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STATISTICAL METHODS FOR ASSESSING AND INTERPRETING GENETIC DISTANCE AND GENETIC DIVERSITY

Document prepared by experts from United Kingdom

STATISTICAL METHODS FOR ASSESSING AND INTERPRETING GENETIC DISTANCE AND GENETIC DIVERSITY

Introduction

Intensive plant breeding has been undertaken over the past 70 years for the major UK crops and it has been suggested that this effort has resulted in a shift in the level of genetic diversity and a possible change in the genetic 'distance' between varieties.

To address these points we are currently undertaking two projects that are assessing and comparing various measures of both diversity and distances in wheat, barley and oilseed rape. In both projects, various types of data, including pedigree information, morphological descriptors, biochemical markers and different types of DNA profiling methods, are being used to derive and compare appropriate indices. In this paper we report preliminary analyses that have been carried out on a limited set of data in one species, with the objective of evaluating certain molecular markers for use in distinctness testing.

The data used are from a wider study of the assessment of diversity at both the molecular and phenotypic level in past and present varieties of wheat, barley and oilseed rape (see Donini, Hayter and Koebner 1996) and consist of analyses of wheat using two second generation PCR techniques (Amplified Fragment Length Polymorphisms [AFLP] and simple sequence repeat micro-satellites [SSR]).

Varieties of winter wheat that achieved NIAB 'Generally Recommended' status in 1934 and at 10 yearly intervals thereafter, have been selected as representing 'successful' and therefore widely grown varieties. Some 55 varieties were selected by this criterion over the past 70 years, with between 5 and 14 varieties for each decade. In addition 11 varieties from Greece, China, India, Japan and New Zealand were included as external reference material.

Although each UK variety has been tested and commercialised, direct distinctness comparisons may not necessarily have been made with material more than say 30 years older. Equally, older material would not have been compared to 'future' varieties.

The Varieties

The numbers of representative commercially widely grown UK varieties of wheat distributed over the past 70 years were as follows:

1934	1944	1954	1964	1974	1984	1994
5	7	5	6	5	13	14

The number of external reference varieties were sampled as follows:

China	3
India	2
Greece	2
New Zealand	2
USA	2

Morphological Data

For the 55 UK varieties UPOV style 1 - 9 descriptors were used based on the standard DUS test evaluations. A small sub-set of all possible characters recorded over the past 70 years was used. Only 14 common characters covered the complete period 1934 - 1994. Such detailed data were not available for the external reference varieties from the rest of the world.

Molecular Data

Six AFLP primer pairs produced an average of 15 polymorphic bands per primer combination with 90 informative bands (probably equivalent to loci) in total. Fourteen SSR's were analysed giving a total of 14 sets of band patterns (loci). Varieties were assigned a score based on the pattern of the banding profile. Seed from all 66 varieties was available and successfully processed. For full details see Donini, Law, Stephenson and Koebner (1997).

Assessment of distinctness

Selected modules of the DUSTX statistical software suite (Weatherup 1974) were modified to compute pairwise distinctness based on the following criteria:

Morphological Characteristics - distinct if pair differ by one UPOV note in at least one character.

AFLP - distinct if pair differ by at least one band in the profile

Microsatellites - distinct if pair differ by at least one band pattern score.

For each type of data the distinctness criteria can be made progressively more stringent by increasing the number of 'characters' in which the single difference must occur.

Results

Overall Level of Distinctness

Comparable molecular marker and morphological data were only available for the 55 UK varieties. The additional external reference 'world' material has been shown to be very

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different from the UK varieties. Results as they relate only to the UK varieties are reported below.

Six AFLP primer combinations have been used, generating between 11 and 19 polymorphisms each with a total of 90 bands formed by combining the information from all primer pairs. Using all 90 polymorphisms, each of the 55 UK varieties and the 11 'world' varieties were separable on the basis of at least one band difference.

As can be seen from Table 1 below, the overall level of distinctness using all the available AFLP bands exceeded that observed from the morphological character set while the discrimination power of the SSR was much lower. Even with an increase in stringency of the distinctness criteria from differences required in more than a single band to that requiring differences in two or more bands, the level of discrimination achieved with the full AFLP data remained impressive.

Table 1. Initial Distinctness Criteria

Distinctness percentage all possible pairs	Single Difference
Morphology (14 common characters)	89.1%
AFLP (6 primer combinations)	100.0%
Microsatellites (14 sets)	45.4%

Table 2. More Stringent Distinctness Criteria

Distinctness percentage all possible pairs	Difference in Two 'characters'
Morphology (14 common characters)	60.0%
AFLP (6 primer combinations)	100.0%
Microsatellites (14 sets)	15.2%

Relationship Between Overall Discrimination Power of AFLP and The Number of Bands

Sub-samples of the original 90 AFLP bands were drawn at random to estimate the likely effect of a reduction in the number of AFLP bands on the overall discrimination power. Samples were selected to give data sizes of 10, 15, 20 30 and 45 bands. This exercise was not intended to form a rigorous 'boot-strap' simulation but to aid in establishing the minimum number of AFLP bands that might be required in future studies.

The results have been summarised graphically in Fig 1. Three increasingly stringent distinctness criteria have been used - from at least a single band to the requirement of differences in at least 3 bands. The overall discrimination percentages for individual sub-samples are plotted and means over sub-samples joined by lines. The reference is provided by the discrimination of the morphological character set of 89.1% (Table 1).

It can be seen that sub-samples of less than 30 bands tend to have a weaker discrimination power compared to the morphological character set although the actual distinctness criteria employed have a marked effect on the exact break-even point. Sub-samples exceeding 50 in number showed no appreciable influence of distinctness criteria and each exceeded the level achieved by the morphological character set.

In this particular example, when discriminating *v* varieties the target number of AFLP bands is likely to be closer to *v* rather than v/2. With an allowance for the partial artificial nature of this data set , i.e. it consists of material expected to be distinct, the target number of AFLP bands required in a more general class of problem is likely to be between *v* and 2*v*. The effect of using too few bands can be clearly seen in Fig 1. as a sharp decrease in discrimination power.

Relationship Between Overall Discrimination Power of AFLP and The Number of Sets of Primer Combinations.

Section on overall level of distinctness above looked at the effects of numbers of AFLP bands on overall level of discrimination. However the number of bands available is not a continuous variable but limited to the amount of information scorable from a gel generated by a particular primer combination. If a few more additional bands are necessary it is likely that a whole additional primer combination will be required. The information contained in the individual primer combinations, based on broadly similar numbers of informative bands, is likely to differ.

To assess the discrimination power that could occur in a practical situation, each individual primer combination has been analysed separately (Table 3) and in linked pairs (Table 4).

The results from Table 3 show discrimination rates in good agreement with the random sub-sampling scheme shown in Fig 1. The small numbers of informative bands (less than 20) have caused the overall discrimination power to be significantly lower than that achieved from the full set of bands.

Table 4 shows that even a modest increase in the band numbers can have a marked improvement in overall discrimination rate.

Table 3. Individual AFLP Primer Combination - Distinctness Criteria Difference in at least a single band

Primer Combinations	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	**Full Set**
Number of bands	12	14	16	11	19	18	**90**
Discrimination Rate %	52.7	40.0	61.8	38.2	80	93.7	**100.0**

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Table 4. Paired AFLP Primer Combination - Distinctness Criteria Difference in at least a single band

Paired Primer Combinations	Set 1&2	Set 3&4	Set 5&6
Number of bands	26	27	37
Discrimination Rate %	100.0	90.9	100.0

Conclusions

In this study the application of AFLP data as a potential tool for establishing distinctness is assessed. AFLP compare very favourable with the existing morphological data when the distinctness criterion is based in a single band difference. Given sufficient bands the effects of increasing the stringency of the distinctness criteria on overall discrimination rates, based on AFLP data, are minimal. The substantially weaker discrimination power from the use of too few AFLP bands is confirmed. There is great scope for this technology and method of analysis to be applied to a wider class of problem.

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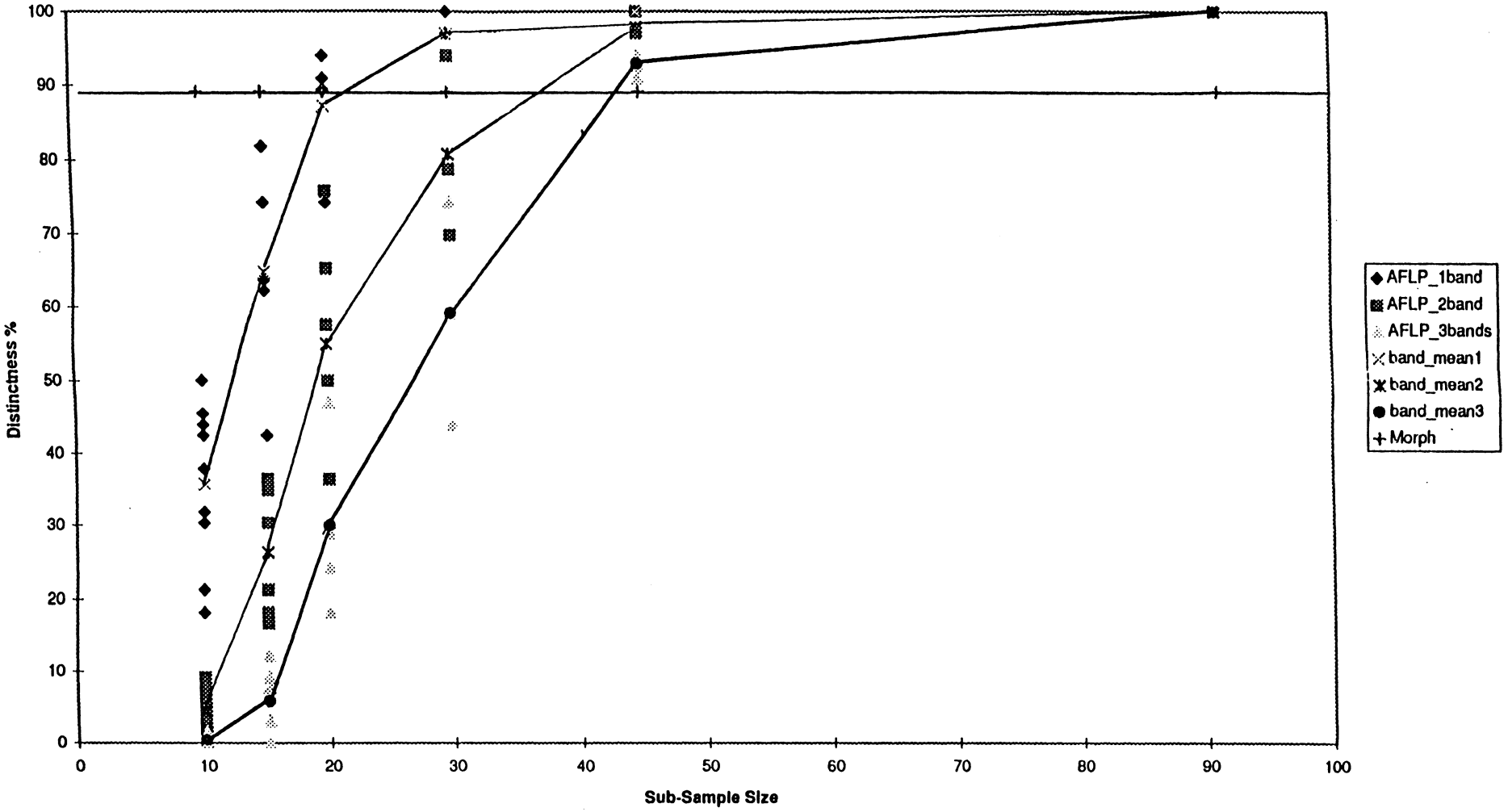

Fig 1. AFLP sub-sampling with 3 Distinctness Criteria
[1 band; 2 bands; 3 bands] Compared Morphological Character Set

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