

Repetitive sequences and their organization on genomic clones of *Zea mays*

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Fourteen recombinant clones from *Zea mays* were studied with regard to their composition of unique and repetitive sequences. Southern hybridization experiments were used to classify restriction fragments of the clones into a unique, middle or highly repetitive class of reiteration frequency. All three classes were often found on the same genomic clone. Cross-hybridization studies between clones showed that a given repeat might be present on several clones, and thus four families of highly repetitive elements were established. Heteroduplex analysis was used to show the arrangement and size of repeats common between several clones. A short interspersion pattern of unique, middle and highly repetitive DNA was found. The dispersed repetitive elements were ~300–1300 bp in length. Analysis of the pattern produced by a given repeat in genomic Southern experiments suggests that some small dispersed repeats may also exist as part of a larger repeating unit elsewhere in the genome.

Key words: *Zea mays*/repetitive sequences/genome organization

Introduction

Genome organization in higher plants has been traditionally studied using three methods: cytology, classical genetic crossing experiments, and DNA reassociation kinetics (C_{ot} analysis). The first two methods have been used to localize some repetitive DNA segments such as centromeric regions and knobs, and unique DNA segments (i.e., functional genes) to a given chromosomal position, while the third method has allowed an overall view of the distribution, percentage and average size of repetitive and unique DNAs. Recombinant DNA technology presents a fourth method which complements the above methods by allowing a finer, detailed analysis. Cloned DNA fragments of specific chromosomal segments can be obtained, the size of specific repetitive units can be identified at the nucleotide level, and finally the organization between unique and repetitive DNA can be analyzed for stretches of several kilobases in length.

The following study is the beginning of such an analysis using 14 recombinant clones of maize (*Zea mays*). Each of these clones was initially chosen because of the presence of an allele of the *Cin1* family of dispersed repeats (Gupta *et al.*, 1983). Thirteen of the clones also contain other repetitive sequences. A simple method is used to classify these repeats into a middle or highly repetitive class of reiteration frequency. Several of the repeats are classified into families due to cross-hybridization between repeats present on several clones. The size and distribution of several repeats is shown by hetero-

duplex analysis. The results are discussed with respect to earlier observations concerning genome organization in plants (for reviews, see Walbot and Goldberg, 1979; Hake and Walbot, 1980; Flavell, 1980; Thompson and Murray, 1981).

Results

Identification and classification of repetitive fragments

The *Z. mays* genome consists of 60–80% repetitive DNA (Flavell *et al.*, 1974), the majority of which is dispersed throughout the genome in a short interspersion pattern with unique or other repetitive sequences (Hake and Walbot, 1980). We therefore expected that the 14 recombinant maize clones which carried the *Cin1* dispersed repetitive element, should also contain other repeats. To identify unique *versus* repetitive segments of the clones, *HpaII* restriction fragments of the clones were separated on an agarose gel, transferred to nitrocellulose and hybridized to sonicated, radioactive total maize genomic DNA (Figure 1). The intensity of hybridization of a given restriction fragment should reflect the presence of the most highly repeating unit present on the fragment. That is, fragments which carry highly repetitive elements should hybridize strongly while the hybridization of unique restriction fragments should not be detectable. This was seen to be the case in Figure 1A, where lane 1 contains *HpaII* restriction fragments of the LC1 clone which is thought to be unique in the maize genome (Shepherd *et al.*, 1982), and lane 2 contains the *HpaII* fragments of the NF1 clone which is homologous to LC1 except for the *Cin1* repetitive element (Shepherd *et al.*, 1982). Hybridization of the genomic DNA probe to the unique restriction fragments of LC1 and NF1 was not observed. Only the 1.35-kb *HpaII* restriction fragment containing the *Cin1* repeat was seen to hybridize (Figure 1a, part b). Thus, *HpaII* restriction fragments of the 14 maize clones were classified into unique *versus* repetitive DNA (for example, see Figure 1B). The repetitive fragments were further divided into middle or highly repetitive classes, depending upon the relative strength of the hybridizing band. Although this system of classification is only a rough estimation of reiteration frequency, it is fast and reliable for the initial analysis of recombinant clones. The general classifications appear to be correct since the *Cin1* repetitive element only hybridized weakly (Figure 1A, part b) and it is thought to be a middle repetitive sequence (Gupta *et al.*, 1983). It was realized that the reiteration frequency of the smaller *HpaII* restriction fragments might be underestimated due to inherent problems of the Southern hybridization method. Thus *HpaII* fragments <200 bp were omitted from the classification system summarized in Table I. It is possible that several clones do not contain any unique sequence (for example, LC102 or LC105) but, as mentioned above, the method may allow some unique sequences to go undetected.

The arrangement of the repetitive or unique fragments on a given clone would require the determination of a *HpaII*

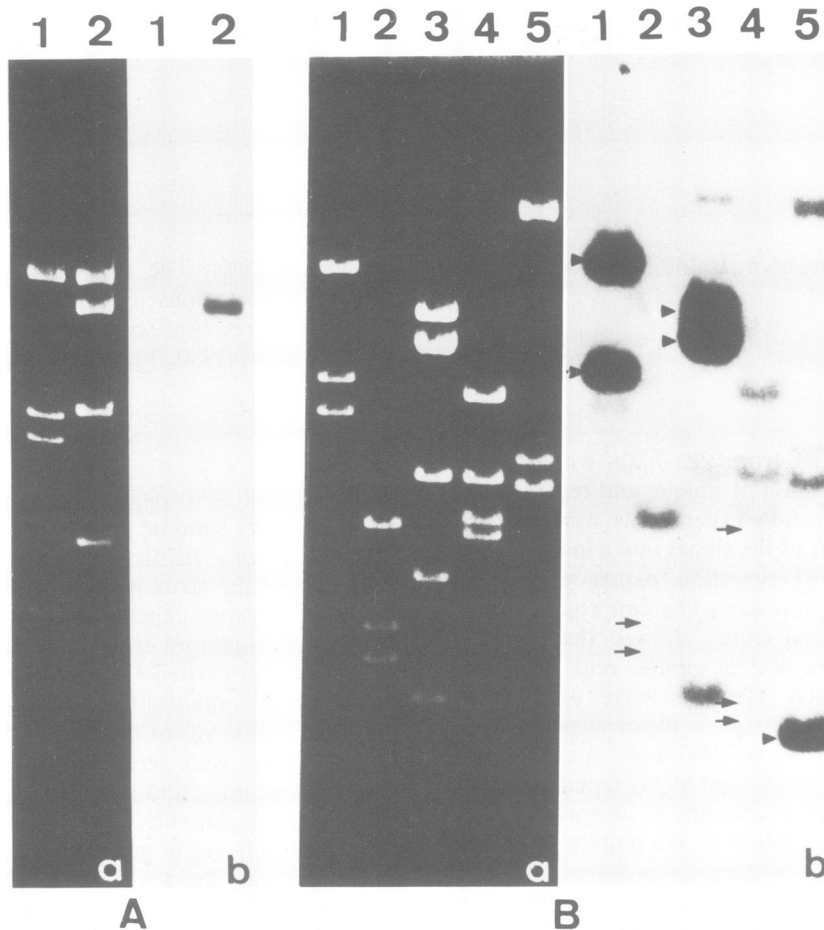


Fig. 1. (A) Electrophoretic separation of *Hpa*II restriction fragments of the LC1 (lane 1) and NF1 (lane 2) clones on a 2% agarose gel (a). These restriction fragments were transferred to nitrocellulose and hybridized to sonicated, ³²P-labelled genomic maize DNA as described in Materials and methods (b). The autoradiogram was developed after 24 h of exposure. (B) Electrophoretic separation of *Hpa*II restriction fragments of the LC clones: LC115 (lane 1), LC112 (lane 2), LC110 (lane 3), LC108 (lane 4) and LC106 (lane 5). The fragments were stained with ethidium bromide and visualized under u.v. light (a), transferred to nitrocellulose and hybridized to sonicated, ³²P-labelled genomic maize DNA as described in Materials and methods. The autoradiogram was developed after 24 h exposure (b). Untailed arrows indicate fragments classified as highly repeated sequences and tailed arrows indicate the unique fragments (which did not appear even after exposure for 6 days). The unmarked fragments were classified as middle repetitive. The smallest fragment in lane 5 (which is classified as highly repetitive) is 350 bp. This fragment was observed on the ethidium bromide stained gel but not apparent in the photograph (part Ba, lane 5).

restriction map; however, since very small *Hpa*II fragments are not classified and since the extent of a repeat on a given fragment is not known, we decided first to classify the repeats into families and then to use heteroduplex analysis to position the repeats on the clones.

Identification of several families of repeats

Early in this work it was noticed that several of the clones cross-hybridized when a non-*Cin1*-containing restriction fragment of one clone was used as a radioactive probe on several other clones. Table II shows the result of such experiments when *Hpa*II restriction fragments containing highly repetitive sequences from either clone LC102, LC103, LC109 or LC114 were purified, pooled, and used as a radioactive probe on the other clones.

For each of the probes, several (but not all) restriction fragments of another clone hybridized. All of the cross-hybridizing fragments had previously been classified as either middle or highly repetitive in Table I, and we therefore concluded that the highly repeating sequence(s) in the probe were also present to some extent on these other clones. Thus, at least four distinct families of highly repeating sequences could be designated: the 102, the 103, the 109 and the 114 family.

Several restriction fragments which were designated as highly repetitive in Table I did not hybridize to any of the four families (Table II, last column). It is not known if each of these other fragments represents a distinct family not present elsewhere on the 14 clones, or if a few of them are similar.

Genomic hybridization experiments

To visualize the hybridization pattern of each clone with genomic DNA, each clone was used as a radioactive probe on *Eco*RI-digested total maize DNA in a Southern hybridization experiment (Figure 2). Since each clone contained the *Cin1* repeat and several clones contained either the 102 or 103 highly repetitive family, non-radioactive LC102 or LC103 insert DNA was often included in the hybridization solution for competition. This competition method worked well (Figure 2, lane c) and allowed a more distinct pattern to be observed for the various clones. In general, the overall repetitive pattern of the various clones was different (for example, lanes a and b which represent probes of LC102 and LC103, respectively). However, several clones did show a similar pattern: the LC113 and LC114 patterns (lanes m and n) are similar and most likely reflect the 114 highly repetitive family which is present on both clones; the LC107 and LC108 hybridization

Table I. Classification of the *Hpa*II restriction fragments of the LC genomic clones according to their reiteration frequency in the genome of *Z. mays*

Clone name	Clone size ^c	Reiteration frequency class ^a		
		Unique	Middle repetitive	Highly repetitive
LC102	2.9			<i>Cin1</i> ^b 0.5, 0.42
LC103 ^d	9.7	0.46, 0.54	1.05, 0.74, 0.68, 0.54, 0.43	1.55
LC104	8.1	0.48, 0.41	0.68, 0.60, 0.43	1.10
LC105	5.1		1.50, 0.34, 0.24	2.20
LC106	7.6		3.75, 1.05, 0.58	0.90 ^e
LC107	2.4	0.44, 0.28	0.42, 0.30	0.25
LC108	6.3	0.71, 0.38, 0.37	0.94, 0.76, 0.25	1.45
LC109	5.2		0.75	0.91
LC110	7.4		0.90, 0.51	0.39
LC111	7.6		2.70, 1.03	0.36
LC112	2.2	0.45, 0.42, 0.30, 0.29		0.74
LC113	9.8		0.96, 0.80, 0.64	2.25 ^e
LC114	7.6		1.90, 1.10, 0.21	2.80
LC115	6.6	0.38	1.30, 0.39	2.80

^a*Hpa*II restriction fragments of the LC-clones were divided into reiteration frequency classes according to the procedure described in Materials and methods and shown in Figure 1B. Fragments < 0.2 kb in size were not included.

^bRestriction fragment(s) which hybridize to the *Cin1*-001 allele of the NF1 maize recombinant clone in a Southern hybridization experiment.

^cThe size of the maize insert contained in the recombinant clones is given in kilobases. Data were taken from Gupta *et al.* (1983).

^dThe restriction fragments for LC103 are actually taken from two subclones (*Eco*RI-*Bam*HI fragments) which together contained the entire 9.7-kb, LC103 insert.

^eThis restriction fragment is a doublet of fragments which were not separated by the 2% agarose gel system.

Table II. Cross-homology of highly repeated sequences among maize genomic clones

Clone ^a	Probe ^b				Unclassified ^c
	LC102 (1.05+0.47)	LC103 (2.5)	LC109 (1.0+0.44+0.43)	LC114 (0.85+0.74)	
LC102	1.05,0.47 ^e				
LC103		2.5			
LC104	0.83	1.3	0.68 ^d ,0.37		1.2
LC105		2.2,0.45			
LC106					0.35
LC107					0.72
LC108					
LC109			1.0,0.44,0.43,0.37		0.91
LC110		1.75 ^e	1.75 ^e		2.1
LC111	3.05				
LC112					
LC113		2.25 ^e ,1.2	2.25 ^e ,1.2	2.25 ^e	1.05
LC114				2.8,1.9 ^d ,1.1 ^d ,0.85,0.74	
LC115	2.8	1.55	1.55		

^aEach LC-clone was digested with *Hpa*II restriction enzyme, fractionated on a 2% agarose gel, transferred to a nitrocellulose filter and hybridized to a nick-translated ³²P-labelled probe (see Materials and methods).

^bHighly repetitive *Hpa*II restriction fragments of a given clone (LC102, 103, 109, 114) were pooled, nick-translated (Rigby *et al.*, 1977), and used as a radioactive probe in the hybridization experiment mentioned above.

^cHighly repetitive restriction fragments which did not hybridize to the probes listed in b.

^dThe fragment is classified as middle repetitive but hybridizes to the highly repetitive probe.

^eTwo fragments of similar size were not separated on the 2% agarose gel.

patterns (lanes g and h) are similar and perhaps reflect a middle repetitive, non-*Cin1* sequence which is common on the two clones; and finally, the LC104 and LC115 patterns (unpublished data) were almost identical and perhaps reflect the 109 repetitive family that is present on both clones (the strong band hybridizing at 1.8 kb in LC104, LC109 and LC113 is

perhaps characteristic for the 109 family). Two clones showed hybridization patterns completely different from any other. The LC106 pattern was extremely repetitive and may reflect the hybridization due to the repeat present on the 0.35-kb *Hpa*II fragment (Tables I and II). The hybridization pattern of LC112 was different from the other 13 clones because it contained only the *Cin1* element on the 0.74-kb *Hpa*II fragment with the remainder of the clone being unique DNA (Table I). Since the *Cin1* hybridization pattern was competed out, no hybridization was observed after 24 h exposure of the film (Figure 2, lane l). However, after prolonged exposure, two unique bands hybridizing at 7.3 and 2.2 kb were observed (data not shown). The 2.2-kb band represents the size of the original *Eco*RI fragment cloned in LC112.

Heteroduplex analysis

Thirteen of the 14 recombinant clones have been shown to contain repeating units other than a *Cin1* element. Heteroduplex analysis was used to localize and determine the approximate size of some of these repeats. The recombinant clone LC104 contains members of three of the highly repetitive families (the 102, 103 and 109 family) as well as containing other middle and highly repetitive sequences (Tables I and II). A heteroduplex structure between LC104 and either LC102, LC103, or LC109 was obtained (Figure 3). Since the location of the *Cin1* repeat had previously been determined for each clone (Gupta *et al.*, 1983), all other heteroduplex structures should be due to repetitive sequences which are common to both clones and present in the same orientation with respect to their *Cin1* alleles. The specific heteroduplexes are described in detail in the legend to Figure 3 and summarized by a line drawing in Figure 4. It is clear only in the case of LC102 that the arrows drawn in Figure 4 correspond to the highly repetitive family and to the *Cin1* repeat (dark arrows). Both clones LC103 and LC109 also contain middle repetitive sequences which may be in common with a sequence on LC104 – thus the non-*Cin1* arrows shown for

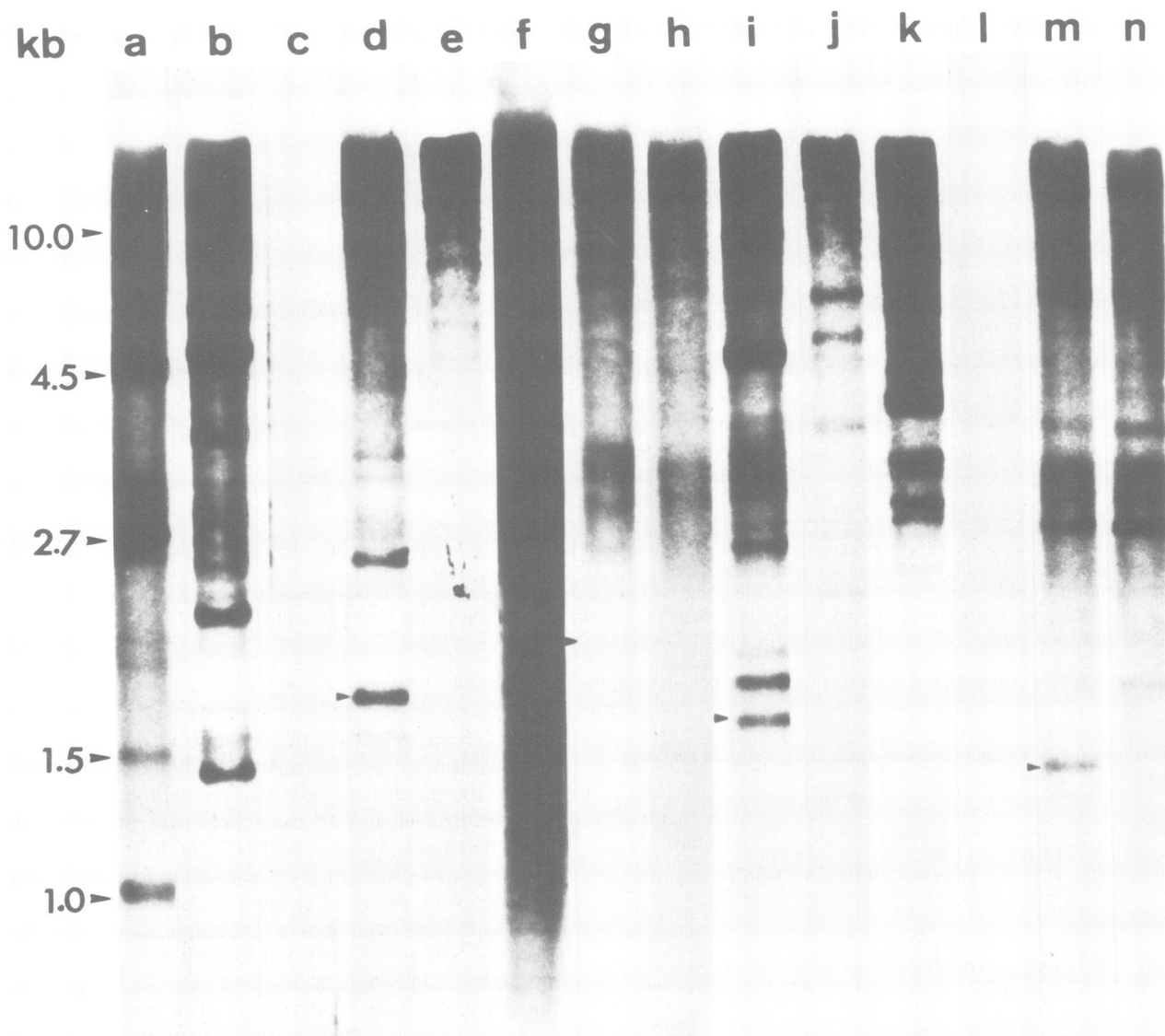


Fig. 2. Hybridization of LC-clones to maize genomic DNA. Approximately 12–15 μg of LC maize genomic DNA was digested with *Eco*RI, electrophoresed on an 0.7% agarose gel, transferred to nitrocellulose (Southern, 1975a) and hybridized to 1 μg of ^{32}P -labelled nick-translated probe. The lanes which were mentioned in the text as showing a similar pattern of hybridization for two different LC-clones were taken from the same gel and are thus directly comparable (lanes m and n, and lanes g and h). Otherwise the lanes were taken from different gels, but the relative size of the fragments corresponds roughly with those shown for lane a. Probes used for hybridization were (a) a non-*Cin1*-containing, 0.61-kb *Hin*I fragment of LC102, (b) a non-*Cin1*-containing, 0.5-kb *Hin*I fragment of LC103 and (d–n) the *Eco*RI maize insert of LC104–LC114, respectively. In order to eliminate the repetitive pattern of the *Cin1* repeat and that of the LC102 or LC103 highly repetitive family, hybridization of LC104, LC105, LC110, and LC113 (lanes d, e, j and m) were competed with non-radioactive LC103 DNA, while the remainder of the clones (including LC104) were competed with non-radioactive LC102 (lanes a and b were excluded). For competition, 100 μg of sonicated *Eco*RI insert of LC102 or LC103 DNA was hybridized to the genomic blot for 6 h in the hybridization buffer prior to addition of the radioactive probe. These competition conditions are seen to be sufficient in lane c, where 100 μg of non-radioactive, sonicated LC103 DNA was used to compete the 1 μg ^{32}P -labelled LC103 probe DNA. Exposure time for the autoradiograms was 24 h with a Kodak intensifier screen. A mixture of λ DNA digested with either *Eco*RI or *Hin*III was used as fragment size markers. The arrows in lanes d, i and m mark the position of a 1.8-kb restriction fragment, while the arrow in lane g marks the 2.4-kb position (see text).

these clones do not necessarily represent members of the 103 and 109 highly repetitive families.

The non-*Cin1* arrows on clone LC102 represent homologous repeats, as do the non-*Cin1* arrows on clone LC103. The repeats represented by cross-hatched arrows on clone LC109 may not be homologous, for no out-of-register pairing was observed. One of these repeats has been drawn contiguous to the *Cin1* repeat, on both LC104 and LC109, but may actually represent an extension of the *Cin1* repeat if the *Cin1* allele on LC104 has greater homology to the LC109 *Cin1* allele than to the original *Cin1*-001 allele of the NF1 clone.

Discussion

The 14 genomic clones analyzed in this paper were all initially chosen because of the presence of a given dispersed repeat, *Cin1* (Gupta *et al.*, 1983). Therefore, they seemed ideal candidates to study directly the genome organization of dispersed repetitive sequences in maize. Studies of genome organization in *Z. mays* by reassociation kinetics (Hake and Walbot, 1980) had previously revealed two interspersion patterns. The first, a short-period interspersion pattern (Davidson *et al.*, 1973), is common to most plant and animal genomes (for review, see Walbot and Goldberg, 1979). In higher plant genomes it consists of short repetitive sequences 50–2000 bp in length inter-

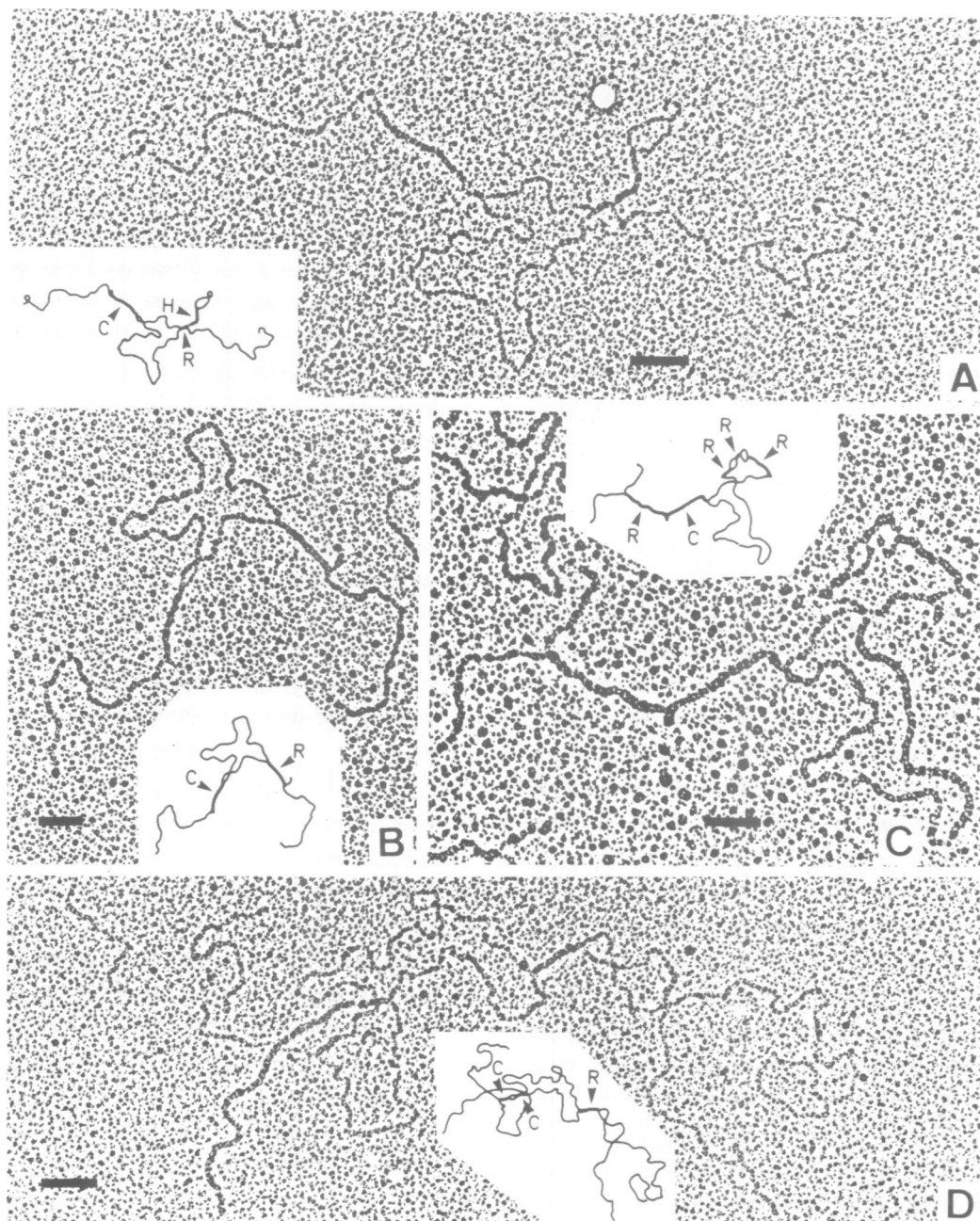


Fig. 3. Electron micrographs showing heteroduplex formation between the *EcoRI* insert of LC104 and either LC102 (A and B), LC109 (C) or LC103 (D). Repeats were located on the clones by measuring the single- and double-stranded DNA lengths using single- and double-stranded ϕ X174 as a standard. The bar in each micrograph represents $0.1 \mu\text{m}$ of DNA. A line drawing of each heteroduplex structure is used to indicate the position of repeated sequences (R) which are present on both maize clones. The position of the *Cin1* repeat (C) on the line drawings corresponds to that previously determined by heteroduplex analysis of the clone with the *Cin1*-001 repeat present on the NF1 clone (Gupta *et al.*, 1983). In A, the LC102 and LC104 clones form a heteroduplex of 430 bp due to the *Cin1* repeat (C), a 107-bp heteroduplex due to another common repeat (R), and a homoduplex (H) or snap-back structure of the LC102 clone. When the formation of the homoduplex structure was inhibited by cleaving the LC102 molecule with *BglI*, the common repeat (R) was seen to increase to 470 bp (B). This indicates that the end of the LC102 molecule also contains sequences homologous to the repeat, but in an inverse orientation. The heteroduplex structure between LC109 and LC104 reveals several double-stranded segments (C). Since the position of the *Cin1* element on LC109 and LC104 had previously been determined (Gupta *et al.*, 1983), the segment containing homology between the *Cin1* alleles was determined (C). However, this double-stranded segment is 797 bp in length, and the *Cin1* element present on LC104 and LC109 had previously been determined to be 440 and 480 bp, respectively. Thus only a portion of this double-stranded region may be due to the *Cin1* elements and another repeat immediately flanking *Cin1* on both clones may extend the double-stranded region (see Figure 4). The remainder of the double-stranded regions are due to other repeats found in common between the two clones (R). Only two heteroduplex structures showing the complete structure shown in C were observed. Unfortunately, both structures also contained a miscellaneous broken fragment from LC104 which hybridized to the long single-stranded loop of LC104 (lower right region of C). Since this double-stranded region does not indicate a heteroduplex formation, but rather an artifact due to broken DNA fragments in the preparation, it was not drawn in the schematic shown in the inset of C. Seven other heteroduplex structures were measured which contained only the long R and C repeat of the schematic and eight molecules containing only the three repeats containing the double 'bubble' structure were measured. We thus believe our analyses of the arrangement of repeats in C and shown in Figure 4 are correct. Clones LC103 and LC104 form two double-stranded heteroduplex regions (D). The *Cin1* repeat was 440 bp in total length with a small single-stranded 'bubble' structure suggesting sequence divergence between the two *Cin1* alleles. The other common repeat (R) was 300 bp in length. Two such repeats seem to be present on the LC103 molecule for one side of the single-stranded loop which is present between the *Cin1* repeat and the 'R' repeat measured either 2.8 kb or 4.8 kb when several heteroduplex structures were measured. The length of the non-hybridizing, single-stranded arm of the LC103 clone was similarly affected, while the portion of the single-stranded loop from the LC104 clone remained a constant 1.5 kb.

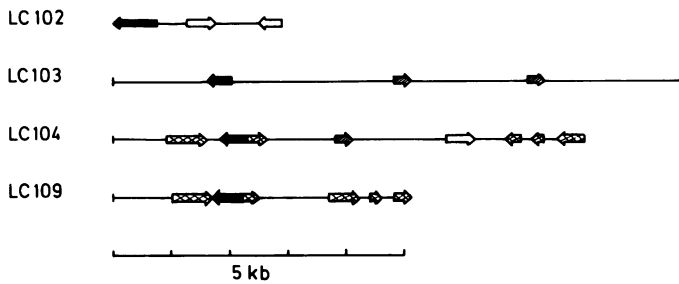


Fig. 4. Schematic drawing of the heteroduplex structures shown in Figure 3. Maps are derived from the measurements of 5–10 molecules of each heteroduplex structure shown in Figure 3. The solid arrow in each map indicates the position and extent of the *Cin1* repeat as determined previously (Gupta *et al.*, 1983). The other arrows denote repetitive sequences (R) of a particular clone. The open arrows on LC102 represent homologous repetitive sequences as do the hatched arrows on LC103. The five cross-hatched arrows shown on LC109 may actually represent five different repetitive units.

persed with short single copy DNA of 200–4000 bp (for review, see Flavell, 1980). In maize the short-period interspersal pattern consists of dispersed repetitive sequences of 500–1000 bp and the average length of the unique DNA segment is 2100 bp (Hake and Walbot, 1980). The original *Cin1*-containing NF1 clone (Shepherd *et al.*, 1982) and the LC112 clone could have arisen from such a short-period interspersal pattern. The 700-bp *Cin1* repetitive element present on the NF1 clone is flanked by a minimum of 2.3-kb unique DNA on either side (Shepherd *et al.*, 1982) while the LC112 cloned fragment consists of a *Cin1* element flanked by unique DNA of at least 1050 bp and 270 bp (this report and Gupta *et al.*, 1983).

The second interspersal pattern which has been described for maize DNA is that of middle repetitive sequences interspersed with highly repetitive sequences (Hake and Walbot, 1980). Repeat/repeat interspersal has been well documented in wheat (Smith *et al.*, 1976), for rye heterochromatin (Bedbrook *et al.*, 1980), and inferred for the pea genome (Thompson and Murray, 1980). The heteroduplex analysis presented in Figure 4 demonstrates the presence of dispersed, repetitive sequences together with the middle repetitive *Cin1* repeat on several clones. Since other repeats as well as unique restriction fragments are also present on the clones, an interspersal of short sequences of various reiteration frequencies is suggested. This is unlike the soybean genome where repetitive sequences which differ significantly in reiteration frequency are not interspersed among each other (Goldberg, 1978; Walbot and Goldberg, 1979).

The presence of small (several 100 bp in length), unique DNA segments interspersed with repetitive sequences has been previously reported only for the pea genome (Murray *et al.*, 1978). It is consistent with the idea that mutational drift of a given segment of a repeat occurs until the segment is highly divergent and is no longer recognizable as part of the repetitive family (Thompson, 1978). However, the small unique DNA segments may also suggest that the initial dispersion events consisted of independent transpositions of the various repetitive units into a unique segment of DNA. Of course, unequal crossing over could then generate permutations such as deletions and amplification of the initial pattern (Smith, 1976). Evidence for transposition was obtained for the *Cin1* family of repeats by DNA sequence analysis (Shepherd *et al.*, in preparation). It would be interesting to

define these other repeats at the nucleotide level by comparing the DNA sequence of several cross-hybridizing restriction fragments, and thus to obtain further evidence for the transposition of small repetitive units.

The dispersed repeats identified on the LC-maize clones may also be present elsewhere in the genome as part of a larger repeating unit (tandem or dispersed). Evidence for this comes indirectly from the genomic hybridization patterns shown in Figure 2. For a given probe, several genomic *EcoRI* fragments hybridize more strongly than others. A strong hybridization signal at a given position could represent an *EcoRI* fragment containing a repeat highly homologous to the probe, several copies of the repeat on a single *EcoRI* fragment, or multiple *EcoRI* fragments of the same size – each containing the repeat. We do not believe that the first explanation is correct, for genomic fragments which are identical to the probe but present as single copy DNA do not hybridize strongly within 24 h in our genomic Southern hybridization experiments (see Figure 2, lane 1). The lack of a strong hybridization band at the position corresponding to the cloned *EcoRI* maize insert (e.g., Figure 2, lane g does not have a strong band at the 2.4-kb position marked by an arrow) also suggests that the first explanation is insufficient. Thus many of the small dispersed repeating units identified on the clones may also be present as part of larger repeating units. In this respect, it would be interesting to digest genomic maize DNA with various restriction enzymes in order to search for a 'ladder' hybridization pattern (Southern, 1975b) when probed with one of the identified dispersed repeats. The finding of such a 'ladder' would indicate that the dispersed repeat was also present as a tandem array (simple or complex). It would also be interesting to try *in situ* hybridization to see if the repeats are more abundant in certain chromosomal positions.

It is thought that further analysis of the dispersed repeats in maize will extend our knowledge of plant genome organization as well as precisely defining elements which may play a role in chromosome condensation, homologue recognition and recombination (Britten and Kohne, 1968; Thompson and Murray, 1981).

Materials and methods

Plant material

Z. mays Line C (LC) is a derivative of a mid-western inbred line W22. Plant DNA was isolated according to the method described previously (Gupta *et al.*, 1983).

Recombinant plasmids

pLC1 (Shepherd *et al.*, 1982) and pLC102-115 (Gupta *et al.*, 1983) are recombinant plasmids containing *EcoRI* DNA inserts from the Line C of *Z. mays* in the *EcoRI* site of the vector pACYC184 (Chang and Cohen, 1978). pNF1 is a recombinant plasmid containing a 5.7-kb *EcoRI* fragment from a Northern Flint line of *Z. mays* (Shepherd *et al.*, 1982). Two subclones of pLC103 were prepared and used for data in Table I. These two subclones were *EcoRI*-*BamHI* fragments of pLC103 (for restriction map see Gupta *et al.*, 1983) which were ligated into *EcoRI*-*BamHI* cleaved pBR328 (Soberon *et al.*, 1980) and transformed into HB101 bacteria (Dagert and Ehrlich, 1979). Plasmid DNAs were isolated according to Birnboim and Doly (1979) and the maize DNA inserts were isolated by preparative agarose gel electrophoresis and electroelution (Wienand *et al.*, 1979).

Sonication of plasmid and genomic DNA

DNA of the undigested recombinant plasmids, pLC102 or pLC103, or genomic Line C maize DNA were suspended in 10 mM-Tris/HCl, pH 7.5, 1 mM EDTA and 20 mM β -mercaptoethanol to the final concentration of 100 μ g/ml. DNA was sonicated to an approximate length of 300–1000 bp as determined by agarose gel electrophoresis. After sonication, the DNA was phenolized, ethanol precipitated and resuspended as required.

Reiteration frequency analysis of the maize clones

1 µg DNA of each maize clone insert was digested with *Hpa*II, fractionated on a 2% agarose gel, transferred to nitrocellulose paper (Southern, 1975a) and hybridized to sonicated and nick-translated (Rigby *et al.*, 1977) genomic DNA (5×10^7 c.p.m./µg DNA) from Line C of *Z. mays*. Hybridization was at 65°C in 3 x SSPE (1 x SSPE = 1 mM Na₂EDTA, 10 mM NaH₂PO₄, 150 mM NaCl, pH 7.0), 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.1% SDS. The filter was washed at 70°C in 2 x SSPE, 1% SDS, and exposed to Kodak XAR-5 film with an intensifier screen at -70°C.

Restriction fragments were classified into three reiteration frequency classes depending upon their hybridization intensity with the genomic DNA probe: highly repetitive sequence – hybridization easily seen after 24 h exposure; middle repetitive sequence – weak hybridization seen within 24 h to a 6 day exposure; unique sequence – no detectable hybridization even after 6 days exposure.

Heteroduplex analysis

Heteroduplexes were made between two Line C clones according to the method described previously (Shepherd *et al.*, 1982).

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