Analysis of adaptive genetic variation

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Outline

❖ Introduction
❖ What?
❖ How to?
❖ Methods and tools
❖ Examples
❖ [Practical issues/Discussion...]
Pre-Introduction

Usefulness
Exportable methodology
Practical cases
Net annual gain/lost 1990-2015
<table>
<thead>
<tr>
<th>Variable (unidad, año)</th>
<th>Total</th>
<th>Dirección del cambio</th>
<th>Cambio anual (%)</th>
<th>Disponibilidad de datos (situación/tendencia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Área de bosque (millones de ha, 2015)</td>
<td>624</td>
<td>↓</td>
<td>-0,49</td>
<td>A/A</td>
</tr>
<tr>
<td>Bosque natural (millones de ha, 2015)</td>
<td>600</td>
<td>↓</td>
<td>-0,54</td>
<td>A/A</td>
</tr>
<tr>
<td>Bosque plantado (millones de ha, 2015)</td>
<td>16</td>
<td>↑</td>
<td>1,34</td>
<td>A/A</td>
</tr>
<tr>
<td>Cambio neto anual del bosque (millones de ha, 2010–2015)</td>
<td>-2,8</td>
<td></td>
<td></td>
<td>A/*</td>
</tr>
<tr>
<td>Cambio neto anual del bosque natural (millones de ha, 2010–2015)</td>
<td>-3,1</td>
<td></td>
<td></td>
<td>A/*</td>
</tr>
<tr>
<td>Cambio neto anual del bosque plantado (millones de ha, 2010–2015)</td>
<td>0,2</td>
<td></td>
<td></td>
<td>A/*</td>
</tr>
<tr>
<td>Existencias en formación en los bosques (miles de millones de m³, 2015)</td>
<td>79</td>
<td>↓</td>
<td>-0,37</td>
<td>A/A</td>
</tr>
<tr>
<td>Existencias en formación en los bosques (m³ por ha, 2015)</td>
<td>128</td>
<td>↑</td>
<td>0,13</td>
<td>A/A</td>
</tr>
<tr>
<td>Carbono en la biomasa por encima y por debajo del suelo (Gt, 2015)</td>
<td>60</td>
<td>↓</td>
<td>-0,43</td>
<td>A/A</td>
</tr>
<tr>
<td>Carbono en la biomasa por encima y por debajo del suelo (toneladas por ha, 2015)</td>
<td>96</td>
<td>↑</td>
<td>0,07</td>
<td>A/A</td>
</tr>
</tbody>
</table>
During the last 25 years the forest biomass Carbon stock has been reduced 11,1 Gt, It equivalents a reduction of 442 millions of tons at year
Introduction

Climate dynamics

Genetics (adaptation)

Human activity
DIFFERENT TIME SPAN BUT SIMILAR EFFECTS

Evolution forces

Forest Management

Other Human activ.

Conservation & Sustainable Use

Genetics: Evolution Forces

• Genetic drift: non directional / random changes in frequency between generations / small pops.

• Natural Selection: Adaptive changes (fitness, repro. success)

• Migration: Gen exchange among populations.

• Reproductive Systems: Gene recombination in successive generations

• Mutation: Gene changes at molecular level

• “Life’s little mysteries” Gene flexibilty (Phen-Plas)

Autorganizative, novelties, ....
Genetic diversity

- Existing variation among “populations”
- As consequence of evolution processes & ...

- One of the most powerful resource for fighting against global change
Environmental intraspecific Variation

(a)

13 Year height (cm)

Degree-days > 5°C

(b)

13 Year height (cm)

Summer—Winter differential (°C)

Adaptive Variation

Contrary to “neutral” variation
Environmental adaptation related traits
Survival
Growth (Low: suboptimal or drought defense)
Water use efficiency

Low economical importance but evolutive/adaptive
Reproductive potential (flowering, mating synchr., seed ripening, etc)

\[ P = G + E \]
\[ V_p = V_G + V_E = V_A + V_{NA} + V_E \]
Use for Conservation FGR

"Quantification"

Structure, distribution & patterns of Adapt. variation inter/intra pop.

Variation in metapopulations

Basic information in reduced $N_e$ populations

Info about threatening level

Genetic diversity based on neutral markers can NOT substitute direct measurements of quantitative variation in adaptive traits and particularly for forest genetic resources conservation

Quantitative Genetics:
**NO** direct relation
phenotype & genotype
Continuous distribution genotypes

Mendel Laws

**What**

![Genetic Material Diagram](image)
What / How to

Goal

Tools for addressing analytical problems without fixed laws

Variability

• Existence

• Dealing and understanding (adaptiveness?)

• Modeling and controlling experimental error
Population genetic (above diag) and Pearson (below diag.) correlations with **n.s. indiv. heritabilities** in diagonal.

Standard errors in small numbers.

<table>
<thead>
<tr>
<th>$h^2_{\text{indiv}}$</th>
<th>c</th>
<th>b</th>
<th>m</th>
<th>t10</th>
<th>t90</th>
<th>dur</th>
<th>budset</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>1.022</td>
<td>-0.249</td>
<td>0.084</td>
<td>0.6077</td>
<td>0.0162</td>
<td>0.1968</td>
<td>0.0295</td>
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<tr>
<td>b</td>
<td>-0.33</td>
<td>-0.027</td>
<td>0.5428</td>
<td>0.198</td>
<td>0.0037</td>
<td>0.5155</td>
<td>0.0225</td>
</tr>
<tr>
<td>m</td>
<td>0.428</td>
<td>0.0250</td>
<td>-0.7951</td>
<td>0.0407</td>
<td>0.013</td>
<td>0.9639</td>
<td>0.0022</td>
</tr>
<tr>
<td>t10</td>
<td>0.4238</td>
<td>0.0252</td>
<td>0.2265</td>
<td>0.0390</td>
<td>0.0076</td>
<td>0.9402</td>
<td>0.0036</td>
</tr>
<tr>
<td>t90</td>
<td>0.2338</td>
<td>0.0290</td>
<td>-0.8971</td>
<td>0.0306</td>
<td>0.0169</td>
<td>0.9654</td>
<td>0.0036</td>
</tr>
<tr>
<td>dur</td>
<td>0.1535</td>
<td>0.0299</td>
<td>0.8655</td>
<td>0.0313</td>
<td>0.0008</td>
<td>0.9654</td>
<td>0.0036</td>
</tr>
<tr>
<td>budset</td>
<td>0.241</td>
<td>0.0289</td>
<td>-0.8655</td>
<td>0.0313</td>
<td>0.0103</td>
<td>0.9654</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

**Fam** standard deviations:

<table>
<thead>
<tr>
<th>$h^2_{\text{fam}}$</th>
<th>c</th>
<th>b</th>
<th>m</th>
<th>t10</th>
<th>t90</th>
<th>dur</th>
<th>budset</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>0.9182</td>
<td>0.0150</td>
<td>-0.1037</td>
<td>0.0303</td>
<td>0.1831</td>
<td>0.0296</td>
<td>0.5324</td>
</tr>
<tr>
<td>b</td>
<td>-0.2723</td>
<td>0.0284</td>
<td>0.8469</td>
<td>0.0264</td>
<td>-0.778</td>
<td>0.0121</td>
<td>0.0798</td>
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<tr>
<td>m</td>
<td>0.434</td>
<td>0.0249</td>
<td>-0.5731</td>
<td>0.0206</td>
<td>0.8265</td>
<td>0.0295</td>
<td>0.299</td>
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<tr>
<td>t10</td>
<td>0</td>
<td>0.0235</td>
<td>0.0028</td>
<td>0.0307</td>
<td>0.6048</td>
<td>0.0194</td>
<td>0.8952</td>
</tr>
<tr>
<td>t90</td>
<td>0.1161</td>
<td>0.0302</td>
<td>-0.7167</td>
<td>0.0149</td>
<td>0.8601</td>
<td>0.0080</td>
<td>0.1138</td>
</tr>
<tr>
<td>dur</td>
<td>0.1288</td>
<td>0.1467</td>
<td>0.1308</td>
<td>0.1364</td>
<td>0.1421</td>
<td>0.1471</td>
<td>0.1361</td>
</tr>
</tbody>
</table>
Provenance Trials
Layout designs

Latinized row-columns design

3 replications

9 columns (long columns)

24 rows

Exp. Unit=8 seedlings
Provenance Trials

\[ Q_{st} = \frac{\sigma^2_{between}}{\sigma^2_{between} + 2\sigma^2_{inside}} \]

\[ r^2_{pop} = \frac{\sigma^2_{pop}}{\sigma^2_{pop} + \frac{\sigma^2_{colum}}{b.r/f} + \frac{\sigma^2_{block}}{b/p} + \frac{\sigma^2_e}{p.n}} \]

\[ h^2_{fam} = \frac{\sigma^2_{fam}}{\sigma^2_{fam} + \frac{\sigma^2_{colum}}{b.r} + \frac{\sigma^2_{block}}{c} + \frac{\sigma^2_e}{f.n}} \]

\[ h^2_{indiv} = \frac{4(or3)x\sigma^2_{fam}}{\sigma^2_{fam} + \sigma^2_{colum} + \sigma^2_{block} + \sigma^2_e} \]

\[ \sigma^2_{prov} + \sum_{i} \frac{\sigma^2_{prov\alpha_i}}{n_i} \]

\[ Q_{st \ pop} = \frac{\sigma^2_{pop}}{\sigma^2_{pop} + 2(4\sigma^2_{fam})} \]

\[ Q_{st \ fam} = \frac{\sigma^2_{fam}}{\sigma^2_{fam} + 2(h^2_{indiv} \cdot \sigma^2_e)} \]
Provenance Trials

Q_{st}

ANALYSIS

DATA
- Simple Future Compatibility

SOFTWARE
- Sintaxis operativity

STATISTIC METHODS
- THEORIA
How to

Genetic evaluation

- Design and Analysis of experiments
  - Basic Principles
  - Conventional and current (IB) designs
  - Software
How to

Genetic evaluation

- Adaptive variation
  - Trials (Spp / prov / prog / clon)
  - Conservation Utility
Planning of an experiment (i)

- Definition of objectives
- Definition of all sources of variation
  - Treatments and their levels
  - Experimental units
- Nuisance factors: blocking, noise & covs
- Setting up the experimental units & treatments
Planning of an experiment (ii)

- Definition of response variable, experimental process and issues foresight
- Set up the model
- Scheme of analysis steps
- Set up sampling size
- Review all foregoing points and modifying if necessary
Stages (i)

- Definition of the problem
- Definition of objectives
- Selection of treatments to test (interactions)
- Selection of the material to test
- Selection of the experimental design (simple)
- Selection of the experimental unit size and number of replications
Stages (ii)

- Control of “surroundings” effects
- Kind of data to be taken
- Selection of statistical tests
- Accomplishment of the experiment
- Analysis and interpretation of results
- Final reporting (conclusions)
Principles (i)

1. Replications. (experimental error basis)
   - Standard Error of Difference
   - Agronomic trails $S ED < 1/3 \text{ diff}$
   - Material selection $S ED < 1/6 \text{ diff}$
   - Knowing $s^2 \& d \implies n$

   \[ S ED = \sqrt{\frac{2\sigma^2}{n}} \]

2. Treatment (broad sense) Randomization

3. Local control of existing variation in trial site
   (Blocking or spatial analysis)

ANOVA/SDA/Robust methods
“typical numbers & expressions”

4 replications  
randomization

25 plants per plot
3 sites
4 treatments

2 border lines
latinization
RCB

single tree plot
5 x 5 m spacing
25 genotypes
Principles (ii)

**Operational Limiting factors**

Number of available effectives
Site constraints (topography, surface ...)
Technical limitations (machinery, ....)
Measurements
Competence, specific needs,
Future treatments, thinnings,...
Spacing, density
Experimental design

Initial assumptions or constraints:

Additivity
Normality
Homocedasticity.

Different treatment errors are independent & distributed \( N(0, \sigma^2) \)

Statistic tests:

\( N: \) Shapiro-Wilks, graphs distrib, freq acum., res * pred
\( H: \) Barlett, Levenne, ratios variances

Transformations

No parametric methods
## Elementary Designs

**Model:** \( y_{ij} = \mu + t_i + \varepsilon_{ij} \)

<table>
<thead>
<tr>
<th></th>
<th>dof</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>EMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>rt-1</td>
<td>a</td>
<td>a/rt-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat</td>
<td>t-1</td>
<td>b</td>
<td>b/t-1</td>
<td>( MS_T / MS_E )</td>
<td>( \sigma^2_e + r \sigma^2_t )</td>
</tr>
<tr>
<td>Error</td>
<td>t(r-1)</td>
<td>c</td>
<td>c/t(r-1)</td>
<td></td>
<td>( \sigma^2_e )</td>
</tr>
</tbody>
</table>

Model \( y = \text{treat}; \)
Elementary Designs

**Model:** \( y_{ijk} = \mu + t_i + b_j + \varepsilon_{ijk} \)

<table>
<thead>
<tr>
<th></th>
<th>dof</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>EMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>rb-1</td>
<td>a</td>
<td>a/rb-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat</td>
<td>t-1</td>
<td>b</td>
<td>b/t-1</td>
<td>( \text{MS}_T / \text{MS}_E )</td>
<td>( \sigma^2_e + b \sigma^2_t )</td>
</tr>
<tr>
<td>Blq</td>
<td>b-1</td>
<td>c</td>
<td>c/b-1</td>
<td>( \text{MS}_B / \text{MS}_E )</td>
<td>( \sigma^2_e + t \sigma^2_b )</td>
</tr>
<tr>
<td>Error</td>
<td>t-1)(r-1)</td>
<td>d</td>
<td>d/t(r-1)</td>
<td></td>
<td>( \sigma^2_e )</td>
</tr>
</tbody>
</table>

Model \( y = \text{treat blq} \);
Experimental design

Possible structure of treatments

Factorial: total combination all x all

Possibility interactions study (GxE)

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk} \]

Reaction norms

No interaction

quantitative interaction

qualitative interaction
Experimental design

Possible structure of treatments

Hierarchical or nested: Impossible combination

\[ y_{ijk} = \mu + \alpha_i + \beta_j(\alpha_i) + \varepsilon_{ijk} \]

Model: \[ y = \text{pop fam}(\text{pop}); \]

Is it important the treatment structure?

other structure + important.............
¿Fixed o Random?

1. Critical decision
2. Not well documented on texts
3. Usually based on subjective statistic agreements

**Fixed**: Levels of factor clearly targeted or selected
*Results & conclusions* from anova are for these levels
*Main aim*: Mean estimation of the variable for each level

**Random**: Levels are a random sample from all possible.
*Results & conclusions* from anova can be extrapolated + levels
*Main aim*: Variability estimation of the variable or factor
or perhaps prediction at a given level

(BLUP)
<table>
<thead>
<tr>
<th></th>
<th>dof</th>
<th>MS</th>
<th>A y B fixed</th>
<th>A y B rand</th>
<th>A:fix B:rand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>abr-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>a-1</td>
<td>MS_A</td>
<td>MS_A/ MS_E</td>
<td>MS_A/ MS_AB</td>
<td>MS_A/ MS_AB</td>
</tr>
<tr>
<td>B</td>
<td>b-1</td>
<td>MS_B</td>
<td>MS_B/ MS_E</td>
<td>MS_B/ MS_AB</td>
<td>MS_B/ MS_E</td>
</tr>
<tr>
<td>AxB (a-1)(b-1)</td>
<td></td>
<td>MS_AB</td>
<td>MS_AB/ MS_E</td>
<td>MS_AB/ MS_E</td>
<td>MS_AB/ MS_E</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>ab(r-1)</td>
<td>MS_E</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \sigma^2_e + c_1 \Phi_\alpha \quad \sigma^2_e + n \sigma^2_{ab} + nb \sigma^2_a \]

¿Fixed o Random?  

How to asses?  

**A PRIORI**

**Scientific Criteria :**
1) is it possible to repeat the factor levels in other site or year?  
2) has it meaning this replication?

Yes + Yes = Fixed

**Statistic Criteria :**
“Random” few levels (3-5) =>weak variance estimation, Better setting as fixed and use the results only at these levels

“Fixed” with many levels (>10) without structure, better setting as random and estimating means by BLUPs

E.M.S. Numeric difficulty
¿Fixed o Random?

GLM

Model \( y = \text{loc} \ \text{blq(loc)} \ \text{var} \ \text{var*loc}; \)

Random \( \text{loc} \ \text{blq(loc)} \ \text{var*loc} \ /\text{test}; \)

1º Calculation as fixed
2º Calculation EMS
3º Repeat F-tests with proper denominators

MIXED MODELS
Incomplete Blocks

Evaluation: high nº genotypes limited material

‘Many genotypes’ means huge blocks # no control

I.B. Not all treat by block, so several blocks are needed for a complete replication

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>B</th>
<th>A</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td></td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

Based on Additivity: $B-C = (B-C)_3$

$= (B-A)_1 - (C-A)_2$

Experimental error independent of treatment
Incomplete Blocks

• Coexist direct & indirect comparisons
• Lost of accuracy on indirect comparisons but experimental error reduction

Resolvable designs
i.e.: g=k bi
α-lattice, latinized, row-columns,...

Complex specific Software

interblock info
I.B. design Efficiency

Objective: To compare genotypes highest accuracy

\[ E = \left( \frac{\text{SED}_{\text{RCB}}}{\text{SED}_{\text{IB}}} \right)^2 \]

\[ \text{nº of extra replications in a RCB to get same accuracy level} \]

\[ \left( \frac{2\sigma^2}{r} \right)^2 = E \]

\[ \left( \frac{2\sigma^2}{rxE} \right)^2 \]

A IB with 4 reps y E=1.5 equals to a RCB with 4x1.5=6 CB

“Efficiency” ~ costs
For simple and RCB
AZARsXXI.exe
Layout software: CyCDesignN
<p>| | | | |</p>
<table>
<thead>
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</tbody>
</table>

- 28 genotypes
- 4 blocks of 7

<table>
<thead>
<tr>
<th>Row-Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

- 3 rep α-lattice

- Latinized Row-column
CycDesigN

CycDesigN is a computer package for the generation of optimal or near-optimal experimental designs. It comprises three modules: CycDesigN is a computer package for the generation of optimal or near-optimal experimental designs. It provides the most comprehensive design generation involved in field, glasshouse and laboratory trials.

The designs include cyclic, alpha and factorial design designs, t-latinized and partially-latinized designs as a result, the algorithms incorporate the most recent CycXover is a sub-system, generates optimal or near crossover experiment involves the application of sec periods.

The observations made on each subject at the end of the experiment, and the carry-over effects on designs are also known as change-over or carry-over your output from a CycDesigN or CycXover session generate either GenStat or SAS code for the analysis CycAnalysis manual. So CycDesigN's focus is on the treatments, as in variety trials.

In contrast, GenStat concentrates on the more traditional and strip plots, criss-cross designs, Latin and Graeco designs. CycDesigN is written in Visual C++ and runs

Current users can download it here.

http://www.vsni.co.uk/software/cycdesign/
Kind of design: Incomp. Block or row-column

Resolvable, if v=ks v treats=s blocks of k treats

Parameters: nºreps, nº i.b., nº treats

Factorial or nested

Latinization and spatial restrictions

Generation of algorithms (starting point)
Block design

- Resolvable
- Design parameters [24,4,4]
- Single factor.. [1]
- Alpha design
- Not latinized
- Not spatial
- Seed: clock

Row-column design

- Resolvable
- Design parameters [30,6,5,3]
- Single factor.. [1]
- Alpha design
- Not latinized
- Spatial: Integer
- Seed: clock
- Two stage

No operative differences for user

Design parameters [120, 10, 3]

Block design

Row-column design

Design parameters
- Number of treatments: 120
- Number of units/block: 10
- Number of replicates: 3

Next>

12 I.B. of 10 treat.

Design parameters
- Number of treatments: 120
- Number of rows: 10
- Number of columns: 12
- Number of replicates: 3

1 REP

NESTED
5 POPULATIONS:
30, 20, 24, 26 y 20
Families respectivel.

FACTORIAL:
2 Factors
10 levels in factor 1
12 levels in factor 2
10 x 12 = 120 treat.
28 genotypes
4 blocks of 7

3 rep α-lattice

Latinized Row-column

Row-Column
Possibility of recoding our levels (treats or genotypes)
**International Seminar 'Strengthening Agroforestry Programs in Higher Education for Food Security in Sub-Saharan Africa'**

Madrid, 27 February - 6 March 2017

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### Design Parameters
- **Number of treatments** = 120
- **Number of rows** = 10
- **Number of columns** = 12
- **Number of replicates** = 3

Latentized by columns

Random number seed for design generation = 46

Two stage

### Average Efficiency Factors (Upper bounds)
- **Row** = 0.95737 (0.896445)
- **Column** = 0.875536 (0.875563)
- **Row-Column** = 0.789571 (0.795142)

### Randomization 1

Random number seed for randomization = 203

### Treatment randomization:

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### Replicate randomization:

| 3  | 1  | 2  |

### Column randomization:

| 11 | 4  | 8  |

Row randomization:

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### Intl. Seminar 'Strengthening Agroforestry Programs in Higher Education for Food Security In Sub-Saharan Africa' – SAPHE

**Madrid, 27 Feb – 03 Mar 2017**

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Data collection

- Maps (file: “design.out”)
- Forms (file: “design.ind”)
  - Specialized software
  - Imagination

- Word & Excel !!!
Templates for data recording

- From file: “design.ind”
  - Open with MS Office Word
  - Return code: ^p
  - Replace ^p by ^p ^p ^p ^p ^p….
  - ^p n-times n:number of plants per experimental unit

- Save as .txt (unformatted text)
Templates for data recording (ii)

- Attention to specifications when saving as .txt:
  - Windows default
  - Unchecked boxes

- After saving change the extension to .csv
Templates for data recording (iii)

- Open with MS Office Excel
- Click in the first column
- Data / text in columns
- Delimited / tab, space & consider consec. sep. as one

- Save as excel file
- Use formulas & regular excel tips
Thanks for your attention