Euclidean space

- The idea is to use singular value decomposition (SVD) on the centered interpoint distance matrix to extract Euclidean dimensions
- SVD: X = U S V, where S is diagonal matrix with diagonal elements s_1, s_2, …, s_n, and U and V are unit matrices (i.e. their determinant is 1 and they span (they are form orthonormal basis) their corresponding spaces)

Definition

Let A be an $n \times n$ matrix

Let $\vec{x}$ and λ be such that

$$A\vec{x} = \lambda\vec{x} \quad \text{with} \quad \vec{x} \neq \vec{0}$$

then λ is called an ***eigenvalue*** of A and

and $\vec{x}$ is called an ***eigenvector*** of A and

Note:

$$\left(A - \lambda I\right)\vec{x} = \vec{0}$$

If $\left|A - \lambda I\right| \neq 0$ then $\vec{x} = \left(A - \lambda I\right)^{-1}\vec{0} = \vec{0}$

thus $\left|A - \lambda I\right| = 0$

is the condition for an eigenvalue.

Diagonalization

Thereom If the matrix A is symmetric with distinct eigenvalues, $\lambda_1, \ldots, \lambda_n$, with corresponding eigenvectors $\vec{x}_1, \ldots, \vec{x}_n$

Assume $\vec{x}_i' \vec{x}_i = 1$

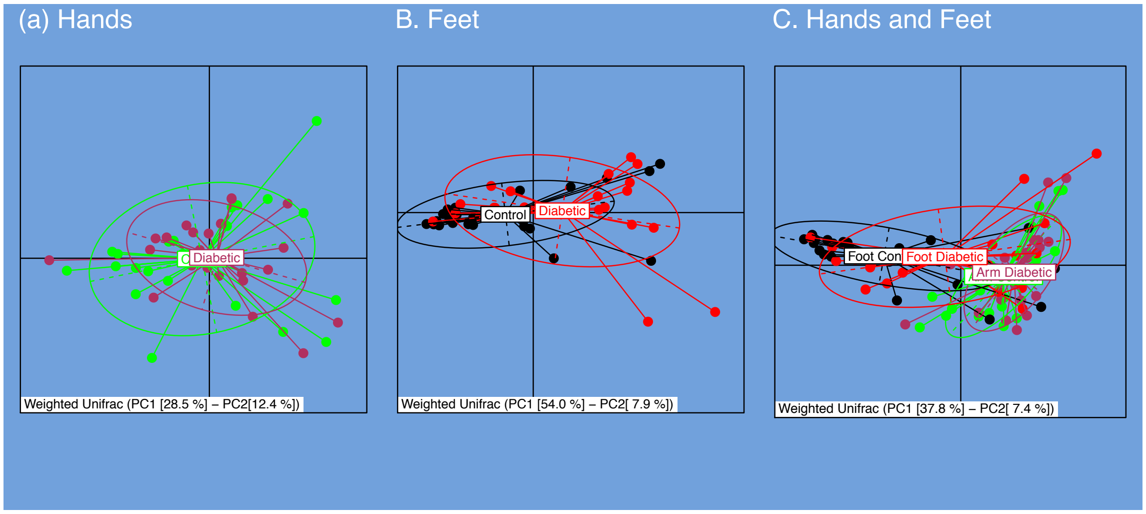
then $A = \lambda_1 \vec{x}_1 \vec{x}_1' + \ldots + \lambda_n \vec{x}_n \vec{x}_n'$

$$= \left[\vec{x}_1, \ldots, \vec{x}_n\right] \begin{bmatrix} \lambda_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \lambda_n \end{bmatrix} \begin{bmatrix} \vec{x}_1' \\ \\ \vec{x}_n' \end{bmatrix} = PDP'$$

PCoA details

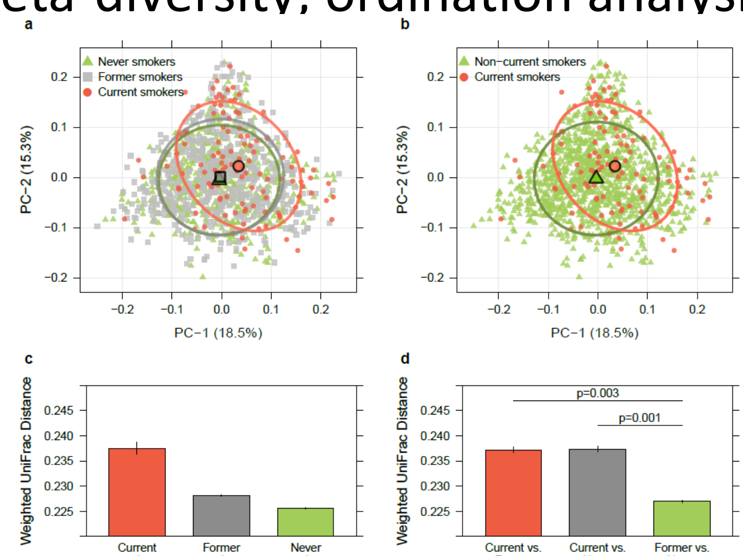
- Algorithm starting from **D** inter-point distances:
  - Center the rows and columns of the matrix of square (element-by-element) distances: **S** = -1/2**H** $\mathbf{D}^{(2)}$**H**
  - Compute SVD by diagonalizing **S**, **S** = **U Λ** $\mathbf{U}^{T}$
  - Extract Euclidean representations: $\underline{X}$ = **U** $\mathbf{\Lambda}^{1/2}$
- The relative values of diagonal elements of **Λ** gives the proportion of variability explained by each of the axes.
- The values of **Λ** should always be looked at in deciding how many dimensions to retain.

Differentiation of microbiota between diabetic and non-diabetic subjects and across body sites



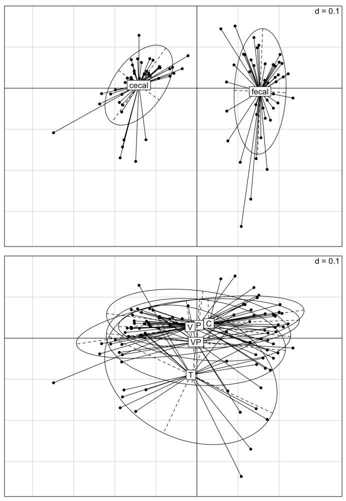
Redel et al. J Infect Dis. 2013 207(7):1105-14

Beta-diversity; ordination analysis

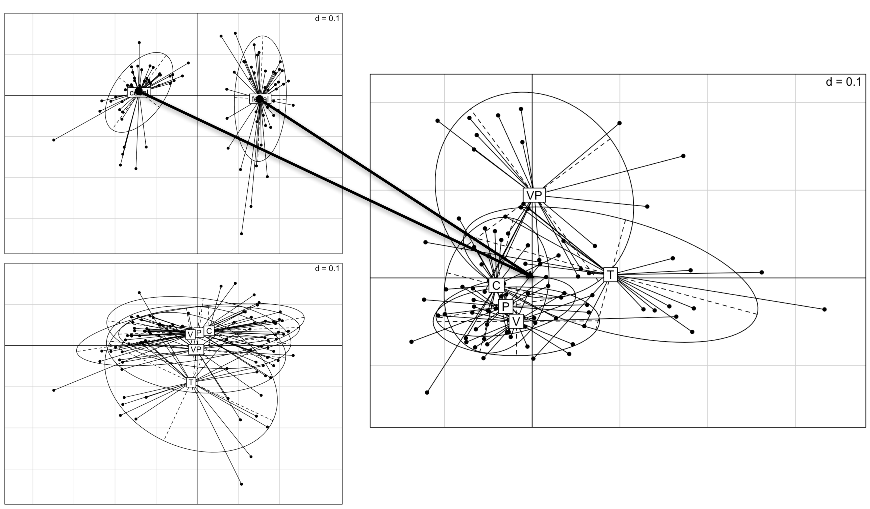


ISME J. 2016 Mar 25. doi: 10.1038/ismej.2016.37

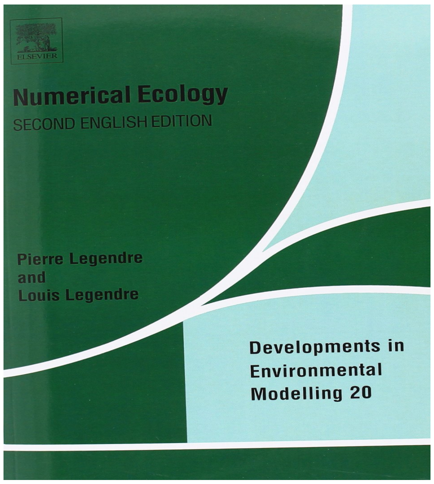
Within class analysis



Within class analysis



Suggested reading



- Susan Holmes "Multivariate Data Analysis: The French Way", IMS Lecture Notes–Monograph Series, 2006.