Measuring Intraspecific Genetic Diversity

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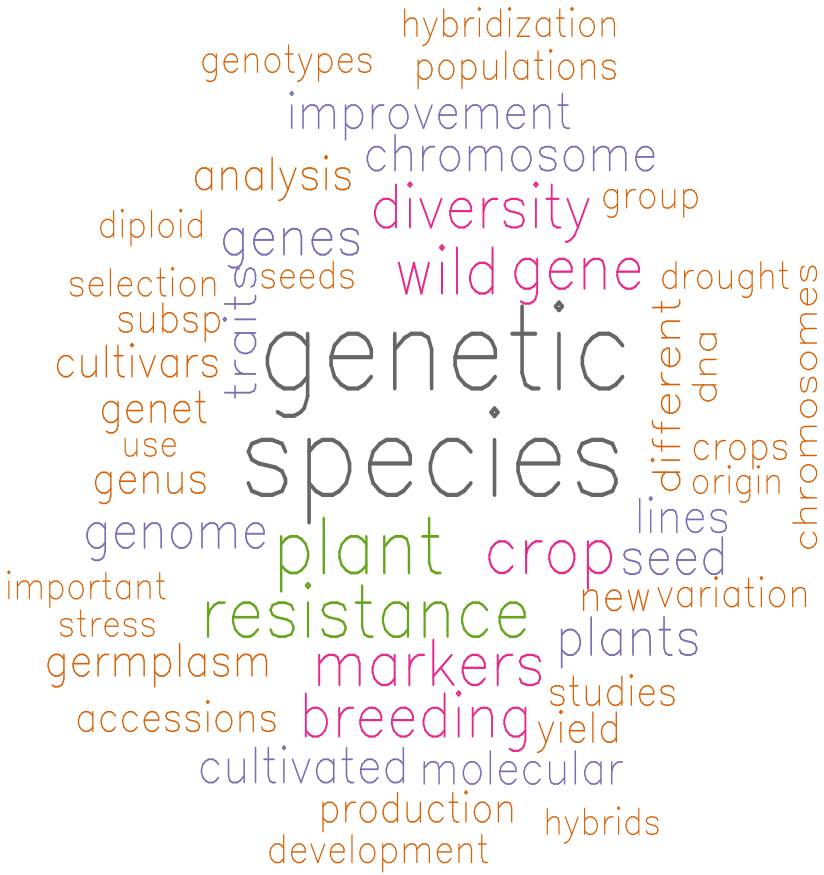


Outline:

1 Introduction
- 1.1 Frequency of Alleles & Genotypes

2 Diversity
- 2.1 Distances & Diversity Indices

3 Intraspecific
- 3.1 Wright Statistics (F)

4 Cluster to identify "groups"

5 BIO-R to make the things easy...

1 Introduction and some Definitions

- **Genotype:**

  Genotype is ... 

- **Markers:**

  Genetic Markers are ... 

- **Population:** ...[CONT]

Populations

- **Population Genetics:** Studies the heredity's mechanisms at the population level.
- **Population:** Set of conspecifics individuals in the *same place and time*, which have the ability to mate (exchange alleles).

In a population, the individual has a transitory importance, what matters are the alleles it has, which will be transmitted to subsequent generations

Reproductive systems

- **Allogamous:** frequency of cross polination is $\geq 95\%$ *e.g.*, maize
- **Autogamous:** frequency of cross pollination is $\leq 5\%$ *e.g.*, wheat.

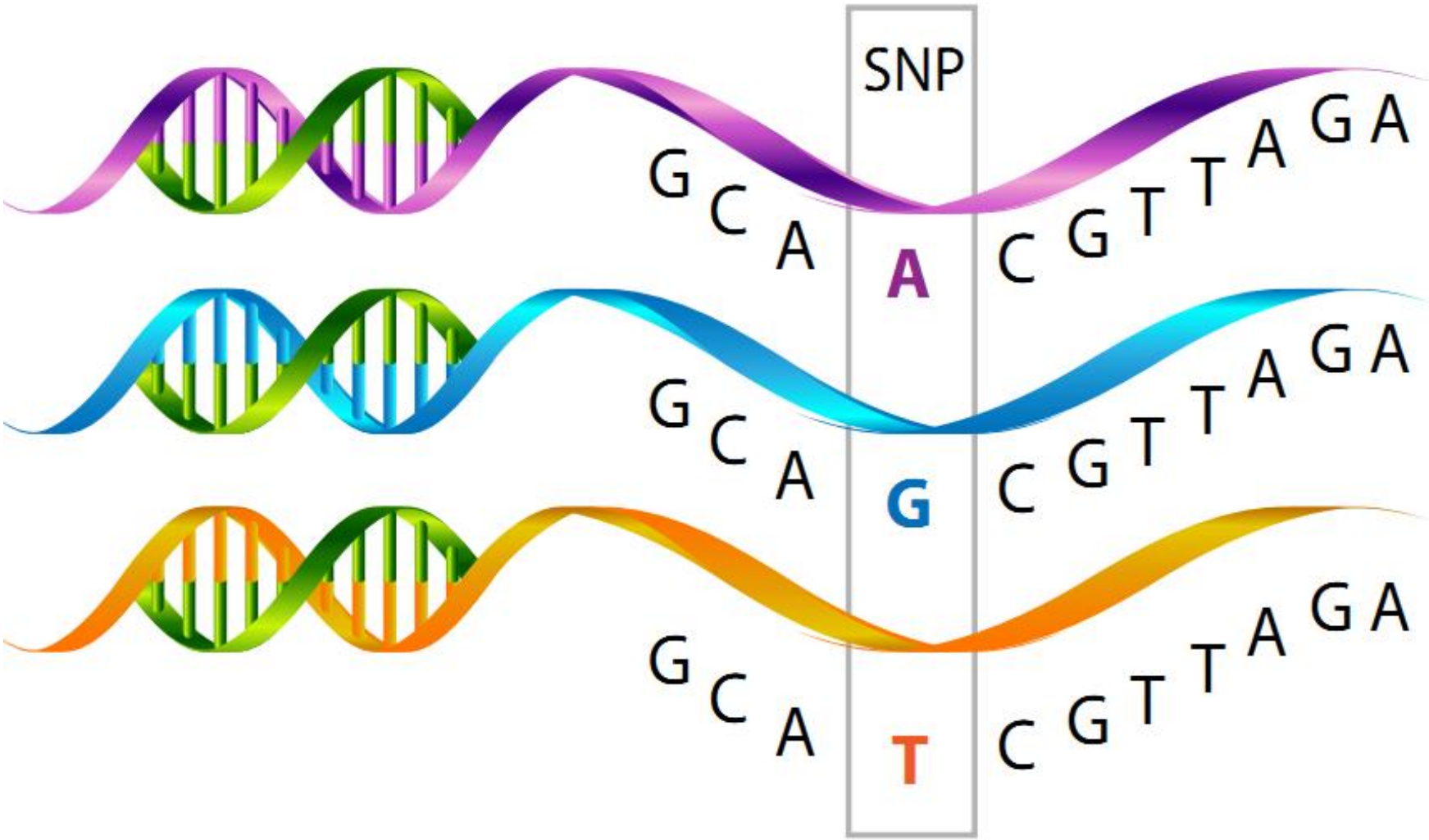
1.1 Frequency of Alleles & Genotypes

Genotype		Phenotype	Observed	Frequency
BB	$\rightarrow$	White	100	0.05
Bb	$\rightarrow$	Yellow	1000	0.50
bb	$\rightarrow$	Red	900	0.45
		Total:	2000	1.00

$$f(B) = p = 0.05 + \frac{0.50}{2} = 0.30$$

$$\begin{aligned} f(b) &= q = 0.45 + \frac{0.50}{2} = 0.70 \\ &= (1 - p) \end{aligned}$$

Genetic Markers



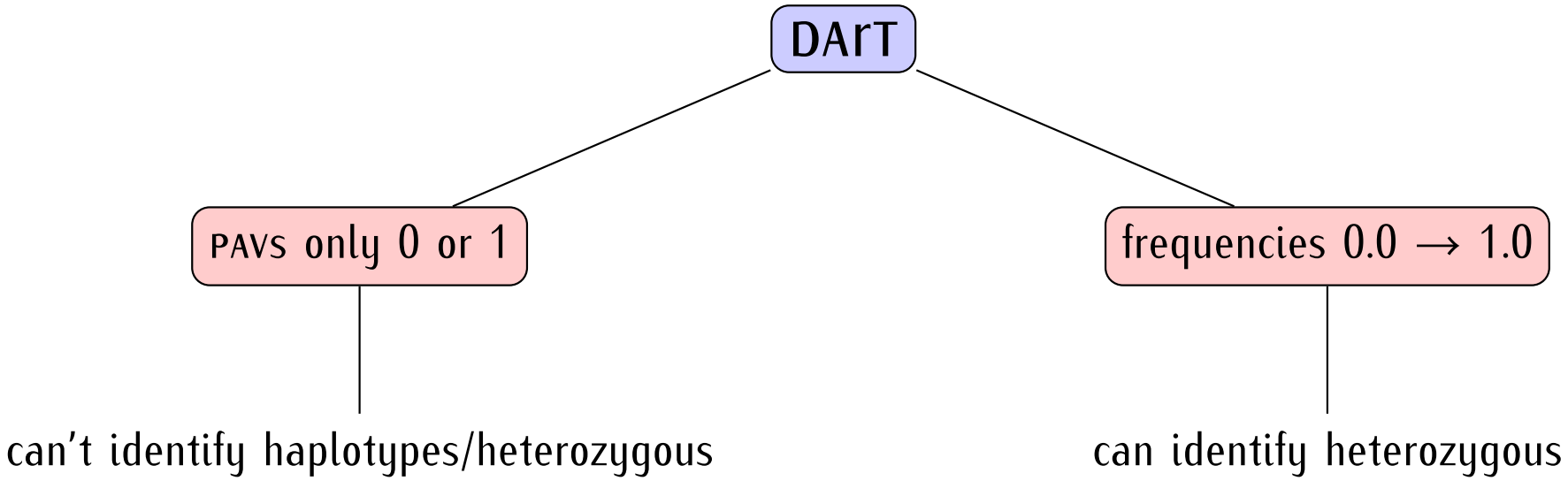
Examples

Marker	Amount	Expression	Polymorphic degree	Specific by locus
Isoenzyme	< 100	codominant	low	✓
RFLP	∞	codominant	medium	✓
RAPD	∞	dominant	medium	–
SSR	∞	codominant	very high	✓
SCAR	∞^*	both	low	✓
AFLP	∞	dominant	high	–
SNP	∞	codominant	very high	✓
DArT	∞	both	very high	✓**

* After the deployment of other kind of markers.

** Clone aren't but markers are.

DArT flexibility...



Maize Bulks w/ frequencies

Marker	Allele	Bulks					
		1	2	3	4	$\cdots$	g
1	1	0.67	*NA**	0.57	*NA*	$\cdots$	0.36
1	2	0.33	*NA*	0.43	*NA*	$\cdots$	0.64
2	1	*NA*	1.00	1.00	1.00	$\cdots$	1.00
2	2	*NA*	0.00	0.00	0.00	$\cdots$	0.00
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\ddots$	$\vdots$
m	1	0.00	*NA*	1.00	0.00	$\cdots$	1.00
m	2	1.00	*NA*	0.00	1.00	$\cdots$	0.00

* *NA* means *Not Available* or missing.

Frequencies, so:

$$p + q = 0.67 + 0.33 = 1.00$$

Maize Genotypes w/ PAV – (presence/absence)

Marker	Allele	Bulks					
		1	2	3	4	⋯	g
1	1	1	*NA*	1	*NA*	⋯	1
2	1	*NA*	1	1	1	⋯	1
⋮	⋮	⋮	⋮	⋮	⋮	⋱	⋮
m	1	1	*NA*	1	1	⋯	1

* *NA* means *Not Available* or missing.

Allele is always 1... the reference allele.

When Genotypes belong to the same bulk, the summary of their PAV generates frequencies

2 Genetic Diversity

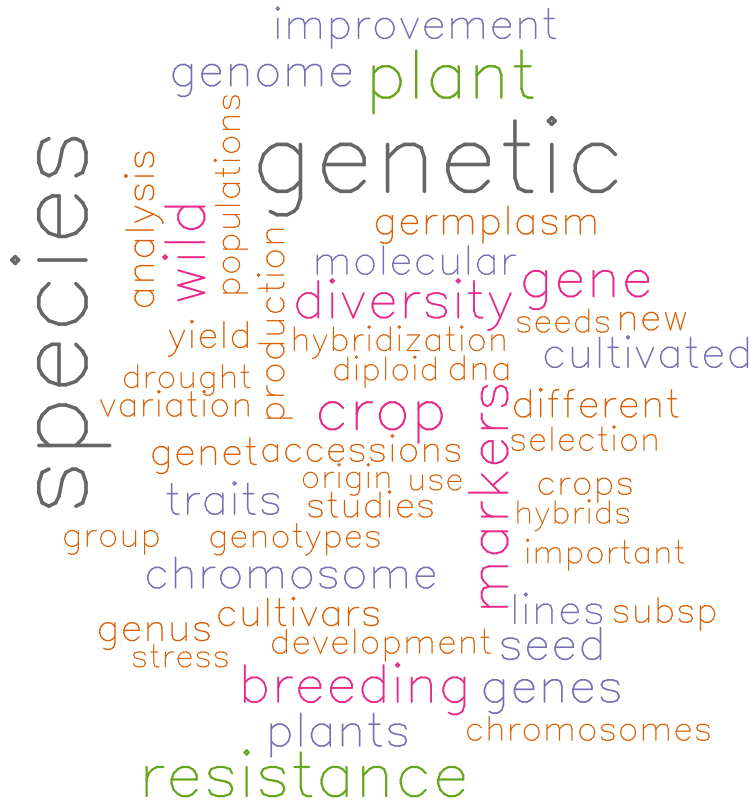
2.1 Genetic Distances

Markers w/ allelic information

For example: SSR, SNP and DArT.

- **Euclidean**;
- **Modified Rogers**; and
- **Cavalli-Sforza & Edwards**.

References: [1, 2, 3]



Euclidean:

$$Ed_{[x,y]} = \sqrt{\sum_{l}^{L}\sum_{a}^{A}(\hat{p}_{x\,la} - \hat{p}_{y\,la})^2} \;,\; \left(0.0 \leq Ed_{[x,y]} \leq \sqrt{2L}^{*}\right)$$

where:

$\hat{p}_{x\,la}$ is the allele frequency for allele a at *locus* l in the genotypes x;

$\hat{p}_{y\,la}$ is the same as above for genotype y;

L is the number of *locus*; and

A is the number of alleles at *locus* l.

*This is the estimator and its respective domain as shown by [3].

Modified Rogers:

$$Rd_{[x,y]} = \frac{1}{\sqrt{2L}} \sqrt{\sum_{l}^{L} \sum_{a}^{A} (\hat{p}_{x\,la} - \hat{p}_{y\,la})^2} \;, \left(0.0 \leq Rd_{[x,y]} \leq 1.0\right)$$

where:

$\hat{p}_{x\,la}$ is the allele frequency for allele a at *locus* l in the genotypes x;

$\hat{p}_{y\,la}$ is the same as above for genotype y;

L is the number of *locus*; and

A is the number of alleles at *locus* l.

For non-diploid species, replace $2L$ by the number of copies.

Cavalli-Sforza & Edwards:

$$Cd_{[x,y]} = \sqrt{\frac{1}{L}\sum_{l}^{L}\left(1 - \sum_{a}^{A}\sqrt{\hat{p}_{x\,la} \times \hat{p}_{y\,la}}\right)}\ , \left(0.0 \leq Cd_{[x,y]} \leq 1.0\right)$$

where:

$\hat{p}_{x\,la}$ is the allele frequency for allele a at *locus* l in the genotypes x;

$\hat{p}_{y\,la}$ is the same as above for genotype y;

L is the number of *locus*; and

A is the number of alleles at *locus* l.

Mean, Variance and Standard Error

$d_{[x,y]}$ is the distance between genotypes x and y, thus:

- **Mean:** $\mu_{d_{[x,y]}} = \frac{1}{\frac{A(A-1)}{2}} \sum_{x<y} d_{[x,y]}$;
- **Variance:** $\sigma^2_{d_{x,y}} = \frac{1}{\frac{A(A-1)}{2}-1} \sum_{x<y} \left(d_{[x,y]} - \mu_{d_{[x,y]}}\right)^2$; and
- **Standard Error:** $S_{\bar{d}_{[x,y]}} = \sqrt{\frac{\sigma^2_{d_{[x,y]}}}{\frac{A(A-1)}{2}}}$.

$\frac{A(A-1)}{2}$ is the 2×2 combination of the alleles i.e., pairs in the distances estimators

Markers w/o allelic information – PAVs

		Genotype x	
		1	0
Genotype y	1	a	b
	0	c	d

- **Simple Matching:** $d_{[x,y]} = \frac{b+c}{a+b+c+d}$;
- **Jaccard:** $d_{[x,y]} = \frac{b+c}{a+b+c}$; and
- **Nei and Li (or Dice):** $d_{[x,y]} = \frac{2(b+c)}{2a+b+c}$.

The same references for the previous cases i.e., [1, 2, 3].

Diversity Indices

A locus is polymorphic if, and only if, the frequency of one of its alleles is ≤ 0.95 or 0.99

Raw statisitcs

- **Polymorphic proportion:** $P = \frac{n_{poly}}{n_{total}}$; and
- **Mean allele number by locus:** $n_a = \frac{1}{L} \sum_l^L n_l$

Others

- **Observed Heterozygosity** (Ho);
- **Expected Heterozygosity** (He);
- **Effective Number of Alleles** (Ae); and
- **Shannon Index** (SH).

Observed Heterozygosity:

It is defined as the percentage of heterozygous loci per individual or the number of heterozygous individuals per locus.

Expected Heterozygosity:

$$He = \frac{1}{L}\sum_{l}^{L}\left(1 - \sum_{a}^{A}\hat{p}_{la}^{2}\right) \quad , (0.0 \leq He \leq 1.0)$$

where:

$\hat{p}_{la}$ is the estimated frequency of the allele a at locus l; and
the other quantities (L and A) were already defined.

NOTE:

$$\sum_{a}^{A}\hat{p}_{la} = 1.0$$

Effective Number of Alleles:

$$Ae_l = \frac{1}{\sum_a^A \hat{p}_a^2}$$

$$Ae = \frac{1}{L} \sum_l^L Ae_l$$

NOTE:

$$Ae_l = \frac{1}{1 - He_l}$$

Shannon Index:

- Total Frequencies:

$$SH_{\text{Total}} = -\sum_{a}^{A} \hat{p}_a \log_{10}(\hat{p}_a) \ , \sum_{a}^{A} \hat{p}_a = 1.0$$

- By Locus Frequencies:

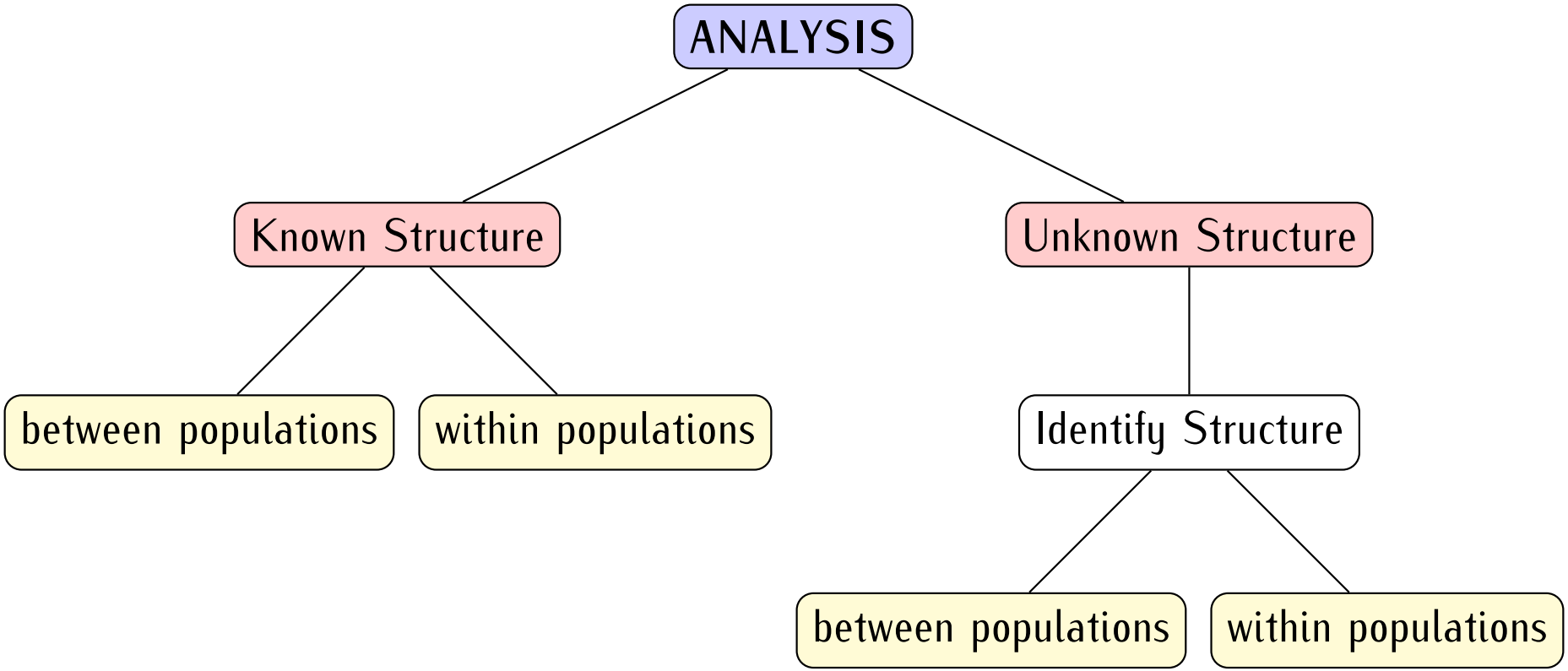
$$SH_{\text{Locus}} = -\sum_{a}^{A} \hat{p}_a \log_{2}(\hat{p}_a) \ , \sum_{a}^{A} \hat{p}_a = L \therefore \left(0.0 \leq SH_{\text{Locus}} \leq L\right)$$

Diversity Indices for PAVs

Statistics . . . :

- Polymorphic proportion ⊘
- Mean number of alleles by locus ⊘
- Observed Heterozygosity (Ho) ⊘
- Expected Heterozygosity (He) ⊘
- Effective Number of Alleles (Ae) ⊘
- Shannon Index (SH) ⊘

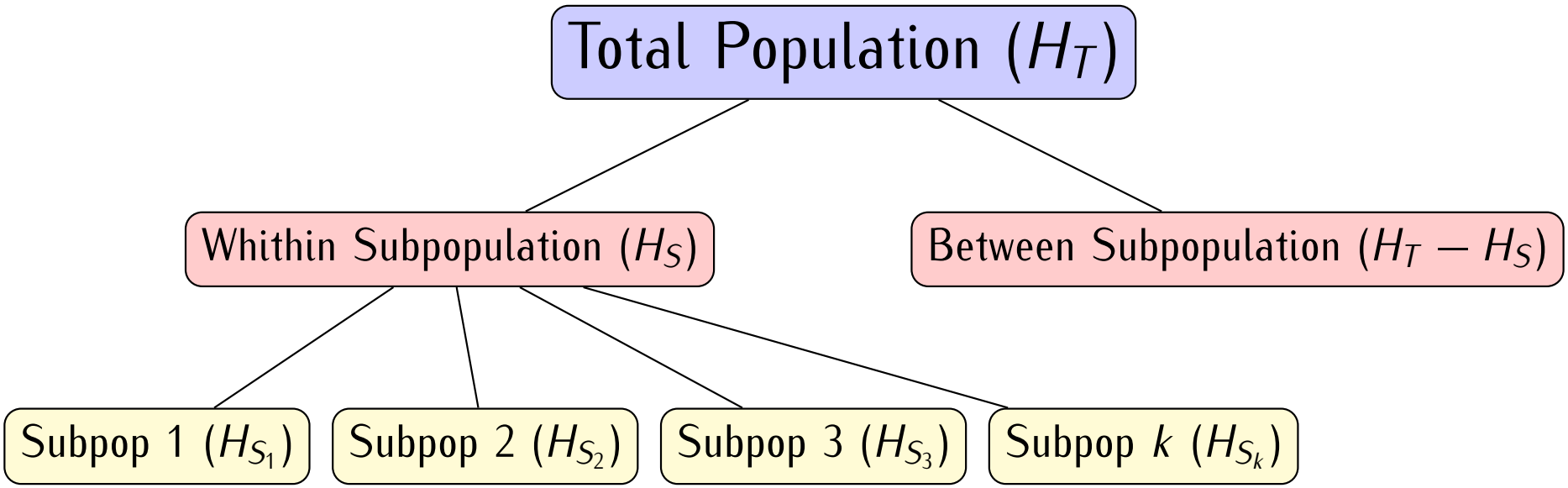
3 Intraspecific Diversity (*1st branch*)



Recapping ...

Parameter	Estimator
Population (by locus)	$He_l(H_{T_l}) = 1 - \sum_a^2 \hat{p}_{a_l}^2$
Population (average)	$He(H_T) = \frac{1}{L} \sum_l^L He_l$
Subpopulation$_i$ (by locus)	$He_{i_l} = 1 - \sum_a^2 \hat{p}_{i_{a_l}}^2$
Subpopulation$_i$ (average)	$He_i = \frac{1}{L} \sum_l^L He_{i_l}$
Within Subpopulation	$H_S = \frac{1}{I} \sum_i^I He_i$
Between Subpopulation	$D_{ST} = H_T - H_S$

$$\text{Total} = \text{Between} + \text{Within}$$



3.1 Wright Statistics (F)

F_{IS} Heterozygosis proportional deviations within subpopulations:

$$F_{IS} = \frac{H_S - Ho}{H_S} \; [-1; 1]$$

F_{IT} Overall heterozygosis proportional deviation (inbreeding coefficient):

$$F_{IT} = \frac{H_T - Ho}{H_T} \; [-1, 1]$$

F_{ST} Heterozygosis proportional deviation between subpopulations:

$$F_{ST} = \frac{H_T - H_S}{H_T} \; [0; 1]$$

Nei, 1987 [4] . . . :

1. Obtain H_T:

$$H_T = \frac{1}{L} \sum_{l}^{L} He_l$$

2. Obtain H_s (by locus):

$$H_S = \frac{1}{L} \sum_{l}^{L} He_{S_l}$$

3. Thus, obtain G_{ST}:

$$G_{ST} = \frac{D_{ST}}{HT} = \frac{H_T - H_S}{H_T} = 1 - \frac{H_S}{H_T}$$

Berg, 1997 [1] . . . :

1. Obtain G_{ST} by locus:

$$G_{ST} = \frac{D_{ST}}{HT} = \frac{H_T - H_S}{H_T} = 1 - \frac{H_S}{H_T}$$

2. Summarize as average:

$$\bar{G}_{ST} = \frac{1}{n_a} \sum_{n}^{n_a} G_{ST_n}$$

3. That is the same as:

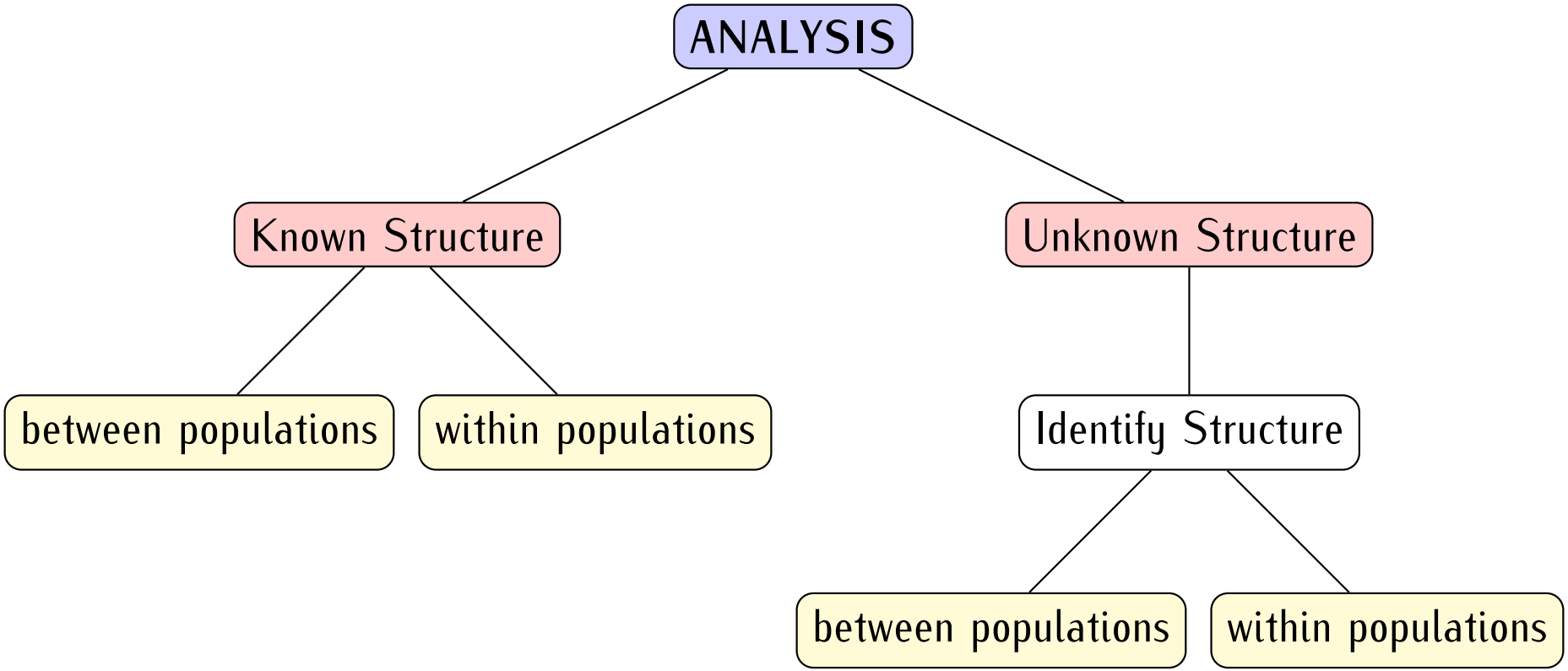
$$\bar{G}_{ST} = 1 - \frac{\sum_{n}^{n_a} \left(\frac{H_{S_n}}{H_{T_n}} \right)}{n_a}$$

Be aware ...

- When we have just one (1) allele: $G_{ST} = F_{ST}$;
- G_{ST} generalizes F_{ST};
- G_{ST} is the proportion of total diversity that is between subpopulations; and
- Thus, $(1 - G_{ST})$ is the proportion of total diversity within subpopulations.
- The meaning of diversity according to F_{ST}:

F_{ST}	means
$[0; 0.05)$	small
$[0.05; 0.15)$	medium
$[0.15; 0.25)$	high
≥ 0.25	very high

. . . Intraspecific Diversity (*2nd branch*)



4 Indentifying Groups

OBJECTIVE:

Minimize within variability → Maximize between variability

References can be consulted for deep understanding:

- Fundation book ...[5];
- Fundation paper in genetics (Nei) ...[4]; and
- More modern reference ...[6].

$$\mathbf{Y}_{n\times p} = \begin{bmatrix} y_{1,1} & y_{1,2} & y_{1,3} & \cdots & y_{1,p} \\ y_{2,1} & y_{2,2} & y_{2,3} & \cdots & y_{2,p} \\ y_{3,1} & y_{3,2} & y_{3,3} & \cdots & y_{3,p} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ y_{n,1} & y_{n,2} & y_{n,3} & \cdots & y_{n,p} \end{bmatrix}$$

NOTE:

Everything starts from obtaining the distance matrix between all 2×2 pairs ...

$$\frac{n(n-1)}{2}$$

$$\boldsymbol{D} = \begin{bmatrix} 0 & d_{1,2} & d_{1,3} & \cdots & d_{1,j} & \cdots & d_{1,n} \\ & 0 & d_{2,3} & \cdots & d_{2,j} & \cdots & d_{2,n} \\ & & 0 & \cdots & d_{3,j} & \cdots & d_{3,n} \\ & & & \ddots & \vdots & & \vdots \\ & & & & 0 & \cdots & d_{j,n} \\ & & & & & \ddots & \vdots \\ & & & & & & 0 \end{bmatrix}$$

$d_{i,i} = 0.0$ and D is symmetric $\therefore d_{i,j} = d_{j,i}$

Clustering Methods

- **Geometrical:**
  - Hierarchical;
  - Neighbor Joining;
  - *k-means* (density search); and others
- **MANOVA:**

$$\text{Total} = \text{Between} + \text{Within}$$

- **Statistical:**

  Mixture and Bayesian (STRUCTURE)

Hierarchical:

1. All distances;
2. Find the most close individuals (smaller distance); and
3. Iterate over that.
    - UPGMA: merge groups with smaller distance;
    - WARD: merge groups that generate a new one with smaller $S.S._W$; and
    - NJ: merge groups if: (*i*) smaller distance & (*ii*) higher distances in comparison to the others.

Bayesian:

The model account for the presence of HW or LD, introduces population structure...find population structure (groups) with the smaller possible disequilibrium [6].

Graphical Representation:

Multidimensional Scaling (MDS)

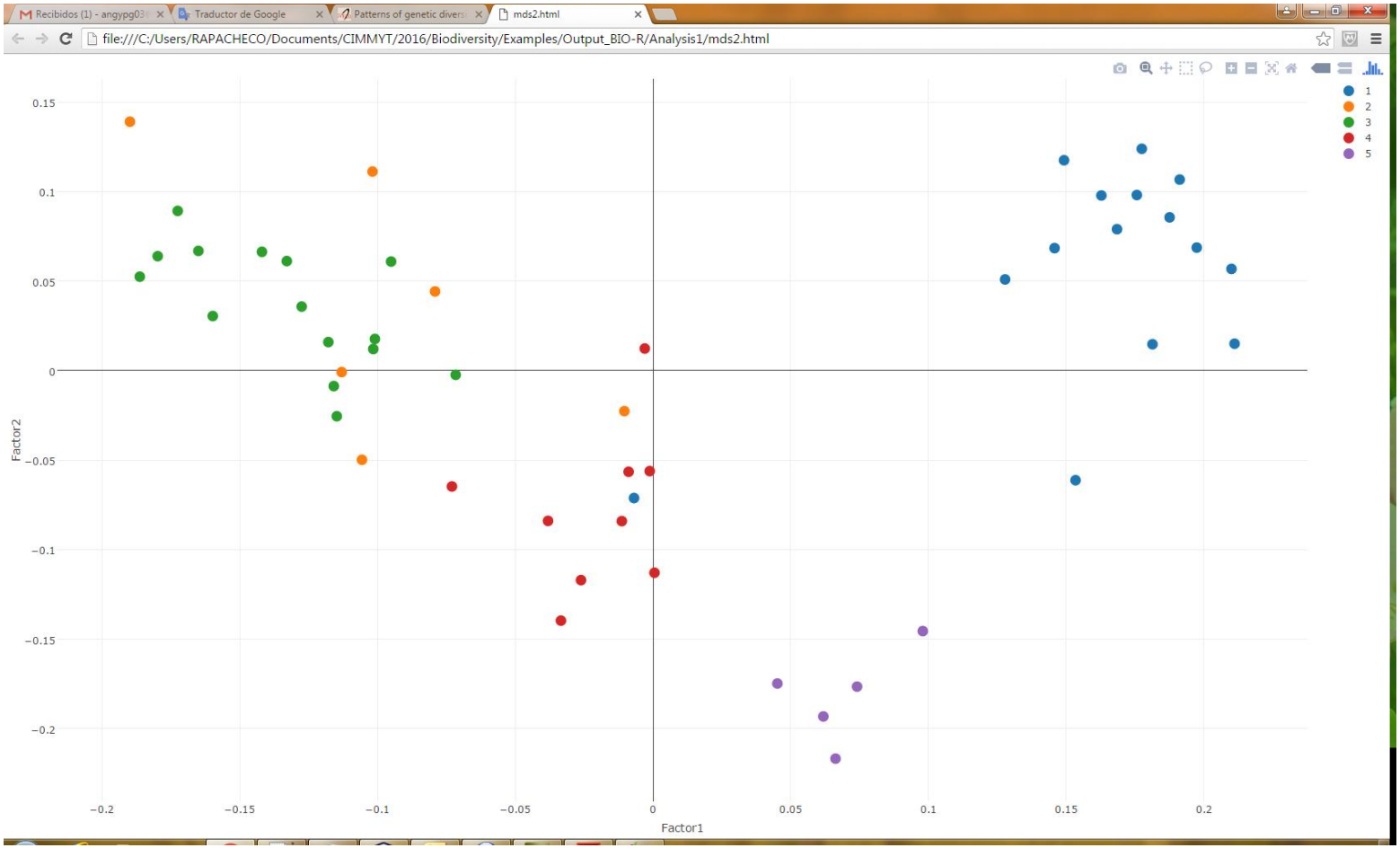
Two concepts:

1. **Similarity** ($s_{[x,y]}$): $d_{[x,y]} = \sqrt{2 \times \left(1 - s_{[x,y]}\right)}$;

2. ***stress*** (S): $S = \sqrt{\frac{\sum_x \sum_y \left(d_{[x,y]} - \mu_{d_{[x,y]}}\right)^2}{\frac{n(n-1)}{2}}}$

NOTE:

Search for the reduced representation (2 or 3 dimensions) that minimizes the $S.S._B$ in the p dimensional space and with minimum the estimated distance.


mds2.html
file:///C:/Users/RAPACHECO/Documents/CIMMYT/2016/Biodiversity/Examples/Output_BIO-R/Analysis1/mds2.html
1
2
3
4
5
0.15
0.1
0.05
0
−0.05
−0.1
−0.15
−0.2
Factor2
−0.2
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−0.1
−0.05
0
0.05
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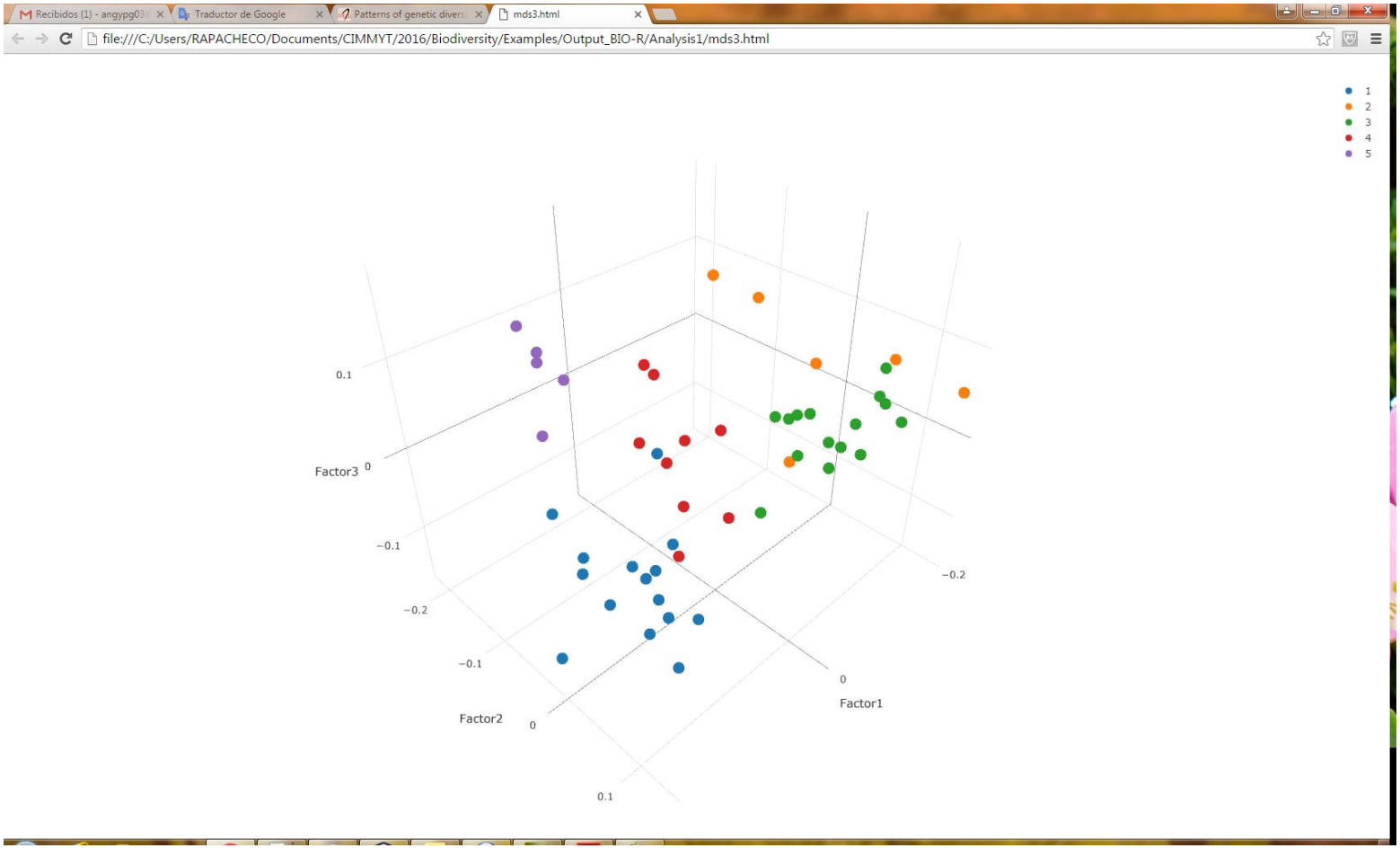
0.2
Factor1


Recibidos (1) - angypg03
Traductor de Google
Patterns of genetic diversi
mds3.html
file:///C:/Users/RAPACHECO/Documents/CIMMYT/2016/Biodiversity/Examples/Output_BIO-R/Analysis1/mds3.html
1
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5
0.1
Factor3
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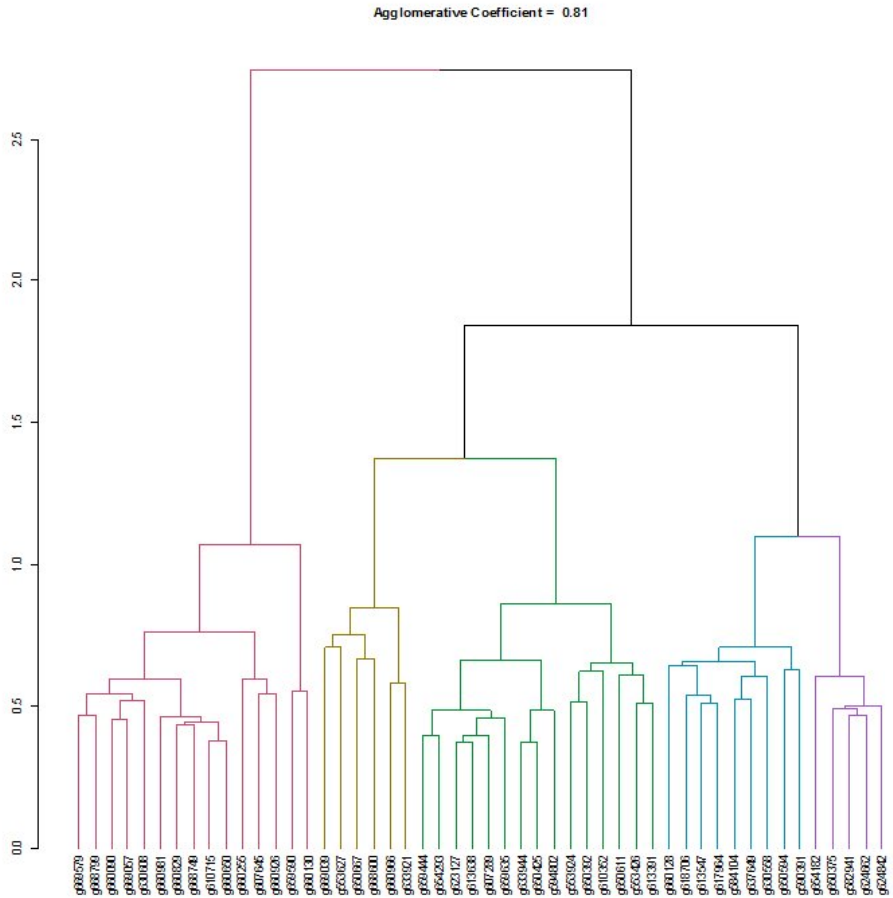
−0.1
−0.2
−0.1
Factor2
0
0.1
−0.2
0
Factor1

Trees *a.k.a.* Dendograms:



5 Software BIO-R and family...

- **Phenotypic data**
  - *Experimental Design:* ADEL, AUDE & STAD;
  - *Individual & Multi-Env:* META ($>$ 1500), AGD ($>$ 1300);
  - *Interaction/Stability:* GEA ($>$ 1800); and
  - *Spatial Analysis:* SPAT.
- **Genotypic data**
  - *kinship:* BROWSE & COP; and
  - *Diversity:* BIO.
- **Fusion data**
  - *Relationship/Interaction:* GEA;
  - *Genome prediction:* BGLR (*R package & application tool); and
  - *Selection Index:* SI ($>$ 300), RINDSEL.
- **Decision:** *Eval* $L \times T$.

Download

- BIO-R is comming soon... **go to:** `http://data.cimmyt.org`;
- Java interface/application for embedding *R* [7] scripts to perform Diversity analysis; and
- heterozygosity, diversity B & W groups, shannon index, number of effective allele, % of polymorphic loci, Rogers & Nei distance, clusters and multidimensional scaling (2 & 3d plots).

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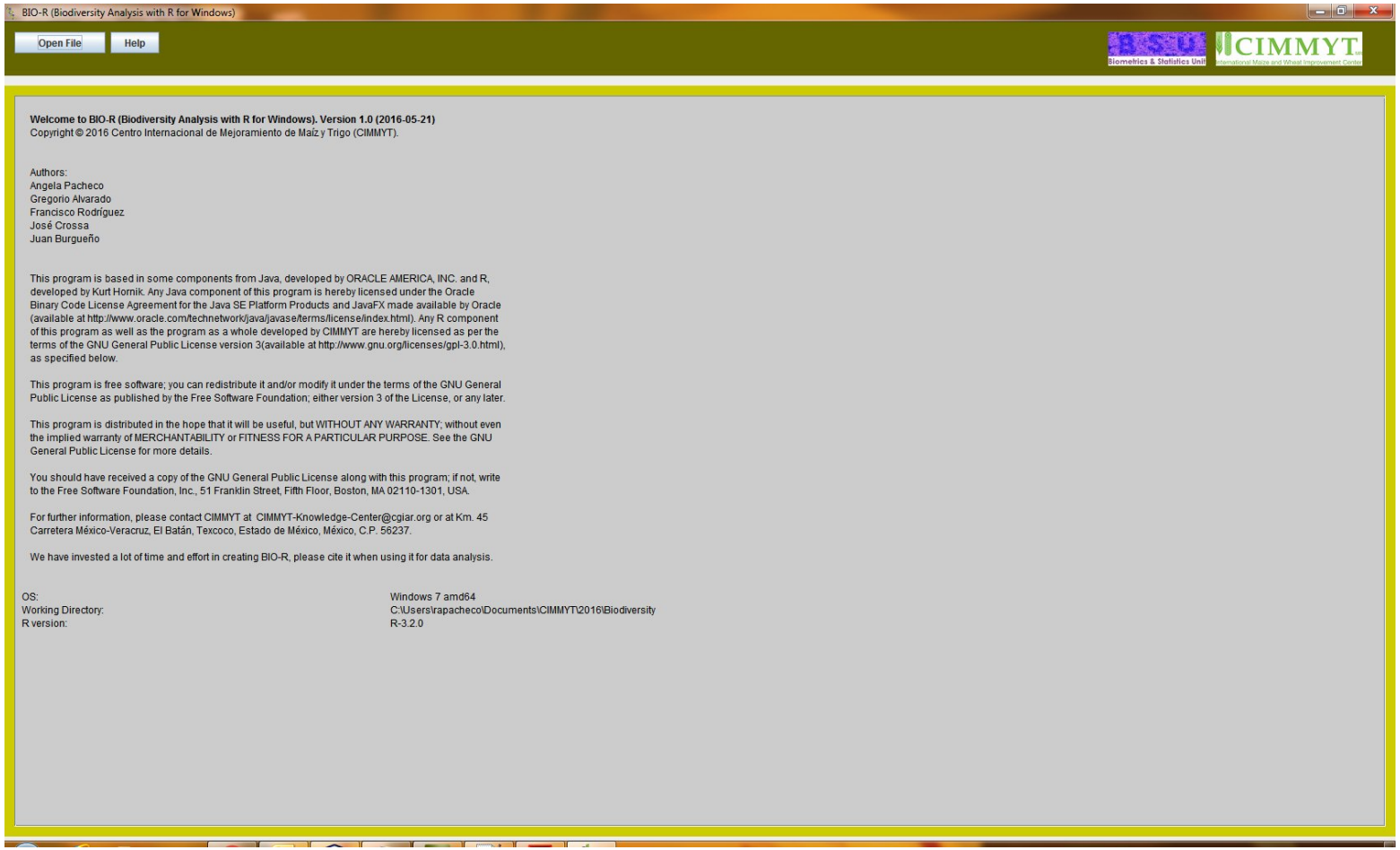
BIO-R (Biodiversity Analysis with R for Windows)
Open File
Help
Biometrics & Statistics Unit
CIMMYT
International Maize and Wheat Improvement Center
Welcome to BIO-R (Biodiversity Analysis with R for Windows). Version 1.0 (2016-05-21)
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Juan Burgueño
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You should have received a copy of the GNU General Public License along with this program; if not, write to the Free Software Foundation, Inc., 51 Franklin Street, Fifth Floor, Boston, MA 02110-1301, USA.
For further information, please contact CIMMYT at CIMMYT-Knowledge-Center@cgiar.org or at Km. 45 Carretera México-Veracruz, El Batán, Texcoco, Estado de México, México, C.P. 56237.
We have invested a lot of time and effort in creating BIO-R, please cite it when using it for data analysis.
OS: Windows 7 amd64
Working Directory: C:\Users\rapacheco\Documents\CIMMYT\2016\Biodiversity
R version: R-3.2.0

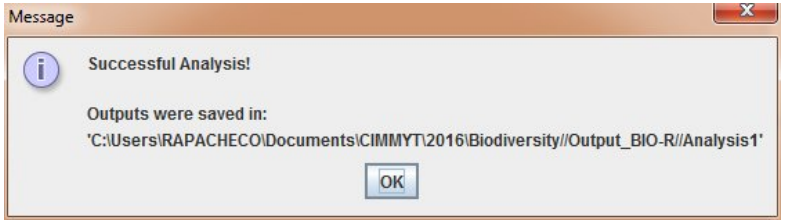
Input data `MyData.csv`

mark	*g1*	*g2*	*g3*	*g4*	...	*gX*
1	*0.67*	*NA*	*0.57*	*NA*	...	*1*
2	*NA*	*1*	*1*	*1*	...	*NA*
3	*1*	*1*	*0.52*	*0.50*	...	*0.80*
4	*1*	*NA*	*0.50*	*1*	...	*NA*
5	*1*	*NA*	*1*	*NA*	...	*1*
6	*0.67*	*0*	*0.71*	*1*	...	*0.33*
7	*1*	*NA*	*0*	*1*	...	*1*
8	*1*	*NA*	*1*	*1*	...	*1*
9	*NA*	*1*	*0.60*	*0.22*	...	*0.71*
⋮	⋮	⋮	⋮	⋮	⋱	⋮
N	*1*	*1*	*1*	*1*	...	*1*

Setting parameters and analysing...

1. *Markers* – selects the column that identify the **markers**;
2. *# Clusters* – type the number of groups to split the population;
3. *Output folder* – type the path of the output folder where results will be saved;
    - It will be created inside the BIO-R's Output folder;
    - You can change the name for different sets; and
    - It is necessary to change the name for each analysis.
4. *Genotypes* – selects the columns that identify the **genotypes**;
5. *Distance* – selects the method to calculate **distances**; and
6. *ColorMDSPlot* – can specify **.csv* file containing additional information for colors in MDS plot (see manual).

Results...



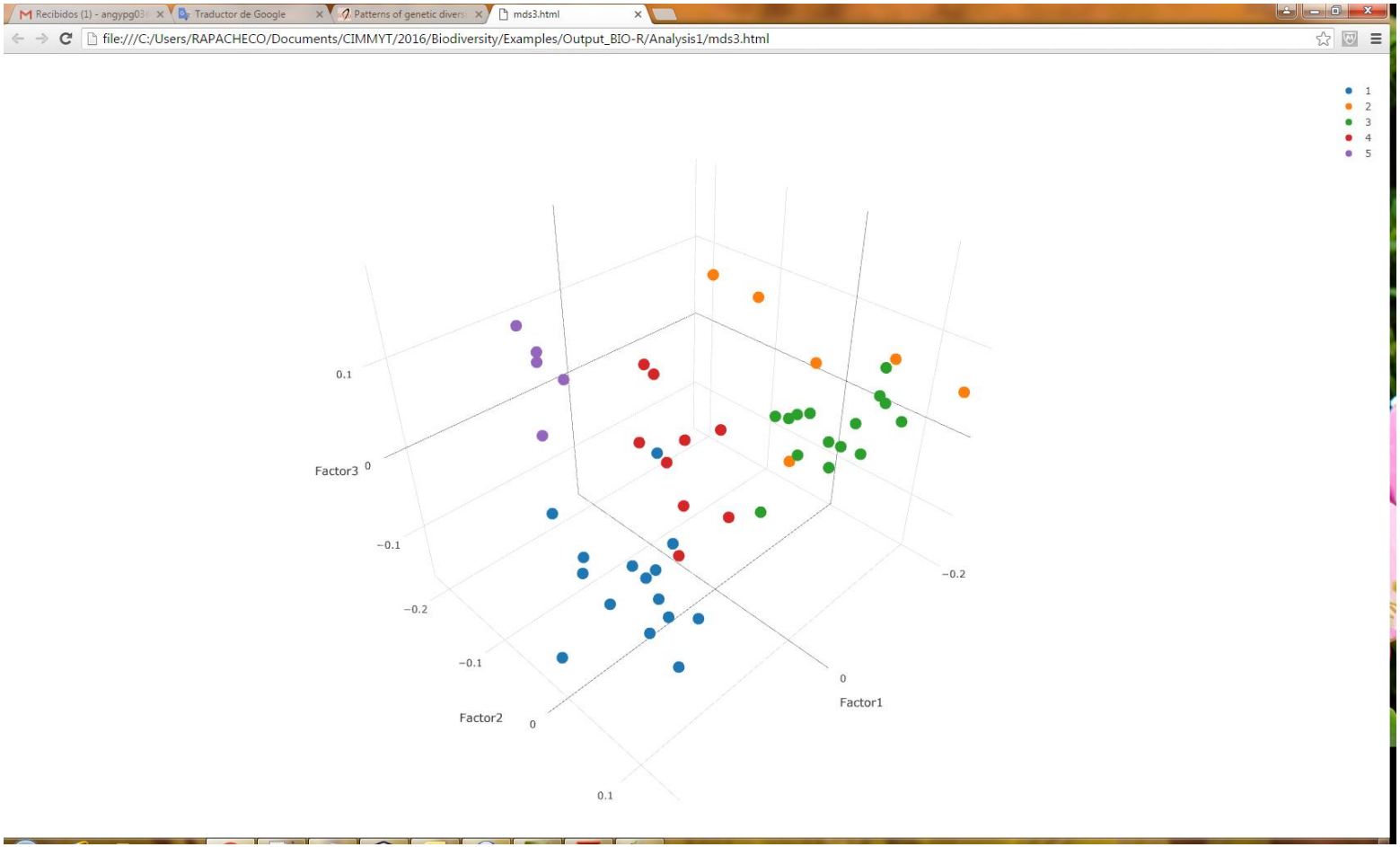
- Output
  - Analysis 1
    - CalculusPerGenotype.csv
    - CalculusPerLocus.csv
    - Dendogram.wmf
    - mds2.html
    - mds3.html
    - MDStable.csv
    - RogersDistances.csv
    - SummaryDiversityAnalysis.csv
    - mds2_files
    - mds3_files

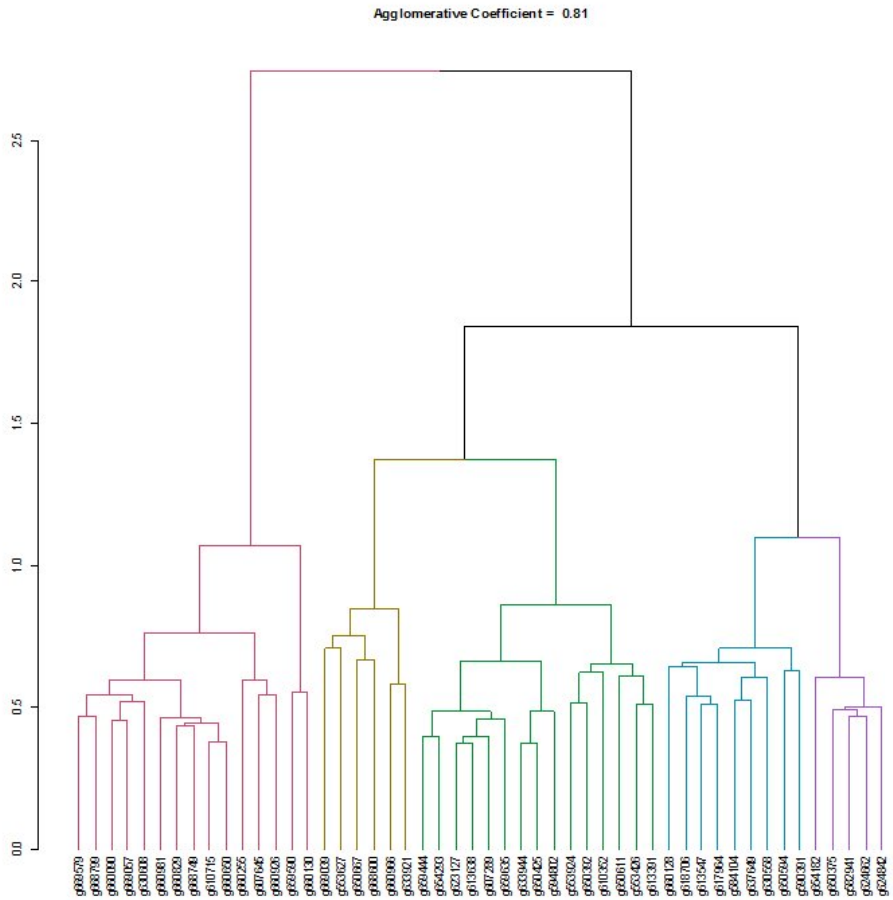
Output : `CalculusPerLocus.csv`

Marker	*He*	*Ho*	*Ae*	*Shannon*	*%NA*
1	*0.50*	*0.29*	*1.99*	*0.95*	*0.22*
2	*0.01*	*-0.05*	*1.01*	*0.02*	*0.08*
3	*0.45*	*0.38*	*1.82*	*0.93*	*0.16*
4	*0.41*	*0.42*	*1.69*	*0.87*	*0.24*
5	*0.03*	*-0.08*	*1.03*	*0.11*	*0.10*
6	*0.40*	*0.43*	*1.68*	*0.86*	*0.20*
7	*0.50*	*0.31*	*2.00*	*0.99*	*0.24*
8	*0.49*	*0.47*	*1.94*	*0.98*	*0.18*
9	*0.45*	*0.47*	*1.82*	*0.93*	*0.14*
⋮	⋮	⋮	⋮	⋮	⋮
N	*0.08*	*-0.03*	*1.09*	*0.26*	*0.12*

Output : CalculusPerGenotype.csv

Genotype	He	Ho	Ae	Shannon	%NA	clusterGroup
1	0.39	0.06	1.63	0.83	0.11	1
2	0.38	-1.46	1.62	0.82	0.37	2
3	0.40	0.51	1.67	0.85	0.03	3
4	0.39	0.03	1.64	0.83	0.19	4
5	0.37	0.39	1.60	0.81	0.03	3
6	0.41	-3.56	1.69	0.86	0.42	1
7	0.39	0.34	1.65	0.84	0.04	3
8	0.37	-0.28	1.54	0.81	0.24	5
9	0.40	-0.70	1.61	0.82	0.32	1
⋮	⋮	⋮	⋮	⋮	⋮	⋮
X	0.38	0.33	1.66	0.84	0.04	3


mds3.html
file:///C:/Users/RAPACHECO/Documents/CIMMYT/2016/Biodiversity/Examples/Output_BIO-R/Analysis1/mds3.html
1
2
3
4
5
Factor3
0.1
0
−0.1
−0.2
Factor2
−0.1
0
0.1
Factor1
0
−0.2


Agglomerative Coefficient = 0.81
0.0
0.5
1.0
1.5
2.0
2.5

Summary: `SummaryDiversityAnalysis.csv`

- **% of polymorphic loci:** 0.94;
- **Exp. Heterozygosity:** 0.30;
- **Std. Dev. of** *He*: 0.01;
- **Obs. Heterozygosity:** 0.22;
- **Std. Dev. of** *Ho*: 0.01;
- **Number of effective alleles:** 1.55;
- **Std. Dev. of** *Ae*: 0.02;
- **Shannon Index:** 0.63;
- **Std. Dev. Shannon:** 0.02 ...

Remarks

- Frequency of Alleles & Genotypes;

  (Population Genetics) The fundamental concept!

- Distances & Diversity Indices;

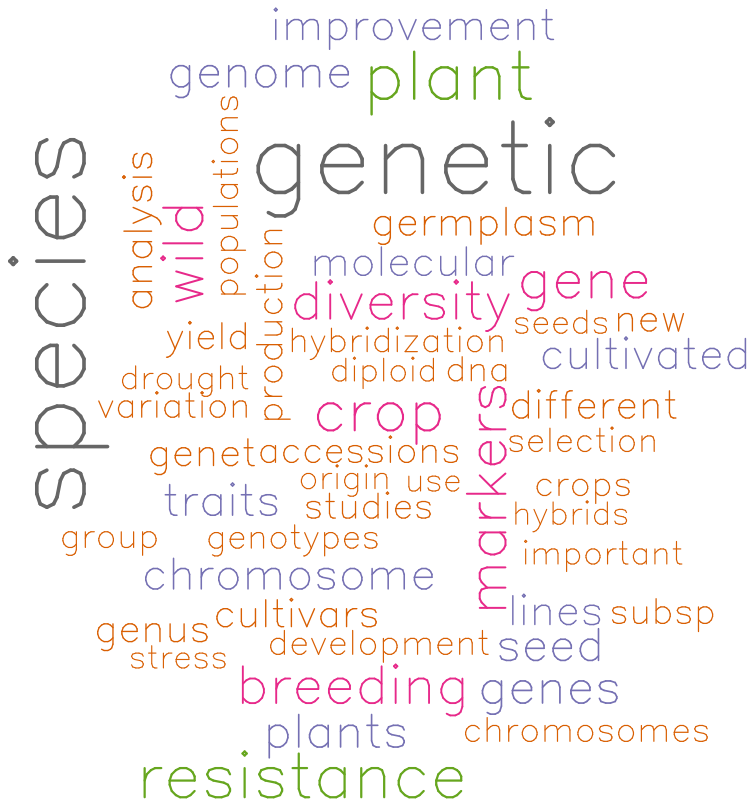
  (Genetic Diversity) Several ways and flavors.

- Wright Statistics;

  (Intraspecific Diversity) Between/Within variability

- BIO-R; and

  (Software) it has been built considering everything you may want



Questions/Suggestions??

For Further Studies:

[1] E. E. Berg and J. L. Hamrick, "Quantification of genetic diversity at allozyme loci," *Canadian Journal of Forest Research*, vol. 27, pp. 415–429, 1997.

[2] S. A. Mohammadi and B. M. Prasana, "Analysis of genetic diversity in crop plants – salient statistical tools and considerations," *Crop Science*, vol. 43, pp. 1235–1248, 2003.

[3] J. C. Reif, A. E. Melchinger, and M. Frish, "Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management," *Crop Science*, vol. 45, pp. 1–7, 2005.

[4] N. Saitou and M. Nei, "The neighbour-joining method: A new method fo reconstructing phylogenetic trees," *Molecular Biology and Evolution*, vol. 4, no. 4, pp. 406–425, 1987.

[5] L. Kaufman and P. J. Rousseeuw, *Finding groups in data: an introduction to cluster analysis*. New York: John Wiley, 1990.

[6] J. K. Pritchard, M. Stephens, and P. Donnely, "Inference of population structure using multilocus genotype data," *Genetics*, pp. 945–959, 2000.

[7] R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2016.

That's all folks!

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