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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR
TECHNIQUES, AND DNA-PROFILING IN PARTICULAR**

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Brasilia, November 22 to 24, 2011

ADDENDUM

A POTENTIAL UPOV OPTION 2 APPROACH FOR BARLEY USING HIGH DENSITY
SNP GENOTYPING

Document prepared by experts from the United Kingdom

Plant Science into practice



National Institute of Agricultural Botany



Presenter Name Carol Norris Date November 2011



A Potential UPOV Option 2 Approach for Barley Using High Density SNP Genotyping

A project co-funded by the Community Plant Variety Office (CPVO)

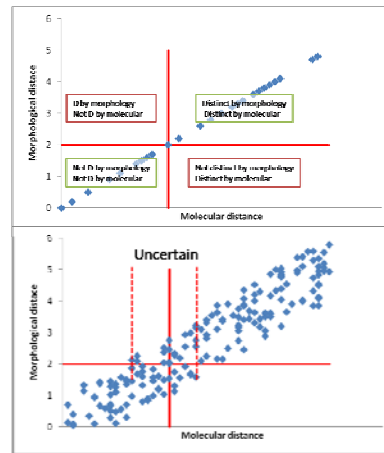
- UPOV Model 2 “Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics”.
- Aim is to ensure the same decisions are made under molecular or morphological testing system.
- Costs of genotyping have decreased, costs of running field trials constant or increasing and better molecular data are available



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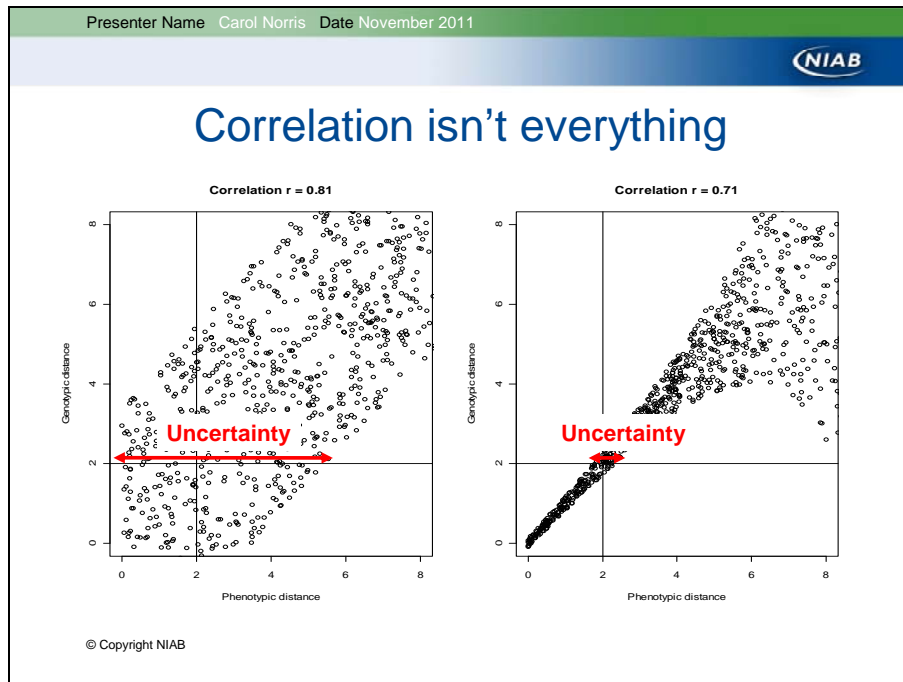
UPOV Model 2

- Ideal: strong positive correlation, no 'noise'
- Identical decisions made by either method
- Reality: uncertainty if the correlation is not perfect
- How much uncertainty is acceptable?
- Can we use Model 2 taking into account this uncertainty?

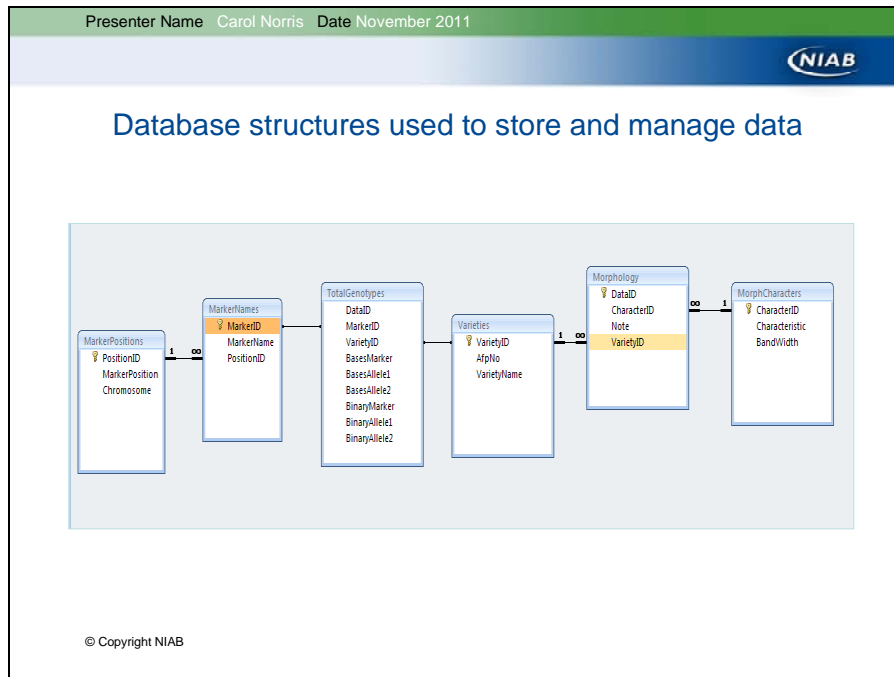


Hypotheses


- Genotypic and phenotypic distance measures for a set of varieties will have a strong positive correlation to each other.
- Varieties shown as 'similar' using phenotypic distances will also be shown as 'similar' using genotypic distances.



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- ## Project resources
- Genotype data from AGOUEB project - Association mapping used to detect associations between SNPs and DUS characteristics
 - 3072 SNP marker loci
 - 500 UK barley varieties
 - Phenotypic data from UK DUS trials
 - 579 winter and spring barley varieties
 - 33 characteristics (some UK)
 - 28 CPVO characteristics
 - 431 varieties with both phenotypic and genotypic data
 - Some varieties rejected – too much missing data
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Dealing with missing phenotypic data

- For each characteristic, missing data were replaced by values drawn at random from the existing data (imputation)
- Missing data were replaced by imputed values to generate complete datasets
- Multiple sets of phenotype data were generated in this way and distance matrices calculated for each of them.
- Distance matrices were pooled and compared to ensure that results were defensible

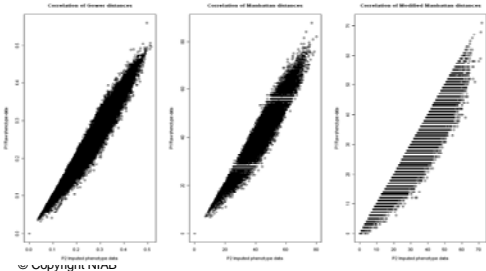
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Validation of phenotypic datasets

- Two datasets used to calculate phenotypic distances
- Raw phenotypic data (P1)
- Data where missing values were replaced by imputation (P2)
- The dataset with missing data (P2) was validated by correlation with the raw phenotype data (P1)



The figure consists of three scatter plots arranged horizontally, each showing the correlation between two datasets (P1 and P2) for different distance metrics. The y-axis for all plots is labeled 'P2 phenotypic dist' and the x-axis is 'P1 phenotypic dist'. The first plot, titled 'Correlation of Genetic distances', shows a strong positive correlation with a correlation coefficient of 0.99. The second plot, 'Correlation of Manhattan distances', shows a strong positive correlation with a correlation coefficient of 0.99. The third plot, 'Correlation of Modified Manhattan distances', shows a strong positive correlation with a correlation coefficient of 0.99. A copyright notice for NIAB is visible at the bottom left of the plots.

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Statistical toolkit

- Phenotypic distances
 - Manhattan distance
 - Modified Manhattan distance
 - Gower distance
- Genetic distance
 - Manhattan distance
 - Euclidean distance

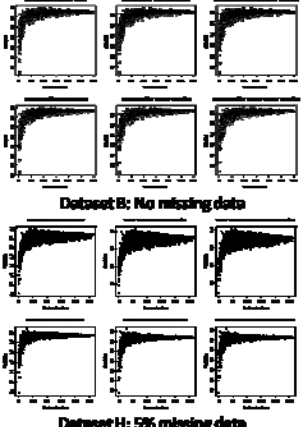
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Do we have enough markers?

- Initially correlations increase with the number of markers used
- As the number of markers increases further, the correlation values plateau
- Once the correlation has reached a plateau, the scatter of correlations around a central value reduces with increasing marker numbers
- Genotypic data divided into subsets
- 300-400 markers needed from Dataset B (no missing data)
- 800-1000 markers needed from Dataset H (5% missing data)



Dataset B: No missing data

Dataset H: 5% missing data

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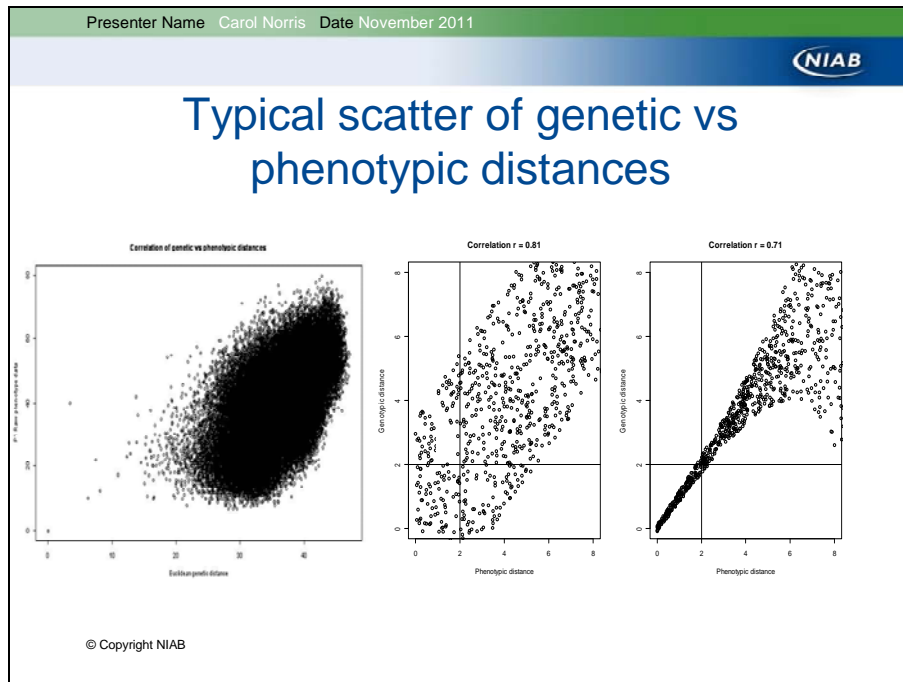
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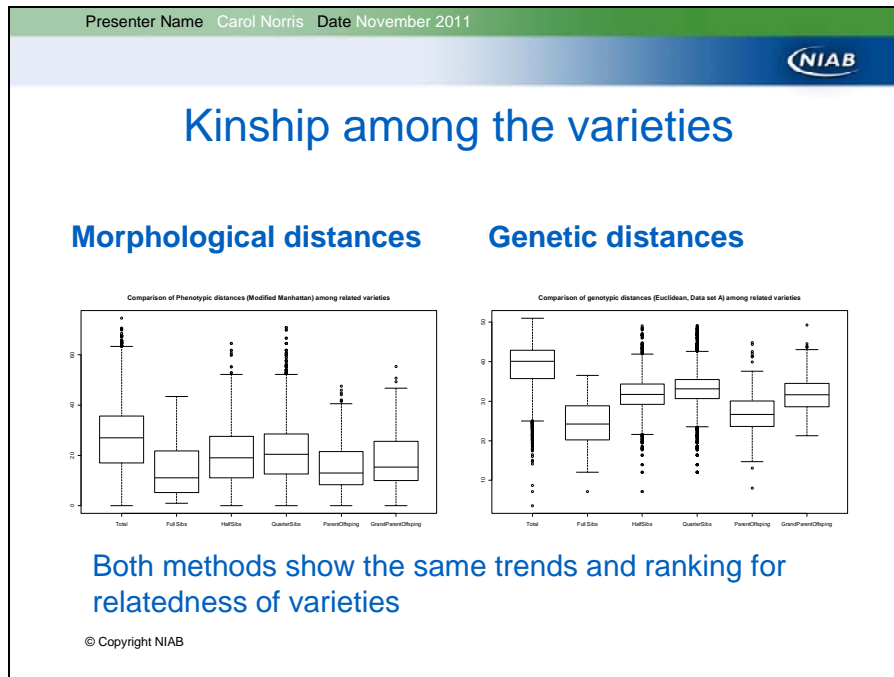
Results

- Correlations between phenotypic and genotypic distances were all positive
- Gower phenotypic distances gave better correlations than Manhattan and Modified Manhattan
- Most correlations were between 0.62 to 0.66 when using Gower's Distance and 0.61 to 0.63 when using Manhattan Distance
- Modified Manhattan Distance gave correlations of 0.58 to 0.60
- Correlations using P2 data were greater than those using P1 data

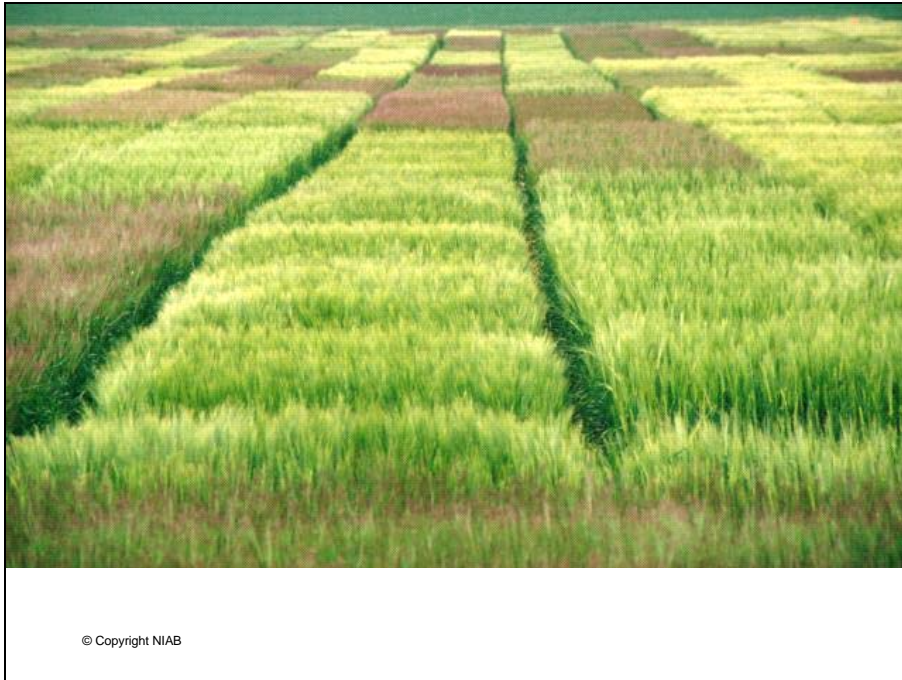
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- ## Looking at pedigree relationships
- Pedigree data extracted from Technical Questionnaires and tabulated
 - All possible full, half and quarter siblings identified
 - Parent-offspring pairs, grandparent-offspring pairs identified
 - Phenotypic and marker-based estimates of relationships were examined
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- ## Next steps
- Genomic prediction of morphology
 - Optimisation of marker selection using spaced markers may give better correlations
 - Testing of decision making – will the same decisions be made with markers as those made with current systems?
 - Report to CPVO in December
 - Too early to make conclusions
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