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Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walpers) using RFLP mapping data

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Abstract. Genome relationships between mungbean (*Vigna radiata*) and cowpea (*V. unguiculata*) based on the linkage arrangement of random genomic restriction fragment length polymorphism (RFLP) markers have been investigated. A common set of probes derived from cowpea, common bean (*Phaseolus vulgaris*), mungbean, and soybean (*Glycine max*) *Pst*I genomic libraries were used to construct the genetic linkage maps. In both species, a single F_2 population from a cross between an improved cultivar and a putative wild progenitor species was used to follow the segregation of the RFLP markers. Approximately 90% of the probes hybridized to both mungbean and cowpea DNA, indicating a high degree of similarity in the nucleotide sequences among these species. A higher level of polymorphism was detected in the mungbean population (75.7%) than in the cowpea population (41.2%). Loci exhibiting duplications, null phenotypes, and distorted segregation ratios were detected in both populations. Random genomic DNA RFLP loci account for about 89% of the currently mapped markers with a few cDNA and RAPD markers added. The current mungbean map is comprised of 171 loci/loci clusters distributed in 14 linkage groups spanning a total of 1570 cM. On the other hand, 97 markers covered 684 cM and defined 10 linkage groups in the current cowpea map. The mungbean and cowpea genomes were compared on the basis of the copy number and linkage arrangement of 53 markers mapped in common between the two species. Results indicate that nucleotide sequences are conserved, but variation in copy number were detected and several rearrangements in linkage orders appeared to have occurred since the divergence of the two species. Entire linkage groups were not conserved, but several large linkage blocks were maintained in both genomes.

Key words: Parallel maps – Molecular markers – Random amplified polymorphic DNA – Restriction fragment length polymorphism

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) and cowpea (*V. unguiculata* (L.) Walpers) are tropical legume species that provide two of the most important and inexpensive sources of dietary protein to the people of Asia and Africa. Despite their importance, research on these species has lagged far behind that of cereals and other vegetables. Both species are members of *Vigna* Savi, a genus belonging to the tribe Phaseoleae, which contains numerous important legume species including the common bean (*Phaseolus vulgaris* L.) and pigeon pea (*Cajanus cajan* (L.) Millsp.) (Baudoin and Marechal 1985, 1988). Mungbean and cowpea are currently believed to represent divergent but parallel evolutionary lineages in the genus *Vigna*. (Baudoin and Marechal 1985; Smartt 1985, 1990). Except for one report of a successful cross between mungbean and cowpea (Stanton 1964), all other attempts to produce viable interspecific hybrids between them have failed (Evans 1976). The strong reproductive barriers have precluded direct comparative genetic studies between these species. However, both are known to share the same basic chromosome number (x = 11) (Joseph and Bouwkamp

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1978; Frahm-Leliveld 1965) and contain a comparable nuclear DNA content of approximately 1.0 pg/2C nucleus (Murray et al. 1979; Arumuganathan and Earle 1991). Thus, it is highly probable that the two species share many orthologous genes (Tanksley et al. 1988) and conserved gene linkages.

The development of DNA marker technologies and mapping strategies have: (1) facilitated the establishment of genetic linkage maps of important cereals (McCouch et al. 1988; Beavis and Grant 1991; Binelli et al. 1992), legumes (Havey and Muehlbauer 1989; Keim et al. 1990; Vallejos et al. 1992; Weeden et al. 1992), vegetables (Landry et al. 1987; Gebhart et al. 1989, 1991; Slocum et al. 1990; McGrath and Quiros 1991; Song et al. 1991; Tanksley et al. 1992), and fruit species (Durham et al. 1992), (2) enabled the tagging of important single and polygenic traits (Barone et al. 1990; Klein-Lankhorst et al. 1990; Paterson et al. 1988; Martin et al. 1991; Michelmore et al. 1991; Ritter et al. 1991; Yu et al. 1991; Edwards et al. 1992; Landry 1992; Young et al. 1992), and (3) contributed to the knowledge of genome organization and evolutionary relationships in several plant species (Song et al. 1990; Gawel et al. 1992; McGrath and Quiros 1992; Wang et al. 1992; Wilde et al. 1992).

Markers which have contributed significantly to the mapping process include isozymes (Tanksley and Orton 1983), restriction fragment length polymorphic markers (RFLPs) (Botstein et al. 1980), random amplified polymorphic DNAs (RAPDs) (Williams et al. 1990), and simple sequence length polymorphic markers (SSLPs) or microsatellites used in animal genome mapping (Dietrich et al. 1992). The success of these markers can be attributed to the following characteristics: they are numerous, selectively neutral, (frequently) co-dominantly expressed (Soller and Beckman 1983; Tanksley et al. 1989) and, in the case of RAPDs and SSLPs, highly polymorphic and easy to assay.

The comparative mapping approach has also facilitated the mapping of orthologous sequences among closely related plant species or genera and added to our knowledge of the organization and evolution of plant genomes in genetically divergent species. This mapping approach allows map construction of a related species by utilizing a common set of molecular markers as hybridization probes. It has proven to be most useful in comparing genomes of reproductively isolated species. For example, an extensive conservation of linkage relationships has been found between tomato and potato (Bonierbale et al. 1988), sorghum and maize (Hulbert et al. 1990; Binelli et al. 1992), *Brassica campestris* and *B. oleracea* (McGrath and Quiros 1991), and pea and lentil (Weeden et al. 1992). At the other extreme, a comparison of tomato and pepper genomes revealed the conservation of nucleotide sequences but not linkage orders between the two species (Tanksley et al. 1988).

We have embarked on a project to construct and compare the linkage maps of mungbean and cowpea using DNA markers. The current maps, although far from saturated, have been utilized successfully to map the resistance genes against bruchid, a seed storage pest (Young et al. 1992), powdery mildew (Young et al. 1993), and mungbean yellow mosaic virus (L. Kumar, in preparation) in mungbean and to identify orthologous linkage blocks controlling seed weight in both species (Fatokun et al. 1992a). We have previously published the linkage maps of mungbean (Menancio-Hautea et al. 1993) and cowpea (Fatokun et al. 1993b). Prior to our reports, no detailed linkage maps existed for either of these species, although a large number of genes and few linkages had been identified (Poehlman 1991; Singh and Rachie 1985). We now present a detailed report on the construction of the linkage maps of mungbean and cowpea using a common set of clones and a comparative analysis of the genome organization of these two closely related, but reproductively isolated, species.

Materials and methods

Plant material

The parental genotypes used to identify polymorphic probes and to generate the F_2 mapping populations in mungbean and cowpea consisted of an improved cultivar as the female parent and a wild but interfertile putative progenitor as the male parent. The choice of parents was based primarily on the segregation of several agronomically important traits such as pest and disease resistance and on information provided by previous RFLP studies in other self-pollinated species, which indicated that adequate RFLPs can be detected by selecting more genetically distant crosses (Bernatzky and Tanksley 1986; Helentjaris et al. 1988; Paterson et al. 1988).

Seeds of mungbean cv 'VC 3890' and a wild relative *V. radiata* ssp. *sublobata*, Acc. TC 1966 and their F_2 progeny were provided by Dr. Doo-Hwan Kim of the Asian Vegetable Research and Development Center (AVRDC), Tainan, Taiwan (Republic of China). The two cowpea genotypes used, 'IT 2246-4', an improved cultivar, and 'TVNI 963', a wild relative *V. unguiculata* ssp. *dekindtiana*, and their F_2 progeny were obtained from Dr. G. Myers of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. For both species, a single mapping population consisting of 58 F_2 plants was derived from selfed F_1 plants.

All plants were grown in St. Paul, Minnesota under greenhouse conditions. Leaves were periodically harvested and used for DNA extraction. Vegetative cuttings were made of each F_2 plant to ensure that there was enough material for DNA analysis and F_3 seed production.

Clone sources

Cloned DNA fragments from soybean (*Glycine max*), mungbean (*V. radiata*), cowpea (*V. unguiculata*) and common bean (*P. vulgaris*) were used as probes for RFLP analysis. These probes were prepared from *Pst*I random genomic libraries as described

previously (Keim et al. 1989; Chase et al. 1990; Young et al. 1992). The soybean clones were a generous gift from Dr. R. Shoemaker, Iowa State University, Ames, Iowa. These clones are designated sgA, sgB, and sgK followed by a number. Dr. C. E. Vallejos (University of Florida, Gainesville, Fla.) kindly provided seven bean clones, which are assigned different numbers preceded by bg4C and bg4D. All other probes, designated cgO and cgP and mgM, mgQ, mgR and mgS, were derived from the cowpea and mungbean libraries, respectively.

Southern blot analysis

Protocols for DNA isolation, restriction enzyme digestion, electrophoresis, blotting, probe preparation and labeling, hybridization, and washing have been described previously (Young et al. 1992). Restriction enzymes *Bst*NI (New England Biolabs), *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Xba*I (Gibco/BRL), and *Hin*fI (Promega) were tested to detect polymorphisms in mungbean. In addition to the same set of restriction enzymes, *Bcl*I (Gibco/BRL) was tested in cowpea. Dependent on the restriction enzyme used, it was necessary to add spermidine (Sigma) in varying concentrations (0.2–4 m*M*) to digest mungbean DNA completely. Hybond N+ (Amersham) was used as the blotting matrix in an alkali transfer procedure. Hybridizations were conducted at 65 °C and washed at medium stringency (2 × SSC, 1 × SSC, and 0.5 × SSC with 0.1% SDS at 65 °C). Blots were re-used up to 7 times after stripping the previous probe by a series of washes in 0.1 *N* NaOH, 0.25 *M* TRIS-HCl, pH 7.5 with 2 × SSC and 0.1% SDS and 0.5 × SSC with 0.1% SDS at room temperature.

Identification of polymorphic clones, nomenclature of loci, and segregation analysis

Cloned DNA probes were hybridized to blots containing individual restriction enzyme digests of total genomic DNA from the two parents. Polymorphic probes were identified as those clones detecting differences in the banding patterns between the parents in at least one of the seven enzymes tested. In cases where more than one probe-enzyme combination revealed polymorphism, the combination which showed the clearest and simplest banding patterns was selected for mapping. Additional bands which were monomorphic in the mapping enzyme chosen but detected as polymorphic by other enzymes, were not mapped. Duplicated loci refer to two or more loci detected by a single clone and designated by the same probe number followed by a small letter (a, b, c, etc.).

Goodness-of-fit to expected monogenic Mendelian segregation ratios was calculated for each locus by chi-square analysis using the StatView II computer program (Abacus Concepts). Pooled chi-square analysis was performed to test the fit to a 1:1 ratio for the number of alleles derived from each parent for probes showing co-dominant distorted segregation ratios. This was done to determine if there is any consistent direction in the overall pattern of the deviation observed.

Linkage analysis

Linkage analysis of the entire set of markers in mungbean and cowpea was performed using MAPMAKER II (Lander et al. 1987). Initially, a two-point group criterion of LOD ≥ 2.5 was used to assign markers to linkage groups. Three-point (LOD = 3.0), multipoint-compare, and multipoint-first order (LOD = 3.0) functions were then used to determine the likely order of the markers in each group. Finally, full multipoint analyses were run to determine the best orders and distances of evenly spaced markers (LOD ≥ 2.0). All remaining markers were tried in every interval using the try function. Probable linkage between groups was presumed when the link LOD score was 3.0. The best orders were confirmed by permuting all adjacent triples of markers by the "ripple" function. Recombination fractions were converted into map distances in centimorgans (cM) using the Kosambi (Kosambi 1944) mapping function.

Results

RFLP mapping in mungbean and cowpea

RFLP levels

From four legume species 162 heterologous and 89 homologous random genomic DNA clones were successfully tested on the two parental DNAs of mungbean digested individually with the eight restriction enzymes. The same set of clones plus an additional 7 cowpea clones were tested on two parental DNAs of cowpea digested with the same set of enzymes plus *Bcl*I.

Approximately 90% of all the probes tested hybridized with the mungbean and cowpea DNAs (Table 1). All bean probes hybridized to the DNAs of both species, while slightly lower levels of hybridization were obtained with the heterologous probes derived from soybean. A slightly higher percentage of hybridization was detected when mungbean probes were hybridized to cowpea DNA than when cowpea probes were hybridized to mungbean DNA. Sequence homology is estimated to be at least 80% (Beltz et al. 1983) at the level of stringency of the wash conditions used.

Polymorphism detected in the mungbean mapping population was nearly twice that observed in the cow-

Table 1. Percentage hybridization, polymorphism, and mapped loci as revealed by clones from different sources

Probe Sources	% Hybridized probes		% Polymorphic probes		% Mapped probes	
	Mungbean	Cowpea	Mungbean	Cowpea	Mungbean	Cowpea
Soybean	88.0 (125/142)	84.4 (119/141)	77.9 (97/125)	49.7 (57/119)	73.2 (71/97)	86.0 (49/57)
Mungbean	100.0 (89/89)	91.0 (81/89)	73.0 (65/89)	30.8 (29/81)	81.5 (53/65)	58.6 (17/29)
Cowpea	85.7 (30/35)	100.0 (43/43)	68.6 (24/30)	34.9 (15/43)	58.3 (14/24)	66.7 (10/15)
Bean	100.0 (7/7)	100.0 (7/7)	57.1 (4/7)	28.6 (2/7)	75.0 (3/4)	100.0 (2/2)
Total	91.9 (251/273)	89.3 (250/280)	75.7 (190/251)	41.2 (103/250)	74.2 (141/190)	75.7 (78/103)

pea mapping population, irrespective of the clone source (Table 1). The percentage of informative probes varied depending on the probe source. The probes which detected polymorphism in mungbean and cowpea were not always identical, but a number of them were common in both species. Approximately 25% of the probes that detected polymorphism between parental pairs of either mungbean or cowpea were not mapped because they did not segregate in the F_2, gave hybridization patterns that were too complex to be analyzed, or could not be resolved satisfactorily. Therefore, a total of 141 genomic probes detecting 151 loci were mapped in mungbean, while 78 probes detecting 85 loci were mapped in cowpea.

Restriction enzyme differences in detecting RFLPs

Differences in detecting RFLPs among the six most frequently used restriction enzymes are summarized in Table 2. A similar trend was observed in both species in the ability of each restriction enzyme to detect polymorphism. Of those clones that were polymorphic, a majority detected polymorphism between the parental genotypes with only one restriction enzyme, while 22.7% and 35.9% of the probes revealed polymorphisms with three or more enzymes in mungbean and cowpea, respectively. Most of the restriction enzymes with a 6-base pair (bp) recognition site, with the exception of *Dra*I, revealed higher levels of polymorphism than those that recognize 5-bp (*Bst*NI) and 4-bp (*Hae*III) recognition sequences. More than 50% of the RFLPs detected between the parents were uncovered with *Eco*RV.

Description of RFLP markers

Based on the banding patterns detected in the parental survey blots and the segregation patterns observed in the F_2 populations, we classified each probe by eye into the following categories: (1) single-copy sequences that showed hybridization to a single segregating band with no monomorphic band (Fig. 1a), or to one segregating band plus one or two additional monomorphic bands (Fig. 1b), or two to three polymorphic bands with no monomorphic bands (Fig. 1c) and (2) multi-copy sequences that hybridized to more than three bands with at least one segregating band plus several monomorphic bands (Fig. 1d). Probes homologous to highly repetitive sequences were also observed, but they were not utilized in map construction. The proportion of single- and multi-copy probes mapped in mungbean were 76% (107/141) and 24% (34/141), respectively; mapped probes in cowpea were 62% (48/77) single copy and 38% (29/77) multi-copy.

Low levels of duplicated loci have been mapped in both mungbean (20/151) and cowpea (13/87), although the majority of the clones hybridized to more than one band. Typically, probes which hybridized to more than one locus showed additional monomorphic bands. Some of these monomorphic bands showed polymorphism with other probe/enzyme combinations used in the parental surveys, but these were not mapped in this study. In other cases, several polymorphic bands were detected by a single probe, but all co-segregated with one another and were treated as a single locus. These loci probably represent genomic sequences in which a restriction site located within the cloned sequence cut the genomic DNA into multiple polymorphic bands. Alternatively, these co-segregating bands could represent tandemly arranged duplications.

Co-dominance was exhibited by a majority of the RFLP markers used. However, 22 and 18 loci were scored as null phenotypes (absence of allelic band) in mungbean and cowpea, respectively (examples are: mgM151b in Fig. 2a and sgA343a and b in Fig. 2b). Null phenotypes in cowpea were equally detected between the improved cultivated parent (9/18 in 'IT 2246-4') and the wild subspecies (9/18 in 'TVNI 963'). In mungbean, a slight bias was detected (14/22) in favor of the improved cultivated parent, 'VC 3890'. A majority of the loci scored as null in both species contained more than one hybridizing fragment. It is possible that the presence of monomorphic bands as well as multiple fragments might have obscured the presence of the alternate alleles. In some cases, loci were scored as a null phenotype so that accuracy would not be compromised.

Significant deviations ($P \leq 0.01$) from expected Mendelian segregation ratios towards one or the other RFLP allele were observed in 12% (18/151) and 22% (19/85) of the loci mapped in mungbean (Table 3) and cowpea (Table 4), respectively. Loci with aberrant ratios were detected by probes derived from all sources. A majority of the loci with distorted ratios biased

Table 2. Polymorphism detected by different restriction enzymes

	Mungbean	Cowpea
% of probes:		
Polymorphic with three or more restriction enzymes	22.7 (44/193)	35.9 (32/89)
Polymorphic with two restriction enzymes	24.9 (48/193)	30.3 (27/89)
Polymorphic with one restriction enzyme	52.3 (101/193)	42.7 (38/89)
% Polymorphic probes revealed:		
*Eco*RV	56.7 (87/153)	53.9 (48/89)
*Eco*RI	38.5 (59/153)	43.8 (39/89)
*Hin*dIII	28.1 (43/153)	34.8 (31/89)
*Bst*NI	25.5 (39/153)	25.8 (23/89)
*Dra*I	25.5 (39/153)	15.7 (14/89)
*Hae*III	22.8 (35/153)	19.1 (17/89)

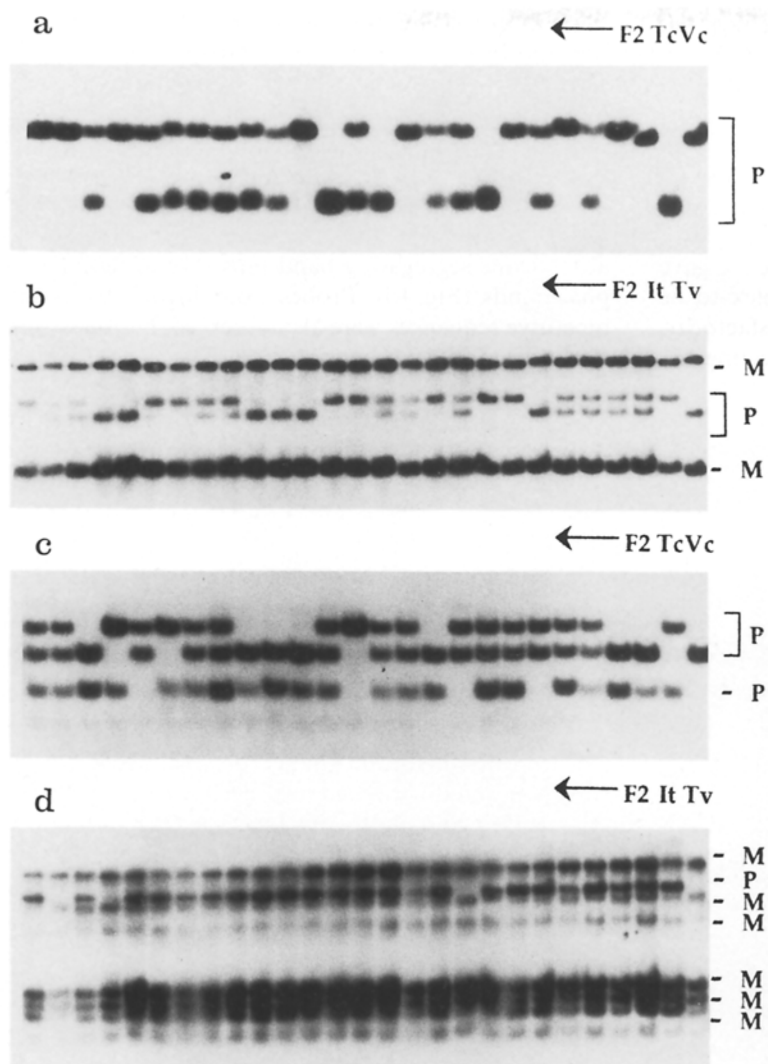

Fig. 1a–d. Hybridization patterns of clones classified as single-copy and multi-copy, as detected by autoradiography. Southern blots of restriction enzyme-digested total genomic DNA of mungbean parents (*Vc* and *Tc*) and cowpea parents (*It* and *Tv*) and their F_2 progeny were probed with single-copy probes revealing one polymorphic (*P*) band (**a**), one polymorphic and two monomorphic (*M*) bands (**b**), and two polymorphic bands (**c**), and with multi-copy probes revealing one polymorphic bands and several monomorphic bands (**d**)

towards one parent were found within the same linkage groups and tended to map close to each other (Fig. 3a, b). Deviations were observed in both single- and multi-copy genomic probes. Two loci (sgA235 and cgO29) showed distorted ratios in both mungbean and cowpea. In both species, a pooled chi-square analysis of co-dominant loci showing distorted ratio indicated highly significant deviation in favor of the alleles of the improved mungbean (56% 'VC 3890' alleles; $\chi^2 = 12.05, P \leq 0.0005$) and cowpea (59% 'IT 2246-4' alleles; $\chi^2 = 24.1$, $P \leq 0.0001$) cultivars.

Description of mungbean and cowpea linkage maps

Random genomic RFLP markers constitute 88.3% (151/171) and 87.6% (85/97) of the loci mapped in mungbean and cowpea, respectively. The maps presented in this paper (Fig. 3a, b) highlight only the positions of the random genomic DNA RFLP markers described in the preceding sections. The same maps consisting of all available markers including 20 cDNA markers in mungbean and 5 cDNA and five RAPD markers in cowpea have been published earlier (Fatokun et al. 1993b; Menancio-Hautea et al. 1993).

The mungbean map

The current mungbean map consists of 171 markers including 151 random genomic DNA and 20 cDNA RFLP loci/locus clusters along with one pest (bruchid) resistance locus arranged into 14 linkage groups. The linked loci span 1570 cM of the mungbean genome with an average distance of 9 cM. Six loci remain unlinked with any other loci. Marker distribution is not even among the linkage groups. Some markers mapped in clusters, while large gaps are still evident in some portions of the map, particularly towards the ends of long linkage groups. Single- and multiple-copy

Table 3. Segregation ratios for loci deviating from the expected Mendelian monogenic ratio (1:2:1 and 3:1) in mungbean

<table>
<tr><th>Linkage group</th><th>Locus</th><th>TC 1966/TC 1966</th><th>TC 1966/VC 3890</th><th>VC 3890/VC 3890</th><th>P</th></tr>
<tr><td>1</td><td>mgM371b</td><td>36</td><td colspan="2">21</td><td>0.0001</td></tr>
<tr><td>1</td><td>mgM456a</td><td>38</td><td colspan="2">19</td><td>0.0001</td></tr>
<tr><td>1</td><td>sgA504</td><td>32</td><td>22</td><td>4</td><td>0.0001</td></tr>
<tr><td>1</td><td>mgR2</td><td>15</td><td>11</td><td>20</td><td>0.0002</td></tr>
<tr><td>2</td><td>sgA235</td><td>7</td><td>31</td><td>19</td><td>0.01</td></tr>
<tr><td>2</td><td>sgA584</td><td colspan="2">33</td><td>24</td><td>0.0001</td></tr>
<tr><td>2</td><td>mgM241</td><td>5</td><td>23</td><td>24</td><td>0.0001</td></tr>
<tr><td>2</td><td>mgA132</td><td>6</td><td>24</td><td>26</td><td>0.0002</td></tr>
<tr><td>3</td><td>mgM177</td><td>19</td><td>16</td><td>22</td><td>0.001</td></tr>
<tr><td>4</td><td>sgB69b</td><td>8</td><td>15</td><td>26</td><td>0.0004</td></tr>
<tr><td>5</td><td>mgS3</td><td>7</td><td>37</td><td>11</td><td>0.01</td></tr>
<tr><td>7</td><td>mgM403</td><td>15</td><td>28</td><td>11</td><td>0.002</td></tr>
<tr><td>7</td><td>sgA363</td><td>15</td><td>15</td><td>20</td><td>0.01</td></tr>
<tr><td>8</td><td>cgO9</td><td>6</td><td>28</td><td>23</td><td>0.0006</td></tr>
<tr><td>8</td><td>mgM273</td><td>7</td><td>26</td><td>23</td><td>0.004</td></tr>
<tr><td>9</td><td>mgM282a</td><td>3</td><td colspan="2">53</td><td>0.0001</td></tr>
<tr><td>10</td><td>sgA946</td><td>18</td><td>17</td><td>22</td><td>0.003</td></tr>
<tr><td>11</td><td>mgM185b</td><td colspan="2">25</td><td>32</td><td>0.004</td></tr>
<tr><td>Unlinked</td><td>cgO29</td><td>14</td><td>16</td><td>28</td><td>0.0002</td></tr>
<tr><td>Unlinked</td><td>mgR34</td><td>21</td><td>12</td><td>14</td><td>0.0004</td></tr>
</table>

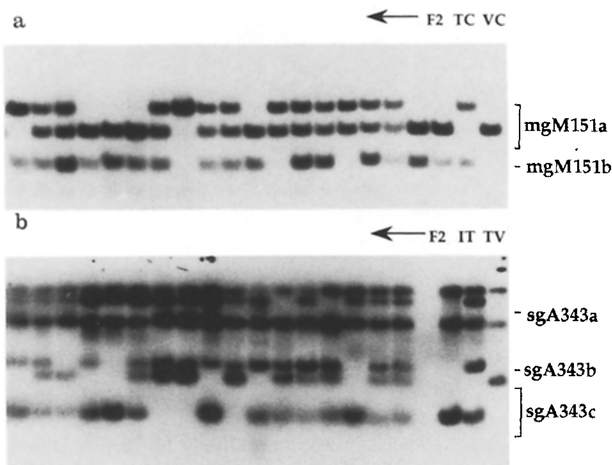

Fig. 2a, b. Segregation analysis of null loci (**a**) mgM151b in mungbean and (**b**) sgA343a and sgA343b in cowpea. Genomic DNA was digested with *Bst*NI and *Eco*RI and hybridized with mungbean clone mgM151 and soybean clone sgA343, respectively. *VC* and *TC* Mungbean parental lines, *IT* and *TV* cowpea parental lines, F_2 segregating progeny

loci were found in all linkage groups. Most single-copy loci were distributed near each other, sometimes interspersed with multi-copy loci. The current number of linkage groups has yet to condense to 11 to coincide with the known basic chromosome number ($x = 11$) of mungbean (Krishnan and De 1965; Joseph and Bouwkamp 1978).

Markers with distorted segregation were found on all but 1 (linkage group 6) of the longest 11 linkage groups, but a majority mapped to linkage groups 1 and 2. Most markers with aberrant ratios favoring the allele of the improved cultivar, 'VC 3890', mapped to linkage group 1. A majority of the loci biased towards the alleles of 'TC 1966' were found on linkage group 2.

Duplicated loci mapped to 10 out of the current 14 linkage groups of mungbean. At least 2 duplicated loci were mapped on linkage groups 1, 3, 9, 10, and 11. Several arrangements of duplicated loci were observed in the mungbean genome. A majority of the duplicated loci were dispersed into different linkage groups. Segmental duplication (Kianian and Quiros 1992) was

Fig. 3a, b. RFLP-based genetic maps of *V. radiata* (**a**) and *V. unguiculata* (**b**). Linkage group numbers are indicated at the *top*. Distances given in centimorgans (cM) using the Kosambi mapping function are shown to the *left* of the *vertical lines*. Loci detected by clones from common bean, cowpea, mungbean, and soybean are indicated by the prefixes *bg*, *cg*, *mg*, and *sg*, respectively; the positions of cDNA and RAPD markers are indicated by *tick marks* on the linkage group, but without marker names. Duplicated loci detected with the same probe are labeled with *lowercase letters*, *a*, *b*, *etc.* and *boxed*. Single-copy clones that gave strictly single-banded patterns are set in *italics*. Markers with distorted ratios ($P \leq 0.01$) are marked with *asterisks*. Markers indicated with *light tick marks* and a *line parallel* to the linkage group could not be ordered with certainty (LOD < 2.0) in the mungbean map. A *hatched line* indicates probable linkage between two linkage groups (LOD ≥ 3.0)

a

1: mgM371b*, mgM456a*, sgA504*, mgM381, mgM228, sgA124, mgM182, cgO103, mgM371a, sgA487, sgA509, sgK286, bg4D7, sgK118, cgP238, sgK227, sgK472b, mgQ22b, mgR2*, sgA517

2: sgA567, sgA235*, mgM411, sgA890, sgA816, sgK24, sgA226, sgA584*, sgK73, sgB53, sgB74, mgM224, sgA71, mgM241*, sgA132*, mgQ43, mgQ45

3: mgM415, mgM177*, cgO26, sgA333, sgA106*, sgA109, mgM26, mgM78, bg4D9, mgM307, sgK472a, mgM454, mgQ22a, sgA841, mgM217, sgA117b, sgA77, sgK70

4: mgM167, cgP137, sgA404, sgA148, sgA81, mgM160, mgM316, mgM244, sgB69b*, mgQ117, sgA7, sgA450, sgA515, sgA955, mgR67

5: mgS3*, sgA162, mgM374, mgQ66, sgA426, cgO91, mgR51, mgR25, sgA262, sgK9, sgA36, sgA131

6: sgA343, sgK411, sgA242, cgO34, sgA711, sgA741, bg4D10b, mgM474, mgR48, mgM392, sgA78

7: sgA708, mgM225, mgM205, mgM236, mgM403*, sgA363*, sgA64, sgK385, mgM247, sgA112, cgO11, mgM208

8: mgQ39, mgM242, sgA121, sgA357, sgA463, mgM100, cgO8, sgA102, mgQ62, mgQ86, cgO9*, mgM282b, cgO53, sgA338, mgM273*

9: sgA257, sgA315, mgM151a, mgM282a*, sgA882, mgR26, sgA352

10: pA186, mgM9, mgM151b, mgM211, sgB32, mgM185a, sgA946*

11: mgM339, sgA60, mgM185b*, sgA117a, mgM456b

12: sgA810, sgB69a

13: sgA204, cgO109

14: mgM398, bg4D10a

MARKERS CURRENTLY UNLINKED

sgA23 sgB46 cgO111
cgO29 mgR34* cgP157

b

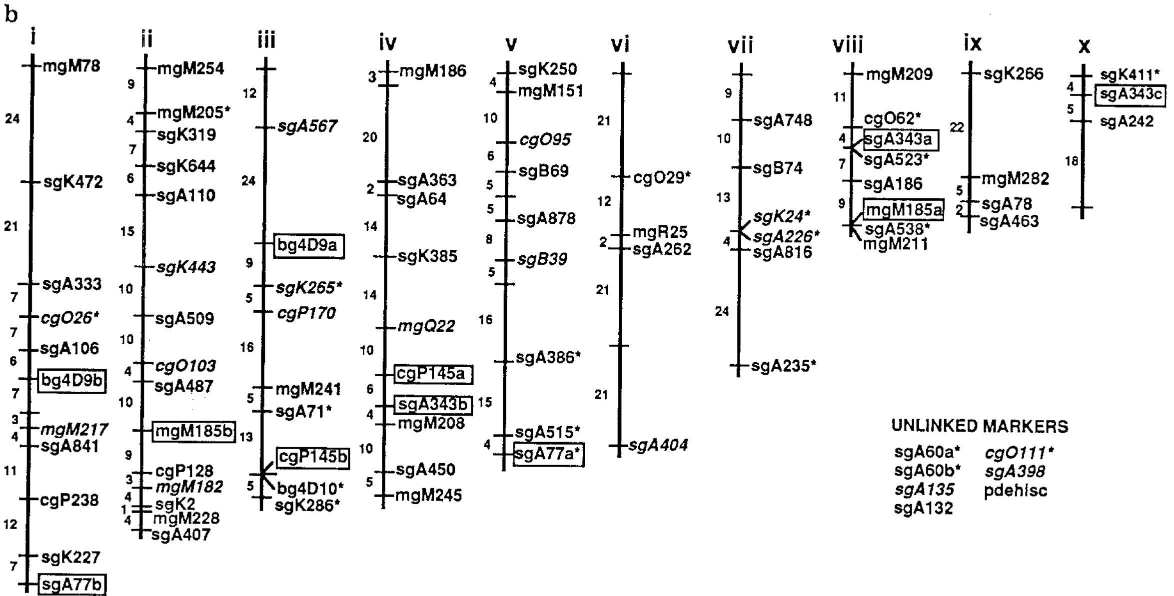

Table 4. Segregation ratios for loci deviating from the expected Mendelian monogenic ratios (1:2:1 and 3:1) in cowpea

Linkage group	Locus	IT 22464/IT 22464		IT 22464/TVNI 963		TVNI 963/TVNI 963	*P*
i	cgO26	2		27		8	0.0001
ii	mgM205		32			24	0.01
iii	sgK265	19		30		7	0.01
iii	sgA71	17		34		5	0.0001
iii	bg4D10	48				7	0.01
iii	sgK286	17		34		7	0.01
v	sgA386	21		30		7	0.01
v	sgA515	19		33		6	0.001
v	sgA77a	17		35		5	0.0001
vi	cgO29	21		12		24	0.0001
vii	sgA235	20		32		5	0.0001
vii	sgA226	24		23		8	0.01
vii	sgK24	24		26		8	0.01
viii	cgO262		47			3	0.001
viii	sgA523	6		39		10	0.0006
viii	sgA538	10			48		0.0001
x	sgK411	7		38		13	0.001
Unlinked	sgA60a	23			33		0.01
Unlinked	sgA60d		53			3	0.0001
Unlinked	cgO111	41		12		5	0.0001

observed only in linkage groups 1 and 3 for a pair of duplicated loci (sgA472 and mgQ22). However, the distance betweeen sgA472b and mgQ22b in linkage group 3 was farther apart than their duplicates in linkage group 1. Furthermore, a single-copy locus (sgM454) separates the duplicated pair in linkage group 3. Non-adjacent duplication (mg371a and b) within the same linkage group 1 was also observed. Linkage group 11 has an interesting organization because most of the markers found in this group are duplications of other loci distributed in other linkage groups.

The cowpea map

The current cowpea map consists of 87 random genomic and 5 cDNA RFLPs, five RAPDs, and two morphological loci/loci clusters arranged in 10 linkage groups. Ten markers could not be linked significantly to any other locus. As in mungbean, linkage group designations were arbitrary and do not coincide with the basic chromosome number of cowpea, which is also x = 11. The 10 linkage groups delimit 684 cM of the cowpea genome. The longest group spans 105 cM, while the shortest group's length is 27 cM. The average distance between adjacent markers is 7 cM, and the markers are more evenly distributed than in the mungbean map.

Of the 10 linkage groups of cowpea, 7 contain single, unique copy loci. Like the mungbean map, single-copy loci tended to map close to each other. Markers with distorted segregation ratios were found on all linkage groups except groups iv and ix. These markers tended to map near each other. A majority of the loci exhibiting an aberrant ratio in favor of the cultivated parent, 'IT 2246-4', were located on linkage groups ii, v, and vii. All mapped duplicate loci in cowpea were dispersed on separate linkage groups. The conservation of linkage arrangement for duplicated loci was not observed in the cowpea map.

Comparison of copy number and linkage arrangement in mungbean and cowpea

A common set of 53 polymorphic probes were genetically analyzed and used to compare copy number and linkage arrangement between the mungbean and cowpea. Among the 53 probes, the copy number of 39 probes (74%) remained the same in both species. A change in copy number between the two species was observed in about 26% (14/53) of the probes. Of these, 9 probes were found to have higher copy number in cowpea than in mungbean. A comparative map (Fig. 4) has been constructed to show the regions of similarities and differences between the two genomes. The most interesting features emerging from the comparison are:

1) Of the 53 loci 49 retained associations between the mungbean and cowpea maps. Complete linkage groups were not conserved, but large blocks were obviously retained in several linkage groups. However, differences in the length of conserved regions were apparent. On average, distances between adjacent markers were greater in the mungbean map than in the cowpea map. Ten regions showed syntenic associations among loci in both maps. The four longest synteny were found between cowpea i and mungbean 3, ii and 1, iv and 7, and vii and 2.

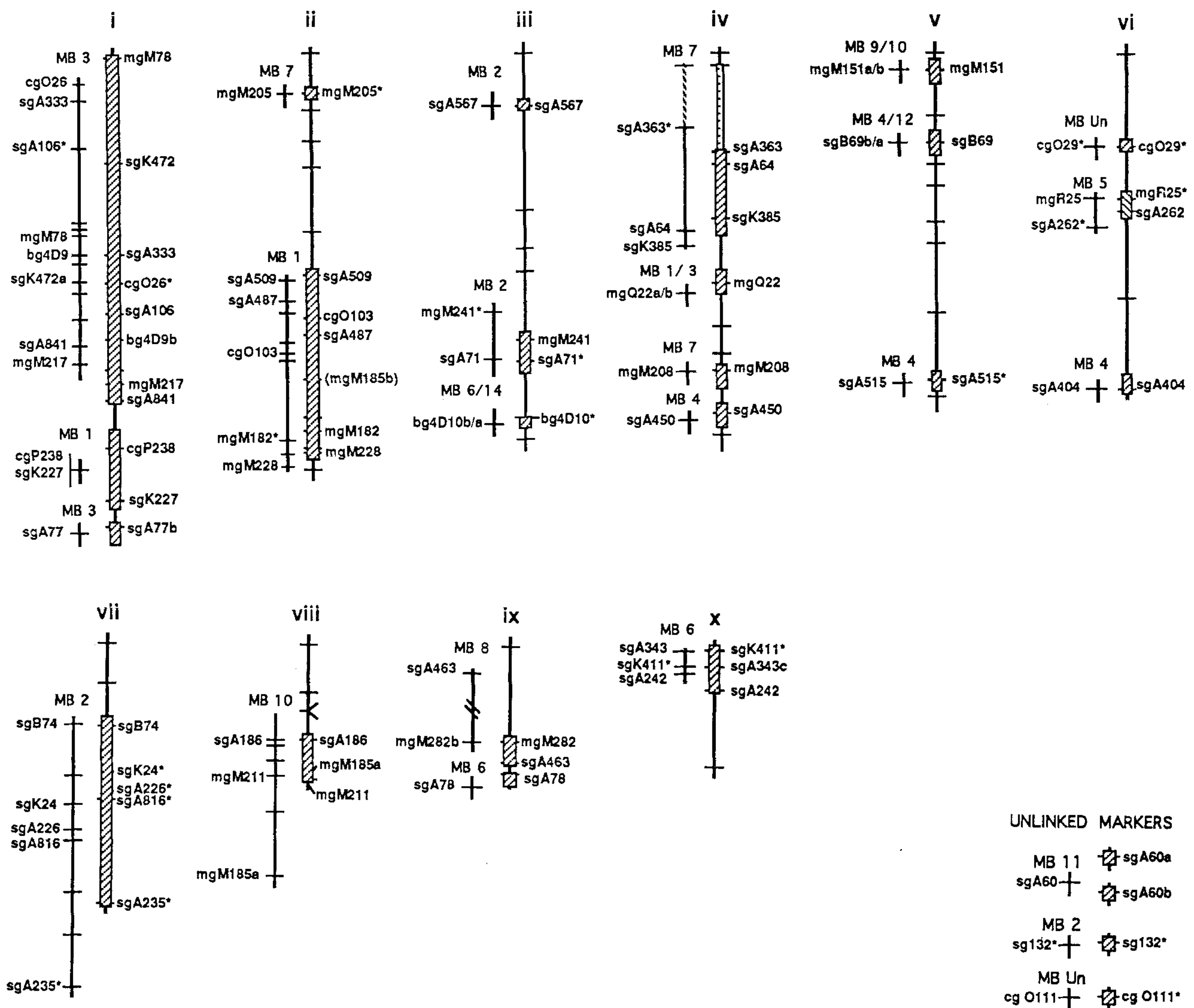

Fig. 4. A comparative map of mungbean and cowpea based on the cowpea linkage groups. Cowpea linkage groups are indicated by *lowercase Roman numerals* labeled at the top of the figure; mungbean linkage groups are identified by the prefix *MB* followed by the linkage group number. Conserved regions between the two species are indicated by *hatched boxes* in the cowpea linkage groups; the corresponding segment in the mungbean linkage groups are indicated to the *left* of the cowpea line. The *open box* in cowpea group iv and *broken line* in mungbean group 7 imply extension of the conserved region based on evidence provided by a mapped cDNA locus (Menancio-Hautea et al. 1993)

2) The linear arrangement/order of loci within conserved linkage blocks either remained unchanged or was substantially rearranged. For example, the sequence of markers from mgM78 to sgA841 in cowpea linkage group i, paralleled by the segment from cgO26 to sgA841 in mungbean group 3, appeared to have undergone considerable rearrangement. These may be due to inversions, deletions, or translocations. In contrast, orthologous blocks of cowpea group ii and mungbean group 1 were co-linear, except where the linkage was interrupted by a possible insertion of a duplicated locus, mgM185b, in cowpea. This is an interesting region because the major seed weight genes of both cowpea and mungbean were mapped within this segment (Fatokun et al. 1992). A group of 5 markers spanning two-thirds of cowpea linkage group viii corresponded to a large internal segment in mungbean linkage group 2. These markers have remained strictly co-linear, but their distances were different in the two genomes.

3) Although a number of linkage blocks of mungbean were conserved in the cowpea genome, some cowpea linkage groups appeared as mosaics of several different linkage groups of mungbean. Loci in linkage

groups 1, 2, 3, 4, and 8 of mungbean appear to be largely involved in detecting orthologous regions in cowpea. For example, linkage blocks of group 1 have been conserved in cowpea linkage groups i and ii, and group 2 were retained in cowpea v and vi.

Discussion

RFLP technology has enabled us to construct the first detailed genetic linkage maps of mungbean and cowpea and to compare directly the genome organization of these closely related, but reproductively isolated, species. We attribute the relative success of our mapping efforts to two important factors: (1) the choice of an intersubspecific cross to generate each mapping population, and (2) the use of heterologous probes from other legume species in addition to the mungbean and cowpea probes. Many of the currently available linkage maps of inbreeding plant species were successfully constructed using more genetically divergent parents, in particular, interspecific combinations, because they have been shown to uncover sufficient amount of polymorphisms (Bernatzky and Tanksley 1986; Helentjaris et al. 1988). In the present study, the choice of an intersubspecific mapping population uncovered adequate polymorphism (75.7%) in mungbean. Lower levels of polymorphism (41.2%) were detected in the intersubspecific cowpea mapping population used, but this was interpreted to be due to the low polymorphism inherent in the cross used and not representative of the extent of genetic diversity observed within *V. unguiculata* (Fatokun et al. 1993b). A more polymorphic mapping population is currently being synthesized to define additional linkage groups and increase the marker density of the cowpea map.

Heterologous probes for use in map construction have been successfully employed in a number of crops (Bonierbale et al. 1988; Hulbert et al. 1990; Weeden et al. 1992). In this study, the use of heterologous probes facilitated map construction, particularly in cowpea, by uncovering a higher proportion of segregating loci. This may be attributed to the ability of the heterologous probe to detect another but slightly divergent copy under the hybridization conditions used. It also enabled us to determine that portions of the genomes of these reproductively isolated species share similar nucleotide sequences. These four species represent three distinct genera within the family Leguminosae. The fact that their genomes share sufficient homologies implies that the information obtained from different mapping projects in these species may be useful to each other.

The mungbean map consists of 171 loci arranged into 14 linkage groups; the cowpea map has 97 markers distributed in 10 linkage groups. Linkage group designations were assigned arbitrarily, and the groups were arranged in order of decreasing length. The current maps represent the arrangement of markers that give the best log-likelihood scores. As a consequence of using this mapping approach, distances between markers may change, and the positions of some of the "non-skeleton" markers may become more certain as more loci are added. With more markers, the linkage group arrangement will necessarily have to change too, if the same basis for assignment of group designations is applied, until such time that each linkage group is mapped onto corresponding chromosomes. A local mapping strategy (Reiter et al. 1992) using additional cDNA clones and RAPD markers is now being used to increase marker density and fill in the large gaps found in the two maps. Although far from saturated, the current maps of both species have already facilitated the localization of a few important characters such as bruchid resistance (Young et al. 1992), genes controlling seed weight (Fatokun et al. 1992), powdery mildew resistance (Young et al. 1993), and mungbean yellow mosaic virus resistance (L. Kumar, in preparation).

A number of notable features of the mungbean and cowpea genomes were revealed while constructing the maps. As expected of a more genetically divergent cross, distorted segregation ratios were observed for 12% and 22% of the mapped loci in mungbean and cowpea, respectively. These values fall within the range reported in a number of plant RFLP studies involving wide crosses (Landry et al. 1987; Bonierbale et al. 1988; McCouch et al. 1988; Gebhardt et al. 1989; Havey and Muehlbauer 1989; Landry et al. 1991; Durham et al. 1992; Vallejos et al. 1992; Weeden et al. 1992). Distorted ratios have been attributed to gametic selection and/or chromosomal rearrangements (Gebhardt et al. 1991; Kianian and Quiros 1992). In this study, a majority of the loci showing distorted ratios mapped within the same linkage groups. Furthermore, skewing in favor of alleles of one or the other parent were observed within these regions. This could indicate pre- and post-zygotic selection for or against a particular allelic combination when the two different genomes are brought together. This is supported by observations of partial sterility in the *unguiculata* × *dekindtiana* cross used (C. A. Fatokun, unpublished results) and other previous reports (Rawal 1975; Lush 1979). Reduced fertility for the *radiata* × *sublobata* cross has likewise been documented (Biswas and Dana 1975; Ahuja and Singh 1977). Alternatively, Zamir and Tadmor (1986) suggested that the markers may be linked to factors such as structural genes or cryptic structural differences between parents. Structural chromosome polymorphism has been previously documented in cowpea (Smartt 1990), while secondary associations of chromosomes have been observed in mungbean (Machado

et al. 1982). Since the sizes of both mapping populations used were small, some of the less pronounced distortions exhibited by some loci may also be attributed to statistical sampling error (Gebhardt et al. 1991). Cytogenetic analyses of the crosses might evaluate the role of chromosomal rearrangements in segregation distortion and altered linkage relationships (Kianian and Quiros 1992).

Our results demonstrate that sequence duplications exist in the mungbean and cowpea genomes. Although only a small number of these duplications were mapped, the data indicate that this number probably represents the minimum level of duplications in these species. Duplicate markers were found to be distributed on more than 1 linkage group, while possible tandem duplications and other linkage arrangements of duplicated loci were detected. A putative duplication of a chromosomal segment was observed in mungbean. Mapped duplications were found mostly in 5 linkage groups. RFLP analysis of duplicated sequences in corn (Helentjaris et al. 1988) and *Brassica* (Slocum 1990; Kianian and Quiros 1992) has been used to provide additional evidence to support the hypothesis of the secondary polyploid nature of these species. It has been suggested earlier (Frahm-Leliveld 1965) that the genome of *Vigna* is comprised of two genomes: one genome contains a basic chromosome number of $n = 5$, and the second genome $n = 6$. More recently, Machado et al. (1982) lent further support to the suggestion that *Vigna* is a secondary polyploid functioning as diploids on the basis of secondary associations between parental chromosomes and split spindle formation. Although a more extensive RFLP analysis of duplicated loci has yet to be carried out in these *Vigna* species, our results are in agreement with the suggestion that *Vigna* is a secondary polyploid.

Insertions/deletions between the genomes of the parental pairs were also detected in both species as evidenced by the presence of loci with null phenotypes. Some null loci mapped adjacent to each other, suggesting large blocks of deletion. The hybridization of clones with parental DNAs digested with several restriction enzymes provided further evidence that insertion/deletion accounts for the RFLPs observed between the parents. Among the 6-cutter restriction enzymes used, *Eco*RV, *Eco*RI, and *Hin*dIII generally produced larger restriction fragments and detected higher amounts of polymorphism in both species than *Dra*I. Furthermore, about 7% of the probes were found to detect RFLPs between the parents with all of the restriction enzymes tested, indicating the presence of deletion and insertion rather than base substitution. These observations parallel the results and conclusions made from rice regarding insertion/deletion (McCouch et al. 1988). At this point we can conclude that insertion/deletion could have played an important role in the evolution of the domesticated species from their wild progenitors.

Comparative mapping provided us an efficient strategy for measuring the changes that occurred in the structure of the mungbean and cowpea genomes since their divergence. The hybridization analysis revealed that the genic complement between mungbean and cowpea appears to be similar at the nucleotide level, although the copy number of some loci has changed between them. The comparative map demonstrated that some linkage blocks remained highly conserved in both species but that the linear arrangement of the markers have changed. Other linkage groups in cowpea consist of segments from different linkage blocks in mungbean. In conjunction with the results discussed in the preceding sections about loci with aberrant ratios, duplications, insertions, and deletions, we can conclude that chromosomal rearrangements may have played a major role in the divergence of the two species. It is interesting to note that the extent of genomic changes found between mungbean and cowpea are greater than those found between potato and tomato (Bonierbale et al. 1988) or sorghum and maize (Binelli 1992), although mungbean and cowpea belong to the same genus while the other crops belong to different genera.

Parallel evolutionary trends have been postulated for mungbean and cowpea (Smartt 1984, 1990). Both species have evolved into similar growth forms as a response to selection pressure under domestication. These include traits such as seed size, photoperiod sensitivity, erect habit, pod shattering, etc. Perhaps the conserved regions controlling the seed weight genes in both species (Fatokun et al. 1992) may be some of the sequences involved during the parallel evolution of these species. It may, therefore, be possible that other regions conserved between the two species contain genes controlling traits associated with the evolution of these two domesticated species from their primitive types. Remarkably similar responses to domestication have also been observed in three important crop species (*V. mungo*, *V. angularis*, and *V. umbellata*) closely related to mungbean (Smartt 1990). These species may provide an excellent system for studying the molecular evolution of homologous characters.

Prior to this work, morphology, cytology, and breeding studies provided most of the information about the genes and genome structures of these two species (see reviews by Smartt 1990; Dana and Karmakar 1990; Poehlman 1991). While these studies have contributed greatly to the improvement of these crops, RFLP analysis may provide efficient methods for breeding better varieties and understanding the evolution of this group of plants. Mungbean and cowpea both possess a vast array of germ plasm already collected and preserved in two international centers and by workers around the world. We are currently utiliz-

ing the map in other crosses to locate and clone the genes for resistance against mungbean yellow mosaic virus and *Cercospora* leafspot diseases. At the same time, we are adding more markers to the maps and synthesizing new mapping populations consisting of recombinant inbred lines for mungbean and cowpea. As the maps become more saturated, more complex traits known to limit the production potential of these crops could be dissected and utilized more effectively in national breeding programs.

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