Theor Appl Genet (2000) 100:607–613

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Are mapped markers more useful for assessing genetic diversity?

Received: 13 November 1998 / Accepted: 17 June 1999

Abstract Genetic diversity within populations of organisms and species is commonly measured using molecular-marker data. It has been claimed that more reliable diversity measurements can be obtained using selected genetically mapped markers to ensure that all regions of the genome are represented in the data sets employed. However, this has not been tested. In the present study, using rice (*Oryza sativa* L.) as a model species, we have shown that the use of unmapped AFLP markers reveals a pattern of diversity that is very similar to that obtained using a range of other marker types and which reflects the known crossability groups within this species. In contrast, we show that use of mapped-marker data can, in some cases, result in highly misleading patterns of diversity; the results obtained are critically related to the choice of parents used in the cross from which the mapping population was produced. For diversity analyses, we propose that it is appropriate to use unmapped markers provided that the marker-type has been shown to have a wide distribution over the genome.

Key words AFLP · Biodiversity · Genetic maps · Genetic resources · Rice

Introduction

Substantial crop biodiversity is being conserved in gene banks around the world, and the number of samples is increasing because of ongoing collecting efforts. The centres of the CGIAR (Consultative Group on International Agricultural Research) maintain more than 500 000 accessions of more than 30 crops while the US Plant Germplasm System stores 380 000 samples of over 8000 plant species. There are more than 95 000 accessions in the International Rice Genebank Collection at IRRI (International Rice Research Institute) which has distributed over 740 000 packets of rice seed throughout the world since 1973 for use in applied research, contributing to improvements in many characteristics of new rice varieties (Jackson and Huggan 1993; Jackson 1994, 1997; Khush 1997).

Efficient use of conserved biodiversity requires information about the degree and distribution of genetic diversity. The advent of molecular technologies resulted in the exploitation of protein- and DNA-based markers for diversity studies. Taking *Oryza sativa* as an example, diversity indices and the patterns of diversity in sets of germplasm have been assessed using isozyme and RFLP (restriction fragment length polymorphism) data (Glaszmann 1987; Zhang et al. 1992). The introduction of the PCR led to the development of a range of new marker technologies and a large number of diversity measurements have been made using PCR-based markers (Newbury and Ford-Lloyd 1997; Westman and Kresovich 1997). In rice, markers such as RAPDs (random amplification of polymorphic DNAs; Virk et al. 1995), ISSRs (inter-simple sequence repeats; Parsons et al. 1997) and AFLPs (amplified fragment length polymorphisms; Mackill et al. 1996; Virk et al. 1998) have been applied.

In almost all cases, workers do not have prior knowledge about the map positions of the markers used for diversity estimation. Some workers in the field have expressed concern about the reliability of diversity measurements achieved using unmapped markers and there have been suggestions that sets of markers should be selected for use on the basis of the degree of genome coverage they afford (Bonierbale et al. 1995; Karp and Edwards 1995; Karp et al. 1996). Karp et al. (1997) stated that much could be gained from a convergence between genetic mapping and diversity studies and that,

Communicated by G. Wenzel

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Table 1 Information about the material used in the present study

Reference number	IRGC number	Name	Isozyme group	Source
1	25840	Benamuri	II	Bangladesh
2	25851	Dhulashaita	II	Bangladesh
3	25868	Jhum Fulbadam	II	Bangladesh
4	64789	Moshur	II	Bangladesh
5	64792	Narikel Jhuri	II	Bangladesh
6	64793	Rakhoil	II	Bangladesh
7	64887	Dagpa Bara	VI	Bhutan
8	64890	Dumja Kaap	n.a.	Bhutan
9	64913	Mandasherpo	VI	Bhutan
10	66513	Guru Muthessa	I	Sri Lanka
11	66529	Podi Niyan Wee	VI	Sri Lanka
12	66540	Cut Keureusek	I	Indonesia
13	66603	Neli	I	Indonesia
14	66612	Pulut Bilemeng	n.a.	Indonesia
15	66669	Sitoru	VI	Indonesia
16	66678	Taria Faigi	I	Indonesia
17	66787	Gochi Boro	VI	Bangladesh
18	66791	Gorbai	II	Bangladesh
19	66817	Moshia Bhadoi	II	Bangladesh
20	67436	Initlog Dalag	VI	Philippines
21	67480	Bilaspuri	I	India
22	67848	Shanka	V	Bhutan
23	71493	Angkarog	VI	Malaysia
24	71501	Baganan Adongko	VI	Malaysia
25	71515	Dayakon	VI	Malaysia
26	71517	Dumpolon	VI	Malaysia
27	71537	Kedayan	VI	Malaysia
28	71544	Kulob	n.a.	Malaysia
29	71545	Kuneng	I	Malaysia
30	71578	Muara	I	Malaysia
31	71596	Pulutan	VI	Malaysia
32	71646	Wangkod	VI	Malaysia
33	73090	Chawal	n.a.	Pakistan
34	74716	Sayari	II	India
35	74720	Anoopa	II	India
36	74773	Ramjawain	I	India
37	77210	Rayada	II	Bangladesh
38	77264	Khandi	I	Bangladesh
39	77272	Lal Bagdar	I	Bangladesh
40	77279	Mukkala Bazal	I	Bangladesh
41	78245	Kam Pai	I	Thailand
42	78250	Khao Gu Lahb	I	Thailand
43	78253	Khao samud	I	Thailand
44	78259	Khao' Mum	VI	Thailand
45	78270	Look Pasom	I	Thailand
46	78275	Neng Nah	VI	Thailand
47	78276	Pah Wean	I	Thailand
48	78357	Nep Bong Ruong Hoa Binh	VI	Vietnam
49	6538	Bamoia 341	III	Bangladesh
50	6541	Bhadoia 233	III	Bangladesh
51	13746	Taothabi	III	India
52	12331	Arc 7229	V	India
53	4021	Binicol	V	Philippines
54	27856	Begumi 302	V	Pakistan
55	328	Azucena	VI	Philippines
56	66970	IR64	I	IRRI

where possible, markers should be chosen according to their distribution to ensure that marker sampling errors are not committed. Laurie et al. (1997) proposed a map-based approach to diversity studies in which markers are selected for use on the basis of their map location.

The advantage of using mapped markers for diversity measurement appears convincing, but there has been no study to test the effects of using mapped and unmapped markers during the measurement of diversity on a single set of germplasm. In the present study we have employed rice as a model system for addressing various issues related to the use of mapped and unmapped markers for diversity assessment. We have used three classes of AFLP markers: (1) a set of unmapped markers; (2) subsets of these markers which are polymorphic between selected accessions and which could therefore be mapped

in a cross between them; these 'hypothetically mapped' markers have been used to model the diversity measurements that would be obtained using a range of mapping populations; (3) a set of markers which have been mapped using a doubled-haploid population (Virk et al. 1998). This has allowed us to answer the following questions: (1) is there any difference in the pattern of diversity revealed using mapped and unmapped markers?; (2) does the degree of genetic relationship between the parents used to produce a mapping population influence diversity patterns revealed using mapped markers?; (3) are there any disadvantages in using unmapped markers for diversity studies?

Materials and Methods

Plant material

The material for this study comprised 56 diverse *O. sativa* accessions from the International Rice Genebank Collection. RAPD data generated for 48 of these accessions have been used by us in other studies (Virk et al. 1996 a,b). A further eight accessions [numbered 49–56 (Table 1)] were added to this characterised germplasm to include the parents of a cross used in our mapping studies and to introduce accessions from rice sub-groups not represented in the 48 accessions previously employed. Using the classification based on isozyme data, 17 of the 56 accessions were designated as indica (isozyme group I) and 17 as japonica (isozyme group VI) types. The remaining 22 accessions belonged to isozyme groups II, III and V (Table 1) (Glaszmann 1987). These designations were made at the Genetic Resources Center, IRRI.

AFLP analysis

Genomic DNA was isolated from a small quantity of fresh leaf tissue (60 mg) following the method described by Virk et al. (1995). The AFLP protocol developed by Vos et al. (1997) was essentially followed, with minor modifications (Virk et al. 1998). Genomic DNA (500 ng) was digested with EcoRI and MseI restriction enzymes prior to ligation with appropriate linkers. The digested and ligated fragments were pre-amplified using the following primers: 5'-GACTGCGTACCAATTCA and 5'-GATGAGTCCTGAGTAAC.

These primers contained one selective nucleotide at the 3' end (A and C, respectively). Four *Eco*RI (E1–E4 with AC, AA, AG and AT selective nucleotides, respectively) and eight *Mse*I end-directed (M1–M8 with CAA, CAC, CAG, CAT, CTA, CTC, CTG and CTT selective nucleotides, respectively) primers were used. Amplification was carried out using 14 primer-pair combinations viz., E1M1, E1M2, E1M8, E2M1, E2M2, E2M3, E2M4, E3M2, E3M4, E3M5, E3M6, E3M8, E4M3 and E4M4.

*Eco*RI adapter-directed primers were end-labelled using γ^{33}P. The pre-amplification and the amplification conditions, as well as the thermal-cycling profile, have been described elsewhere (Virk et al. 1998). A small quantity of denatured products (3 µl) was loaded onto a 5% denaturing polyacrylamide gel with 7.5 M urea. Electrophoresis was performed at a constant temperature of 50° C for 2 h and, after drying, the gel was exposed to Kodak Biomax film for 3–4 days.

Data analysis

The AFLP bands were scored as present (1) or absent (0). The similarity matrices obtained using the simple matching coefficient were subjected to UPGMA (Unweighted Pair-Group method using arithmetic averages) clustering (NTSYS-pc; Rohlf 1992) and represented in the form of dendrograms. The Mantel test (Mantel 1967) was used to ascertain the significance of the correlation coefficients between pairs of similarity matrices.

Use of unmapped and mapped markers for diversity measurement

AFLP analysis was carried out across 56 diverse rice accessions using 14 primer combinations to produce unmapped, polymorphic bands. The 56 accessions included the parents used to produce a doubled-haploid mapping population (Maheswaran et al. 1997); these were IR64 (indica) and Azucena (japonica). In a previous study employing the same primer combinations, a set of AFLP markers had been mapped using this population (Virk et al. 1998). The presence/absence of these mapped markers was recorded for each of the 56 accessions and dendrograms representing relationships between accessions were produced.

Influence of the mapping population on diversity measurement when using mapped markers

One accession was taken at random from each of the five rice groups allowing, in all possible combinations, ten hypothetical crosses between the accessions to be made and ten segregating mapping populations to be modelled. In each case, the 299 unmapped AFLP marker-data set was examined to identify those bands that are polymorphic between each pair of hypothetical parents. These ten sub-sets of markers represent those that would theoretically be mappable in each of the ten segregating populations. Each sub-set of 'hypothetically-mapped' markers was then used to determine variation within the set of 56 diverse rice accessions.

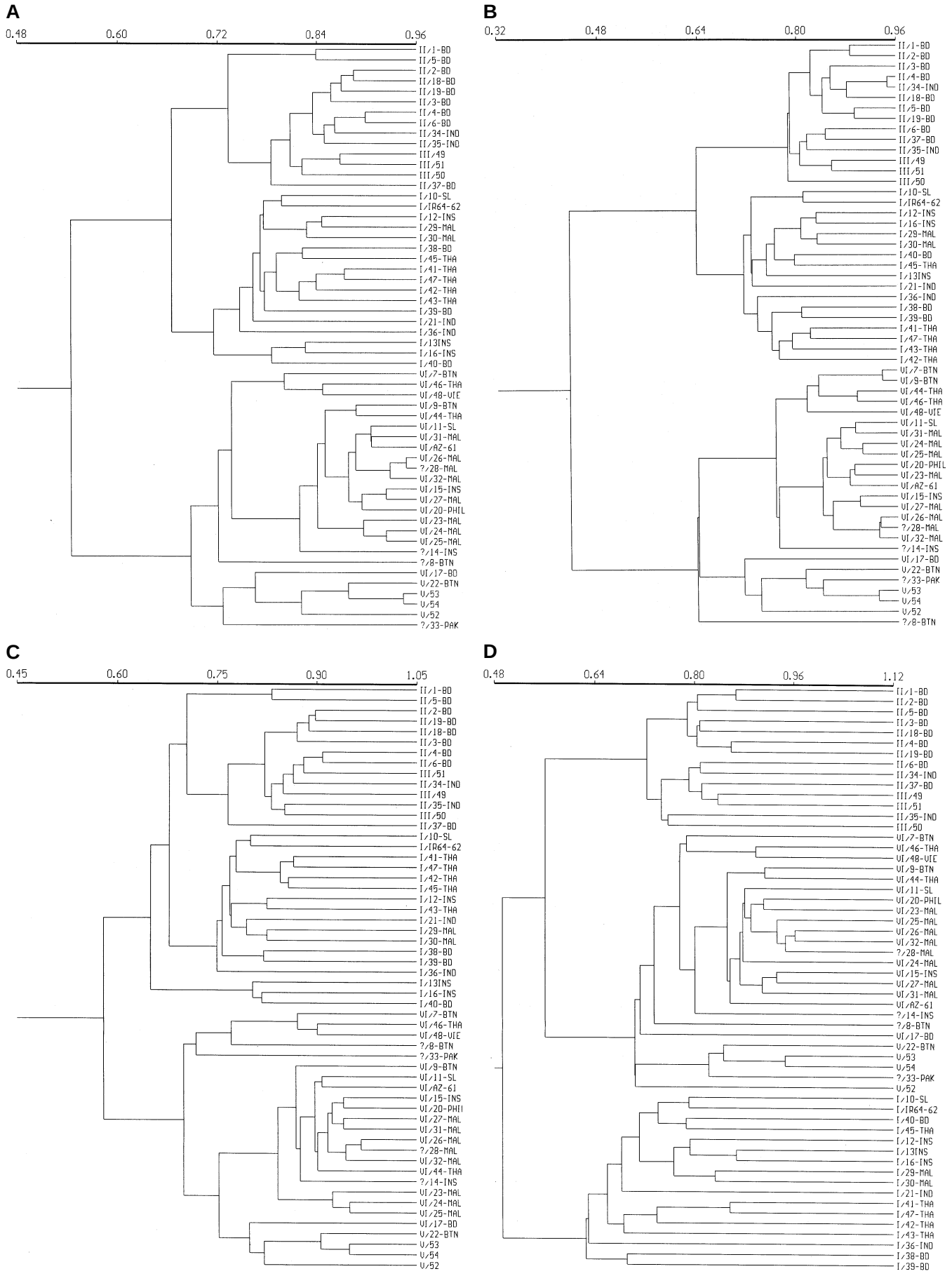
The effect of genomic location of markers on diversity estimation

In order to detect possible effects of the genomic distribution of markers on the patterns of diversity that they reveal, we analysed the effect of using sub-sets of AFLP markers mapped using the doubled haploid population (Virk et al. 1998) and selected on the basis of their map location or their potential exposure to genetic recombination. On these bases the following sub-sets of markers were used for diversity measurements:

(1) markers mapped on the short arms (55) and markers located on the long arms (64) of chromosomes,
(2) markers (44) showing segregation distortion in the doubled-haploid population,
(3) markers located within 25 cM of the centromere (52; Virk et al. 1998).

Results

Assuming that those bands that have been mapped using the doubled-haploid population are homologous with co-migrating bands in diverse rice accessions (see Discussion), these data allow us, for the first time, to compare aspects of diversity measurements using mapped and unmapped markers on the same set of germplasm. The dendrograms produced using 299 unmapped and 122 mapped AFLP markers both classified accessions according to the well-established isozyme groups of Glaszmann (1987). Not only do both mapped and unmapped AFLP marker data sets classify rice accessions into appropriate isozyme groups, but the patterns of diversity revealed are similar. This is reflected in the very high correlation (r=0.93) obtained when the two similarity matrices were compared. Average similarity indices


A
0.48
0.60
0.72
0.84
0.96
II/1-BD
II/5-BD
II/2-BD
II/18-BD
II/19-BD
II/3-BD
II/4-BD
II/6-BD
II/34-IND
II/35-IND
III/49
III/51
III/50
II/37-BD
I/10-SL
I/IR64-62
I/12-INS
I/29-MAL
I/30-MAL
I/38-BD
I/45-THA
I/41-THA
I/47-THA
I/42-THA
I/43-THA
I/39-BD
I/21-IND
I/36-IND
I/13INS
I/16-INS
I/40-BD
VI/7-BTN
VI/46-THA
VI/48-VIE
VI/9-BTN
VI/44-THA
VI/11-SL
VI/31-MAL
VI/AZ-61
VI/26-MAL
?/28-MAL
VI/32-MAL
VI/15-INS
VI/27-MAL
VI/20-PHIL
VI/23-MAL
VI/24-MAL
VI/25-MAL
?/14-INS
?/8-BTN
VI/17-BD
V/22-BTN
V/53
V/54
V/52
?/33-PAK
B
0.32
0.48
0.64
0.80
0.96
II/1-BD
II/2-BD
II/3-BD
II/4-BD
II/34-IND
II/18-BD
II/5-BD
II/19-BD
II/6-BD
II/37-BD
II/35-IND
III/49
III/51
III/50
I/10-SL
I/IR64-62
I/12-INS
I/16-INS
I/29-MAL
I/30-MAL
I/40-BD
I/45-THA
I/13INS
I/21-IND
I/36-IND
I/38-BD
I/39-BD
I/41-THA
I/47-THA
I/43-THA
I/42-THA
VI/7-BTN
VI/9-BTN
VI/44-THA
VI/46-THA
VI/48-VIE
VI/11-SL
VI/31-MAL
VI/24-MAL
VI/25-MAL
VI/20-PHIL
VI/23-MAL
VI/AZ-61
VI/15-INS
VI/27-MAL
VI/26-MAL
?/28-MAL
VI/32-MAL
?/14-INS
VI/17-BD
V/22-BTN
?/33-PAK
V/53
V/54
V/52
?/8-BTN
C
0.45
0.60
0.75
0.90
1.05
II/1-BD
II/5-BD
II/2-BD
II/19-BD
II/18-BD
II/3-BD
II/4-BD
II/6-BD
III/51
II/34-IND
III/49
II/35-IND
III/50
II/37-BD
I/10-SL
I/IR64-62
I/41-THA
I/47-THA
I/42-THA
I/45-THA
I/12-INS
I/43-THA
I/21-IND
I/29-MAL
I/30-MAL
I/38-BD
I/39-BD
I/36-IND
I/13INS
I/16-INS
I/40-BD
VI/7-BTN
VI/46-THA
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?/8-BTN
?/33-PAK
VI/9-BTN
VI/11-SL
VI/AZ-61
VI/15-INS
VI/20-PHI
VI/27-MAL
VI/31-MAL
VI/26-MAL
?/28-MAL
VI/32-MAL
VI/44-THA
?/14-INS
VI/23-MAL
VI/24-MAL
VI/25-MAL
VI/17-BD
V/22-BTN
V/53
V/54
V/52
D
0.48
0.64
0.80
0.96
1.12
II/1-BD
II/2-BD
II/5-BD
II/3-BD
II/18-BD
II/4-BD
II/19-BD
II/6-BD
II/34-IND
II/37-BD
III/49
III/51
II/35-IND
III/50
VI/7-BTN
VI/46-THA
VI/48-VIE
VI/9-BTN
VI/44-THA
VI/11-SL
VI/20-PHIL
VI/23-MAL
VI/25-MAL
VI/26-MAL
VI/32-MAL
?/28-MAL
VI/24-MAL
VI/15-INS
VI/27-MAL
VI/31-MAL
VI/AZ-61
?/14-INS
?/8-BTN
VI/17-BD
V/22-BTN
V/53
V/54
?/33-PAK
V/52
I/10-SL
I/IR64-62
I/40-BD
I/45-THA
I/12-INS
I/13INS
I/16-INS
I/29-MAL
I/30-MAL
I/21-IND
I/41-THA
I/47-THA
I/42-THA
I/43-THA
I/36-IND
I/38-BD
I/39-BD

have also been calculated for 299 unmapped and 122 mapped markers and these are also similar (0.64 and 0.57, respectively; $P>0.05$).

A criticism of the comparisons made above is that a much larger number (299) of unmapped markers than mapped markers (122) have been used in the data sets employed to assess diversity. This reflects the typical availability of such markers in diversity studies. For practical reasons, not all the unmapped markers can be mapped using any single mapping population, and even if they are mappable this involves significant effort. However, in order to compare the quality of diversity information obtained per unmapped or mapped marker, a subset of 121 unmapped markers was used to produce a dendrogram from a similarity matrix as above (Fig. 1C). This initial subset represented the bands produced using 3 of the 14 primer combinations. A very similar pattern of diversity was observed and the correlation between the matrices based on 122 mapped and 121 unmapped markers is high ($r=0.80$; $P<0.01$). Since it is possible that the selection of a sub-set of markers produced using only three primer combinations may bias the data set, we selected a further set of unmapped 122 markers (from 299) entirely at random. In this case the correlation between the matrices produced using the 122 mapped and unmapped markers was even higher ($r=0.91$).

A feature of the use of mapped markers that might influence the pattern of diversity revealed is the degree of genetic relationship between the parents from which the mapping population has been developed. This is because, in opting to use mapped markers, the sub-set of markers that are polymorphic between the parents of the initial cross are being selected. The 56 diverse accessions used in this study have been assigned to five of the six isozyme groups (group IV is not represented) both by direct isozyme analysis and by the AFLP analyses shown above (Fig. 1A–C). The major division within this germplasm is between groups I, II and III and groups V and VI. In the present investigation described above, we have used a cross between an indica (group I) rice and a japonica (group VI) rice, and the use of markers polymorphic between these parents (and hence mappable) results in a pattern of diversity similar to that obtained using unmapped markers ($r=0.93$; $P<0.01$).

It is possible to determine the effect of using other sub-sets of markers mapped using segregating populations derived from crosses involving parents from different combinations of rice groups. Comparison of the dendrograms (data not shown) obtained from ten sets of data generated for those hypothetically mapped markers revealed clear differences. We have defined the 'accepted' pattern of diversity as that which is consistently obtained using unmapped markers of several differing types (AFLP, RAPD, RFLP, isozymes) and which broadly reflects crossability (Chang 1976). This 'accepted' pattern was clearly revealed when: (1) the markers mapped using the doubled-haploid population derived from the IR64 (indica)×Azucena (japonica) cross, and (2) hypothetically mapped markers derived from putative crosses between accessions occupying the two major divisions of the germplasm (i.e. an accession in groups I, II or III crossed with one in groups V or VI) were employed. In contrast, however, when sub-sets of markers derived from putative crosses between accessions occupying more-closely related groups were used, quite different diversity patterns were observed. For example, use of hypothetically mapped markers defined using putative crosses between accessions of groups I x II resulted in a pattern of diversity in which accessions belonging to groups II and III cluster with japonica (group VI) rather than indica (group I) rices (Fig. 1D). The same distortion of the pattern of diversity was observed when using hypothetically mapped markers defined using putative crosses between accessions of groups I×III and V×VI (data not shown).

Correlations were calculated between the similarity matrices obtained using each of these ten data sets of hypothetically mapped markers and with those obtained using both the 299 unmapped markers and the 122 mapped markers (Table 2). Where hypothetical parents were members of distant groups (e.g. I×VI, III×V etc.) the correlation between the similarity matrix and that de-

Table 2 Correlation between similarity matrices obtained from different data sets

Data set		Unmapped markers (299)	Mapped markers (122)
Hypothetically-mappable markers in various crosses			
Cross[a]	Isozyme group	Correlation	
43×3	I×II	0.77	0.73
43×51	I×III	0.77	0.73
43×52	I×V	0.92	0.91
43×27	I×VI	0.91	0.95
3×51	II×III	0.67	0.56
3×52	II×V	0.88	0.88
3×27	II×VI	0.88	0.89
51×52	III×V	0.86	0.85
51×27	III×VI	0.87	0.88
52×27	V×VI	0.77	0.77
Markers representing different genomic locations			
Markers on short arms		0.89	0.97
Markers on long arms		0.90	0.96
Markers in the vicinity of centromeres		0.88	0.94
Markers away from centromeres		0.89	0.96
Markers with distorted segregation		0.89	0.94
Markers with normal segregation		0.90	0.98

[a] see Table 1 for details of accessions and their source country

◀ **Fig. 1** Dendrograms of 56 accessions of rice generated by UPGMA cluster analysis of **A** 299 unmapped AFLP markers generated using 14 primer combinations, **B** 122 mapped AFLP markers, **C** 121 unmapped AFLP markers generated using three primer combinations, and **D** 93 hypothetically mappable AFLP markers defined from a potential cross between accessions numbered 43 and 3 from isozyme groups I and II respectively (see Table 1 for details of accessions and their source country)

rived from the unmapped markers was high (0.86–0.92); the amount of variation explained across the 56 accessions is typically above 50%. Where hypothetical parents were members of more-closely related groups (e.g. I×II, V×VI etc.) the correlation between the similarity index matrices and that using the unmapped markers was lower (0.67–0.77). Notably, the amount of variation explained across the 56 accessions is much less and, in one case (using hypothetical parents from groups II and III), fell to 4%.

Tests were performed in which sub-sets of mapped markers were selected based upon their chromosomal location (see Materials and methods). In all cases the observed diversity patterns were similar to those revealed using unmapped markers. The correlations between the similarity matrices obtained using these sub-sets of mapped markers with those obtained using 299 unmapped markers was very high (usually around 0.9; see Table 2). It appears that neither the distribution of markers across the genome nor distorted segregation had a significant impact on diversity measurement.

Discussion

During the analyses carried out in this study, it was assumed that bands mapped using the doubled-haploid population were homologous with co-migrating bands in diverse rice accessions. We have provided no direct evidence that this is the case, although limited sequencing work that we have carried out on co-migrating AFLP fragments from distantly related rice genotypes has shown that these are homologous. If more than a small proportion of co-migrating bands were not allelic in our analyses, then one would not expect the very high level of similarity in the patterns of diversity revealed using mapped and unmapped markers. Neither would one expect the close correlation between the patterns of diversity revealed by our AFLP analyses and those previously obtained using isozyme, RFLP and RAPD markers.

The frequency with which co-migration of AFLP bands is due to allelism has been assessed in studies using several species. Fifty AFLP markers mapped in a RIL rice population obtained from a cross between IR74 and FR13 A were shown to map to the same linkage groups and in the same order as those obtained using a DH population obtained using IR64 and Azucena (Nandi et al. 1997). Eighty nine per cent of co-migrating AFLP markers were shown to occupy similar map locations in different potato genotypes (Rouppe van der Voort et al. 1997). Of the remainder, close re-examination of autoradiograms showed subtle mobility differences for half of the apparently non-allelic markers (presumably due to the differences in base composition of amplified fragments); the authors suggested that at least some of the other apparently non-allelic markers may be explained by structural differences (loss of synteny) between the potato genotypes. Over 96% of co-migrating AFLP fragments were shown to map to similar genomic regions in three different segregating populations of barley (Waugh et al. 1997). Similarly, it was concluded that co-migrating AFLP bands from different *Arabidopsis* ecotypes were likely to correspond to the same locus (Alonso-Blanco et al. 1998). Hence, there is a lot of evidence that co-migrating AFLP bands, amplified from the genomes of closely related genotypes (within the same species), are highly likely to be allelic.

The first conclusion from our study is that unmapped AFLP bands reveal patterns of variation that are consistent with those obtained using other marker types and which correspond to expectations from previous studies (Ford-Lloyd et al. 1997). This conclusion should apply to other species of plant, fungus or animal, provided that rice possesses no characteristics that make it a special case. There is strong evidence that *O. sativa* is split into two major classes of genotype (groups I, II and III and groups IV, V and VI) (Ford-Lloyd et al. 1997). This simplifies the interpretation of data and makes rice a useful model system for study, but it is unclear whether this simple pattern of diversity could influence general conclusions about the value of different classes of marker. Another notable characteristic of rice is that the AFLP markers that separate genotypes into the two most-distant groups (indica and japonica) are distributed across the whole of the rice genome (Virk et al., 1998). Insufficient information is available in the literature to determine whether this is unusual. The important point, however, is that AFLP markers have been shown to be distributed widely across the genome; in plants, this has been shown for rice (Maheswaran et al. 1997; Virk et al. 1998), barley (Becker et al. 1995: Waugh et al. 1997), *Arabidopsis* (Alonso-Blanco et al., 1998), sugar beet (Schondelmaier et al. 1996), soybean (Keim et al. 1997), and *Eucalyptus* (Marques et al. 1998). Hence, in using AFLP markers one is sampling the whole genome.

The second conclusion from our study is that there appears to be no advantage in using mapped markers for assessing diversity. From a practical point of view, use of mapped markers would greatly hinder progress in diversity measurements within those species for which no linkage map is available and/or suitable marker types have not been mapped. More importantly, even where such material already exists we have shown that the pattern of diversity revealed using mapped markers is very dependent on the parents of the mapping population used. Misleading information on genetic relationships could be obtained if mapped markers using a population produced from a cross between closely related genotypes were employed. We expect this will apply not only to rice but also to other plants and animals. While there is a proposal to utilise marker systems which provide full coverage of the genome being studied in terms of diversity, the set of markers employed should not be chosen on the basis of their polymorphism between parents of a mapping population, especially where the parents have a narrow genetic base.

Acknowledgements This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. We are very grateful for the technical assistance of Amy Juliano and Fay Hughes. The experiments described in this manuscript comply with the current laws of the UK.

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