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Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms

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Summary. Genetic linkage maps were constructed for both maize and tomato, utilizing restriction fragment length polymorphisms (RFLPs) as the source of genetic markers. In order to detect these RFLPs, unique DNA sequence clones were prepared from either maize or tomato tissue and hybridized to Southern blots containing restriction enzyme-digested genomic DNA from different homozygous lines. A subsequent comparison of the RFLP inheritance patterns in F2 populations from tomato and maize permitted arrangement of the loci detected by these clones into genetic linkage groups for both species.

Key words: Restriction fragment length polymorphisms – Genetic linkage maps – *Zea mays* L – *Lycopersicon* species

Introduction

When genomic DNAs from two genetically distinct individuals are digested with a restriction enzyme, electrophoresed, blotted onto a membrane, and probed with a radioactively-labelled DNA clone, polymorphisms in the hybridization patterns sometimes result due to sequence differences between the individuals. The term "restriction fragment length polymorphism" (RFLP) has been coined to describe this variation.

Randomly-dispersed RFLPs were first proposed as a new source of genetic markers in humans, where the low amount of genomic variability, as well as other factors, complicate the application of conventional genetic analyses (Botstein et al. 1980). The actual feasibility of utilizing RFLPs, detected either by clones of known identity or by randomly-isolated clones, as genetic markers for the diagnosis of human diseases/disorders has since been demonstrated (Little et al. 1980; Phillips et al. 1980; Gusella et al. 1983).

The use of RFLPs as genetic markers in plants could also offer an alternative approach to specific problems which previously have been unamenable to conventional genetic techniques. Tanksley (1983) and Beckmann and Soller (1983) have reviewed the potential usefulness of such markers in basic plant genetic studies, as well as in plant improvement programs. Many of these proposed uses of RFLPs are identical to previously-developed approaches utilizing isozymes as "molecular" markers. While isozyme loci have proven to be quite useful in several instances, more extensive application of this approach is limited primarily by insufficient numbers of marker loci and their general lack of informativeness. In contrast, a potentially unlimited number of RFLPs exist, which could circumvent this difficulty and should allow much wider use of the molecular marker approach. Additionally, genetic analysis by RFLPs possesses other advantages, such as their usefulness in determining the exact genotype at a chromosomal locus, their lack of measurable effects on phenotype, and the absence of environmental effects upon their evaluation. These characteristics make them well-suited for particular applications, such as the evaluation of heterozygosity/homozygosity levels in individual plants or lines and the evaluation, by linkage, of traits with obscure or difficult to mesure phenotypes.

As a first step in evaluating the actual feasibility of using RFLPs in these applications, several investigators have examined the degree of genetic variability detectable by RFLPs in plants. Riven et al. (1983); Burr et al. (1983), and Helentjaris et al. (1985) have reported significant variability among different maize inbred lines when either repetitive or single-copy DNA sequences were used as probes. Variability also has been detected in tomato (Helentjaris et al. 1985; Tanksley et al. 1985), barley (Saghai-Maroof et al. 1984), and pea (Polans et al. 1985) when DNA clones were used as probes for plant polymorphisms.

As stated above, one of the foremost attributes of RFLPs is the presence of an almost unlimited number in an organism, such that one could potentially "saturate" the genome with informative loci. In order for these large sets of RFLPs to be used efficiently as genetic markers, knowledge of their in-

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dividual genomic locations or at least the spatial arrangement of the RFLP loci would be extremely useful. Such information would facilitate initial analyses by avoiding the repeated use of tightly-linked loci yielding similar information. Identification of a set of markers known to be dispersed throughout the plant's genome would also insure that no significant areas of the genome were omitted. Lastly, if a loose linkage between a RFLP marker and the expression of an important agronomic trait was detected, the investigator could then systematically test other RFLP markers from the same linkage group to identify an even tighter linkage relationship, as opposed to having to proceed randomly through the entire set of markers.

Accordingly, our objective in this study was to both identify relatively large sets of polymorphic DNA markers in maize and tomato as well as to determine whether they can be efficiently arranged into genetic linkage groups.

Materials and methods

Preparations of clones

cDNA libraries were prepared from maize and tomato leaf tissue mRNA (Gubler and Hoffman 1983) and screened for inserts by a rapid plasmid method (Holmes and Quigley 1981). Details of the methods used are as previously described (Helentjaris et al. 1985). A second source of unique DNA sequence clones was prepared by digesting leaf tissue DNA with a restriction enzyme, HindIII, electrophoresing it in an agarose gel, and removing fragments between 500 and 1,000 base pairs (bp) in size (Maniatis et al. 1982). These fragments were subcloned into the plasmid vector, pUC12, previously digested with HindIII and treated with bacterial alkaline phosphatase, and the resulting recombinants were used to transform the bacterial host, JM83. Clones containing highly repetitive sequences were detected by probing colony lifts (Grunstein and Hogness 1975) with radio-labelled total genomic DNA and were discarded. The remaining "unique" sequence clones were stored at –70 °C in freezing media, and then later grown up in large scale plasmid procedures as described previously (Helentjaris et al. 1985).

Plant DNA preparation, restriction enzyme digestion, and blotting

Procedures for the preparation of total plant genomic DNA, digestion by restriction enzymes, agarose gel electrophoresis, and Southern blotting have been described previously (Helentjaris and Gesteland 1983; Helentjaris et al. 1985). Nylon membranes used as the blotting matrix were obtained from AMF CUNO.

Nick tanslation of probes and hybridization conditions

Plasmid DNAs were nick translated to a specific activity of $0.5–2.0 \times 10^9$ cpms/μg (Maniatis et al. 1975). Prehybridization, hybridization, washing, and autoradiography were exactly as described previously (Helentjaris and Gesteland 1983; Helentjaris et al. 1985). For reuse, blots were washed for 20 min in 0.2 N NaOH and then for 20 min in 0.5 M Tris pH 7.5, 0.1X SSC, 0.1% SDS, all treatments at room temperature.

Source of plant lines

Maize lines, H427, 761, and their F1 and F2 generations were obtained from T. Murphy at Northrup King. *L. hirsutum f. glabratum* P.I.1134417, *L. esculentum* var. 'Manapal', and their F1 generation were obtained from Dr. G. Kennedy of North Carolina State Univ. F1 plants were self-pollinated to produce F2 generation individuals for the tomato analysis.

Results

Selection of informative clones

The first step required in the assembly of a linkage map based upon RFLPs is to construct a source of unique DNA sequence clones and to determine which clones are effective in revealing polymorphisms. We initially utilized cDNA clones prepared from leaf tissue mRNA as an enriched source of unique sequence clones for both maize and tomato, because mRNAs that possess poly-A tails are primarily transcribed from low-copy number genes (Goldberg et al. 1973). In order to determine which clones were actually useful in detecting RFLPs, they were individually subjected to an initial hybridization screen against genomic DNAs prepared from leaf tissue which had been digested with a restriction enzyme, electrophoresed, and blotted onto a nylon membrane. This screen was designed to identify those clones which fulfilled our criteria for further use in that they 1) yielded a strong hybridization signal, 2) revealed a small number of fragments, and 3) at the same time revealed polymorphisms among different homozygous lines. For the tomato screen, the blot contained DNAs prepared from five *Lycopersicon* species of interest to us, including *L. esculentum* and *L. hirsutum,* all digested with either BglII or HindIII. Two examples of such a *Lycopersicon* screen were illustrated previously in an earlier report (Helentjaris et al. 1985). Clones whose evaluation yielded a simple, strong hybridization signal and distinguished the *Lycopersicon* species, especially *hirsutum* from *esculentum,* were retained. For the maize screen, the blot contained DNAs prepared from two maize inbred lines, H427 and 761, and F1 plants derived from cross-pollinating them, all digested with either BglII, HindIII, or SstI. Maize clones were considered useful if their hybridization demonstrated a simple, strong pattern and distinguished the two inbred lines.

More than half of the maize cDNA clones tested did not meet the first criteria; i.e. a weak hybridization signal resulted which hampered further analysis. This problem was encountered much less frequently with tomato cDNA clones. This was apparently not due to a difference in the average size of the cloned maize inserts as compared to tomato inserts (data not shown) nor to peculiarities in the maize library construction, as several libraries were prepared and all produced the same result. The reason for this difficulty in obtaining useful maize cDNA clones is unclear.

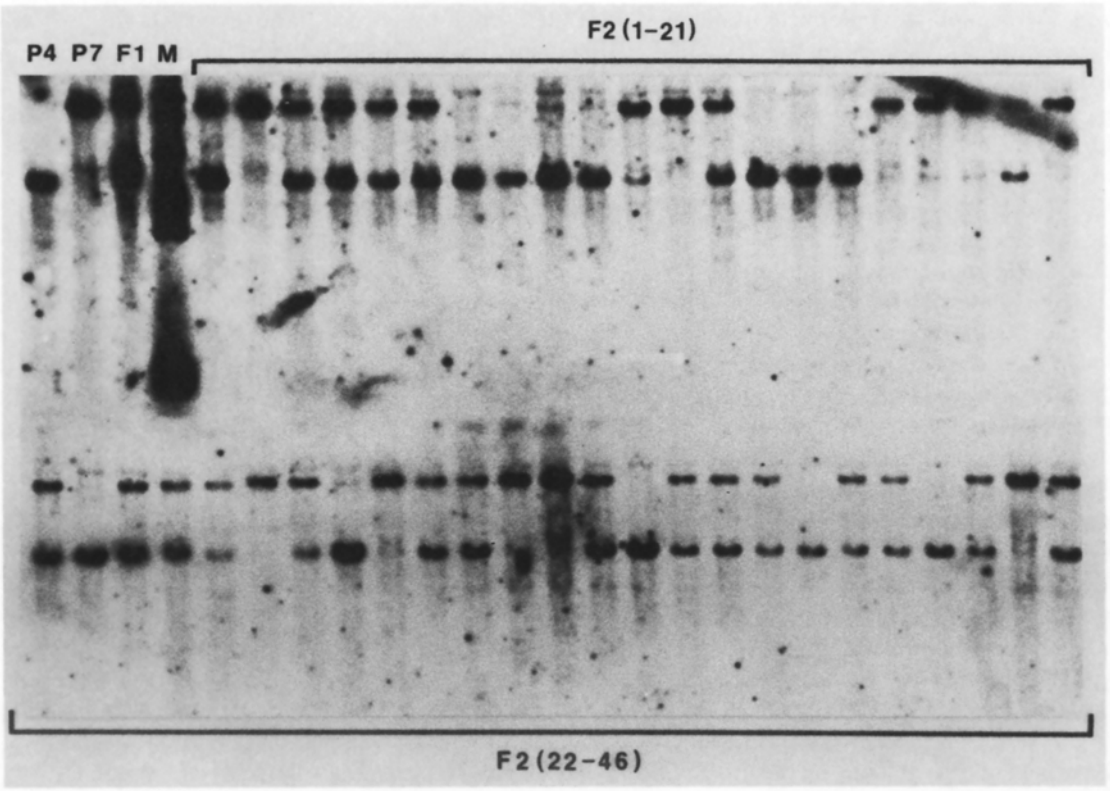

Fig. 1. Segretation of RFLPs in a F2 population. Genomic DNAs prepared from H427 ("P4"), 761 ("P7"), a F1 plant ("F1") produced by crossing them, and 46 F2 plants ["F2 (1–21)" and "F2 (22–46)"] produced by self-pollinating the F1 were digested with HindIII, electrophoresed in an agarose gel, blotted, and hybridized to a radio-labelled maize clone, No. 23. "M" denotes molecular weight markers

As an alternative means of obtaining useful clones for the maize research, we constructed a genomic library consisting of HindIII-digested maize DNA fragments, size-selected to 500–1,000 bp. The "unique" sequence clones from this library, as identified by probing colony lifts with radio-labelled total DNA (see Methods), were also put through the initial screen described above to determine if they met our selection criteria for informative clones. Despite the added difficulty of identifying and removing repetitive sequence clones from the genomic bank, this source eventually proved to be more efficient for maize by consistently providing clones with stronger hybridization signals. Informative genomic clones were then utilized subsequently exactly as were the informative cDNA clones.

Inheritance analyses of RFLP loci

Once informative clone-restriction enzyme combinations were identified, the loci detected by the RFLPs could be used as markers in many different types of genetic analyses. To date we have isolated over 100 clones (RFLP loci) from both maize and tomato which satisfy all of our criteria for informative clones. As stated earlier, efficient utilization of sets of RFLPs of this size requires a knowledge of the relative spatial arrangement in the genome of the loci detected by these clones. A comparison of segregation patterns of RFLP markers in F2 populations derived from homozygous parental lines is one method of obtaining data which would permit the arrangement of loci detected by them into a genetic linkage map. We have begun this process for both maize and tomato by examining the inheritance patterns of our RFLP loci in F2 populations of 50 individuals. Due to the technical complexities involved in the RFLP analysis, it was not practical to use significantly larger populations for the segregation analysis, such as those that can number into the tens of thousands for the analysis of morphological markers (Emerson et al. 1935). Subsequent data presented here will demonstrate that a population size of 50 individuals is sufficient for the construction of linkage maps and provides a degree of accuracy required for many applications. In maize, the two inbreds, H427 and 761, were cross-pollinated to create a F1 generation. F1 generation plants were subsequently self-pollinated to produce segregating F2 populations. Because of the limited variability within the *esculentum* species (Helentjaris et al. 1983; Tanksley and Bernatsky

1985), we utilized a F2 population derived from an interspecific-cross between a *L. esculentum* line ('Manapal') and a *L. hirsutum* line ('PI1134417').

In both maize and tomato, genomic DNAs were prepared individually from fifty of the F2 plants for each species, as well as from the parental lines and F1 generation plants. The DNAs were digested with the enzyme of choice, as determined by the initial screening process, and then electrophoresed and blotted onto nylon membranes. An example of the type of data obtained after hybridization of a unique sequence clone to these genomic DNAs is shown in Fig. 1. A distinctive fragment, presumably corresponding to the homozygous diploid allele, was observed in each parent. Both fragments also were observed in the heterozygous F1 plant, as would be expected for codominant alleles of a single locus. Through examination of the 46 individual F2 plants shown in this figure, seemingly random inheritance of the two RFLP alleles can be seen, with all three expected genotypes clearly present in this F2 population. For example, the homozygous 761 (P7) parental genotype is exemplified by the second F2 individual, the homozygous H427 (P4) parental genotype is seen in the seventh and eighth F2 individuals, and the heterozygous genotype is seen in the first F2 individual.

Before using the data gathered in the segregation analysis to arrange these loci into genetic linkage maps, we felt it was necessary to examine the mode of inheritance of these sets of RFLP loci in both maize and tomato. Each of the clones revealing polymorphisms was hybridized against an identical set of F2 individuals and the inheritance data for the entire group of RFLPs were tabulated separately for maize and tomato. Genotype data and chi square goodness-of-fit to a 1:2:1 Mendelian inheritance ratio for the first fifty loci tested for each species are shown in Table 1. (Corresponding data for the remaining loci are available upon request.) Examination of the maize data set revealed that only six of these 50 loci deviated significantly from the expected 1:2:1 Mendelian segregation ratio, with a resulting average allele frequency for parent 761 of 0.495 and for parent H427 of 0.505. The analyses of variance of the chi squares totaled for all loci and partitioned into two components, pooled and heterogeneity, indicated that the inheritance of this group of loci, as a whole, closely approximated a 1:2:1 ratio, but that some individual loci did deviate from this expected ratio (calculations not shown). In general, the data showed that the loci detected by the RFLPs in maize were inherited with values close to the expected allelic frequencies.

In contrast to the intraspecific maize population, data gathered from the interspecific *Lycopersicon* population demonstrated that 17 of the fifty RFLP loci deviated from the 1:2:1 ratio expected for two codominant alleles of a single gene (Table 1). Moreover in this case, the analysis of variance of the pooled chi squares for these loci, when partitioned, indicated that the tomato RFLP loci as a group did not fit the expected 1:2:1 ratio (calculations not shown). In all cases but one, clone No. 11, the lack of goodness-of-fit to a 1:2:1 segregation ratio was due to higher than expected numbers of *hirsutum* homozygous genotypes when compared to *esculentum* homozygous genotypes. The resulting average gene frequencies summed over all 50 loci was 0.574 for *hirsutum* parent alleles and 0.426 for *esculentum* parent alleles. Recently, the fertilization abilities of pollen grains from *L. esculentum* and an accession of *L. hirsutum* were compared (Zamir et al. 1982). In pollen mixtures of the two species, the pollen from *hirsutum* was observed to be substantially more successful in effecting fertilization at low temperatures. Thus it is possible that gametophytic selection might have occurred to some degree under the greenhouse conditions used for pollination in this study, and that gametes containing specific portions of the *hirsutum* genome were more competitive. Although the exact reason for this segregation distortion is unknown, it is of interest to note that in an unrelated F2 population derived from a cross between *L. esculentum* and another non-cultivated *Lycopersicon* species, *L. pennellii*, some of the same loci again showed aberrant segregation favoring the undomesticated parent (data not shown). This distortion must be considered when examining linkage data for this population, as it resulted in higher standard errors for the calculated recombination frequencies and consequently reduced the resolution obtained when compared to similar data gathered in the maize population.

To verify further that linkage data derived from our F2 populations of fifty individuals would prove accurate, we examined the linkage data for three RFLP loci detected by clones for the maize genes; bronze (*bz-1*), shrunken (*sh-1*), and waxy (*wx*); all shown to be linked on chromosome 9 through conventional analysis (Sheridan 1982). Using cloned DNAs corresponding to these genes, we were able to detect polymorphisms using the same preliminary screening analysis described above. Cloned copies of these genes were then hybridized to DNA from our maize F2 population and the inheritance data was tabulated. Maximum likelihood analysis (Mather 1951) was used to calculate recombination values for the loci detected by these RFLPs and this data is shown in Table 2. The observed estimates of recombination values between these RFLP loci are well within the standard errors of recombination frequencies previously determined by conventional analysis (Emerson et al. 1935; Rhoades 1952). While we cannot precisely map closely neighboring loci, due to

Table 1. Observed segregation and Chi-square goodness of fit analyses to 1:2:1 ratio for 50 different RFLP loci in a maize and tomato F2 population

Maize Genotypes					Tomato Genotypes				
RFLP locus number	homozygous 'H427'	hetero-zygous	homozygous '761'	X^2 (1:2:1)	RFLP locus number	homozygous 'PI134417'	hetero-zygous	homozygous 'Manapal'	X^2 (1:2:1)
1	8	28	6	4.86	1	19	16	12	6.87**
2	17	16	12	4.86	2	17	18	6	6.51**
3	4	30	11	7.17**	3	12	20	8	0.80
4	15	18	7	3.60	4	17	16	5	8.52**
5	12	23	10	0.20	5	15	21	7	3.00
6	11	21	14	0.74	6	13	20	12	0.60
7	12	23	11	0.04	7	19	23	4	9.78**
8 (bz - 1)[a]	9	23	12	0.50	8	14	23	5	4.23
9	11	21	14	0.74	9	14	24	9	1.09
10	7	29	10	3.52	10	11	24	10	0.24
11	11	23	12	0.04	11	8	30	5	7.14**
12	15	21	6	3.86	12	16	21	8	3.04
13	12	20	12	0.36	13	15	20	9	2.00
14	12	17	11	0.95	14	17	21	7	4.64
15 (sh - 1)[a]	9	24	13	0.78	15	19	20	7	7.04**
16 (wx)[a]	11	29	5	5.36	16	16	20	9	2.73
17	17	19	9	3.93	17	14	22	10	0.78
18	12	19	14	1.27	18	16	23	5	5.59
19	8	27	9	2.32	19	18	13	14	8.73**
20	14	15	14	3.93	20	9	28	10	1.77
21	9	22	14	1.13	21	9	23	13	0.73
22	11	24	10	0.24	22	17	18	8	4.91
23	12	22	12	0.09	23	15	20	9	2.00
24	11	19	11	0.22	24	19	17	11	6.32**
25	10	21	8	0.44	25	18	18	11	4.66
26	8	22	16	2.87	26	20	27	2	13.73**
27	11	17	13	1.39	27	16	15	5	7.72**
28	10	24	11	0.24	28	16	19	8	3.56
29	8	21	17	3.87	29	13	24	8	1.13
30	11	19	13	0.77	30	13	24	8	1.31
31	8	26	10	1.64	31	18	13	14	8.73**
32	15	24	7	2.87	32	13	24	8	1.31
33	7	31	8	5.61	33	7	29	10	3.52
34	14	26	4	6.00**	34	20	13	7	13.35**
35	8	23	14	1.62	35	20	16	7	10.67**
36	18	18	9	5.40	36	11	21	4	3.72
37	12	26	7	2.20	37	12	20	13	0.60
38	7	27	11	2.51	38	17	21	9	3.26
39	8	32	5	8.42**	39	20	22	2	14.73**
40	14	17	15	3.17	40	19	24	3	11.22**
41	2	26	16	10.36**	41	7	28	9	3.45
42	7	27	10	2.68	42	9	23	14	1.09
43	7	31	8	5.61	43	20	17	9	8.39**
44	10	11	13	4.77	44	12	24	9	0.60
45	8	22	16	2.87	45	18	21	7	5.61
46	13	25	5	4.12	46	9	24	10	0.93
47	13	15	12	2.55	47	7	21	11	1.05
48	14	19	13	1.44	48	17	18	5	7.60**
49	8	23	9	0.95	49	12	14	8	2.00
50	7	33	6	8.74**	50	11	16	10	0.73

** Indicates Chi-square value greater than would be expected by chance at the 0.05 level of significance
[a] Cloned genes associated with this RFLP locust

our inability to obtain recombination frequencies with small standard errors in these populations (nor did we intend to in this study), the type of analysis presented here can still be used to accurately arrange RFLP loci into preliminary linkage groups. While we consider collection of this type of data as a reasonable prelude to later construction of more detailed maps, even simple maps based upon inheritance analysis can greatly facilitate the use of these marker loci for many types of genetic analyses.

In order to organize all of the analyzed RFLP loci into their respective linkage groups, we initially cal-

Table 2. Recombination frequencies among 3 known cloned genes on chromosome 9 in maize

Gene pair	Genotypic classes				Recombination frequency [a]	Published recombination frequency [b]
	Parental		Recombinant			
	++	--	+-	-+		
sh-1 : bz-1	40	47	0	1	1.14 ± 1.27	1.6
bz-1 : wx	37	34	3	12	17.04 ± 4.05	19.3
sh-1 : wx	38	34	3	12	18.18 ± 4.11	20.9

[a] Maximum likelihood estimate of proportion of recombinant chromosomes, and standard error $((p(1-p))/N)^{1/2}$
[b] (After Rhoades 1952 and Emerson et al. 1935)

culated all two locus recombinational values using maximum likelihood analysis (Mather 1951). These values were used to arrange loci into linkage groups, to determine their approximate order within their respective groups, and lastly to establish relative map distances between them. The linkage maps so derived for maize and tomato are presented in Figs. 2 and 3, respectively. From the linkage data alone, we were able to arrange the 113 maize RFLP loci into 13 distinct linkage groups with six loci unlinked to any others. The tomato map consists of 104 RFLP loci organized into 20 linkage groups with four loci yet unlinked to any of the others. It was not surprising that we found more linkage groups than there are chromosomes (thirteen groups in maize where 1N = 10 and 20 groups in tomato 1N = 12), as some of these RFLP linkage groups are very compact and it is quite likely that some of them represent distant segments on the same chromosome. Maize-C for instance consists of four loci contained within a total map distance of only 9 units. Due to our small population sizes and consequently larger standard errors, we believe our maximum resolution is probably less than 30 map units for maize and even less in the case of tomato due to the skewed segregation observed with that population. With this limitation and an average size of greater than 100 map units per maize chromosome (Sheridan 1982), one would still expect to find more than one linkage group per chromosome, despite our sets of greater than 100 loci. With a marker set of just 50 randomly-located loci, however, one would statistically expect to obtain at least one marker locus from each of the 10 chromosomes in maize, so with these much larger sets, it is likely that each of the 10 maize chromosomes and 12 tomato chromosomes are represented by at least one of our linkage groups.

During the course of this study, we also have established the chromosomal identity of four of the linkage roups in the maize map through the use of clones of genes with known chromosomal locations: chromosome No. 9 (as above), chromosome No. 1 (Alcohol dehydrogenase 1, *Adh1*), chromosome No. 3 (anthocyaninless, *a1*), and chromosome No. 4 (Alcohol dehydrogenase 2, *Adh2*). Extension of this approach to identify the chromosomal origin of all of our linkage groups will be limited, as few other cloned genes with known chromosomal locations are currently available in maize and tomato. Other methods of identification will be required and are already under investigation by us, such as analysis of linkage of RFLP loci to morphological or isozyme loci and analysis of monosomics, trisomics, or various translocation stocks available in each species.

Discussion

This paper reports our results to date in constructing a set of informative DNA clones and arranging the RFLP loci detected by the clones into preliminary genetic linkage maps for both maize and tomato. Utilizing unique sequence clones as probes, we have confirmed that polymorphisms are readily detectable in these species, such that relatively large sets of RFLP loci can be produced with moderate effort. We also have shown that through a reasonable effort, the loci detected by these RFLPs can be arranged into linkage groups with relatively accurate determination of gene order and recombinational intervals between them. We have since confirmed the accuracy of the methods reported here through a number of subsequent procedures. Using analysis of the inheritance of RFLP alleles in monosomic maize lines, we have been able to assign all of our linkage groups to particular maize chromosomes (Helentjaris et al., in press). In no instance did we find loci that were first shown to be tightly linked through analysis of F2 populations and then later determined to have originated from different chromosomes by the monosomic analysis. In analyses of linkage of the maize

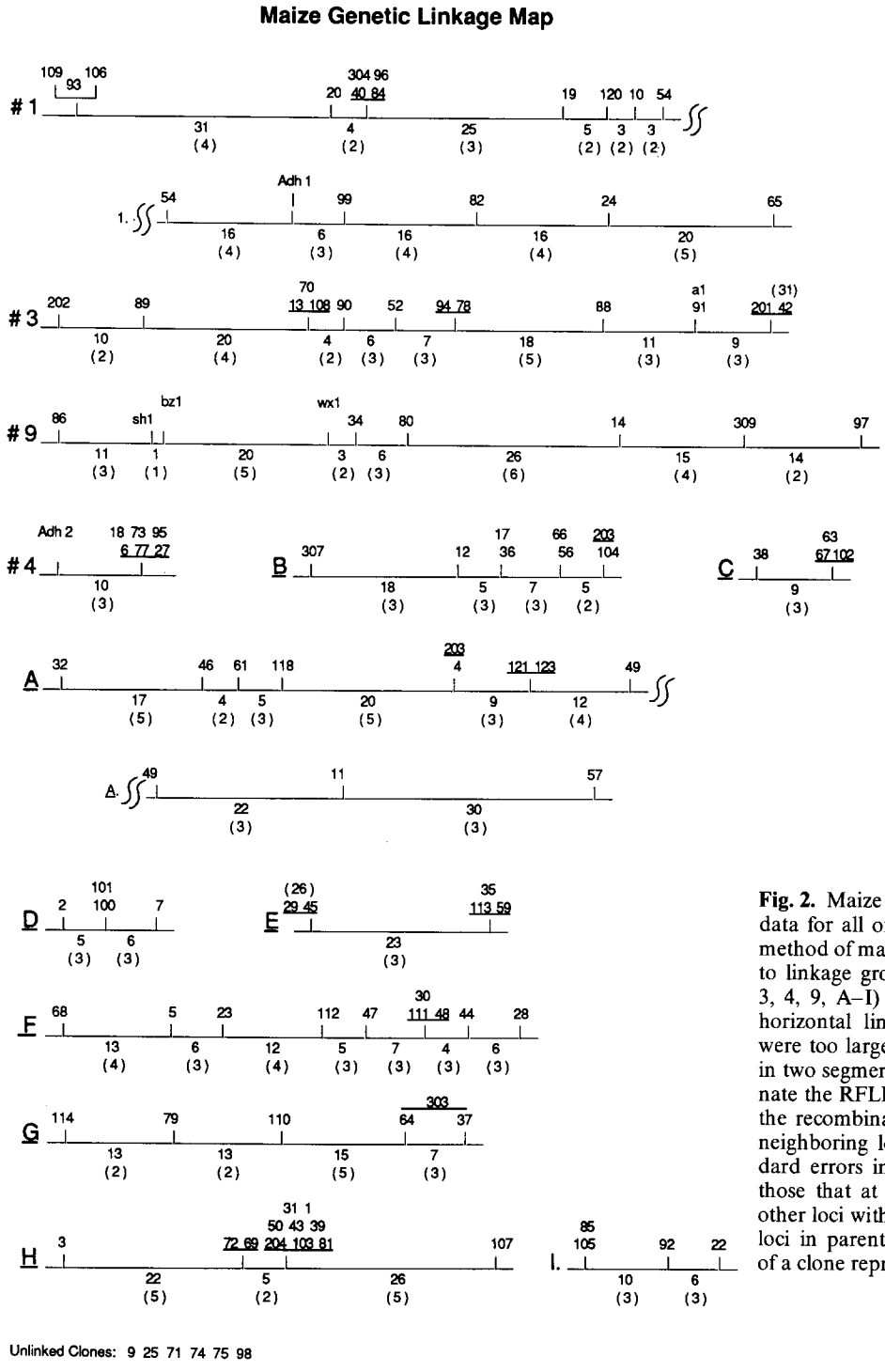

Fig. 2. Maize genetic linkage map. Segregation data for all of the RFLPs were analyzed by the method of maximum likelihood and arranged into linkage groups. The different groups (Nos. 1, 3, 4, 9, A–I) are denoted schematically by the horizontal lines. Linkage groups No. 1 and A were too large to fit in one piece and are shown in two segments. Numbers above the lines designate the RFLP loci; those below the lines denote the recombinational values in per cent between neighboring loci with their corresponding standard errors in parentheses. Unliked RFLPs are those that at this time are yet unlinked to any other loci within the limits of our analysis. RFLP loci in parenthesis represent duplicate isolation of a clone representing that locus

RFLP loci to morphological markers with known genomic locations (Helentjaris et al. 1986) or to isozyme loci (unpublished data with M. Edwards and C. Stuber), we again found no significant anomalies with respect to the data gathered during this reported study. Clearly, further efforts in this direction, when combined with larger population sizes to increase resolution and the use of various translocation stocks, such as the B–A translocations (Beckett 1978), will lead to better correlation between the RFLP linkage map and the maps derived using cytological data, morphological markers, and isozyme loci.

Using the marker sets and the linkage information generated in this study, we already have begun to examine the

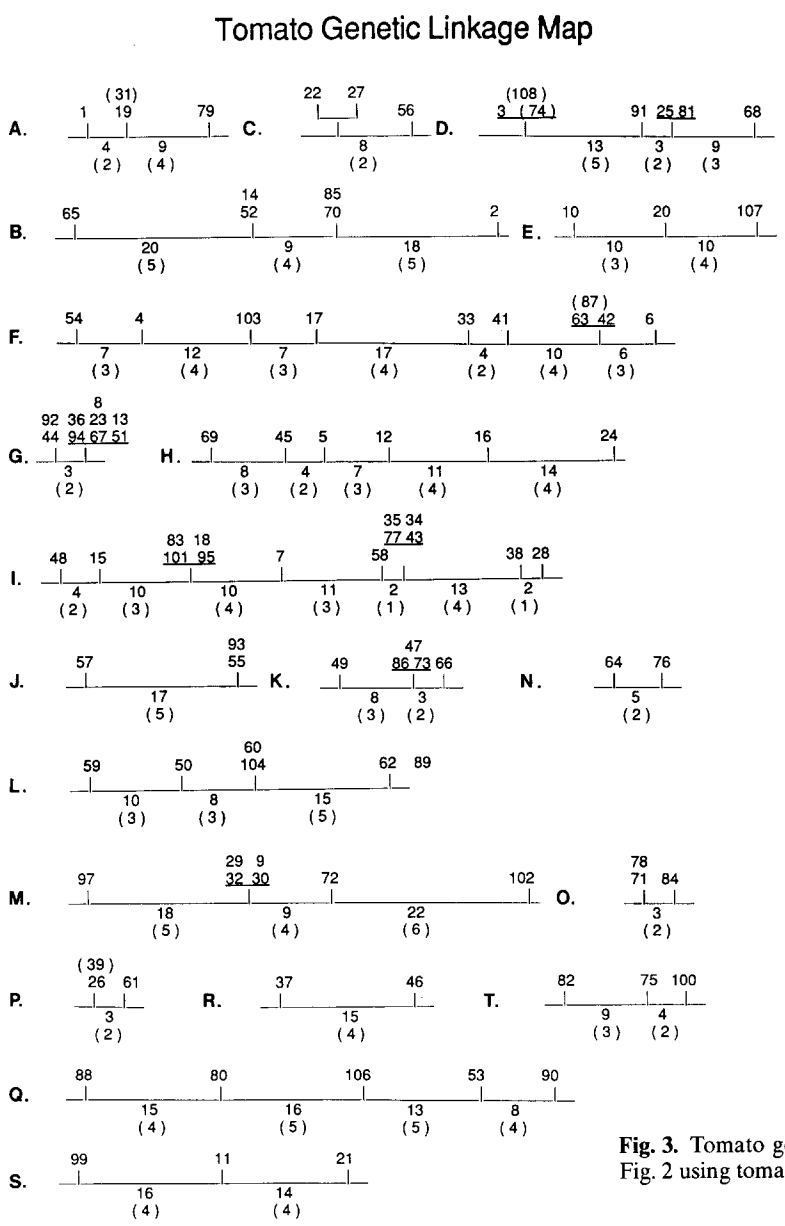

Fig. 3. Tomato genetic linkage map. These data were obtained as in Fig. 2 using tomato RFLPs. All other nomenclature is the same

ability of RFLP analysis to facilitate study of different genetic problems. For example, with maize we have found that clones often hybridize to related sequences at more than one genomic location. The presence of duplicated genes in maize (Rhoades 1951) and how this relates to the origin of maize, if it is actually an allopolyploid (Anderson 1945), has long been a controversial area of maize genetics. A comparison of the location of individual RFLP loci that we have detected so far has revealed that large areas of the maize genome, in one case a segment larger than 50 map units, have been duplicated elsewhere in the genome (Helentjaris et al., unpublished data). Further investigation of these and other duplicated regions in maize may reveal more about the genomic architecture of maize and its possible origins. We also have explored the use of these loci as markers in the analysis and genetic dissection of traits with a complex inheritance in plants. In one study of insect resistance found in one isolate of *L. hirsutum*, a multilocus trait in tomato, we identified three different genomic locations with RFLPs, each of which could individually account for about 15–19% of the phenotypic variance observed for this trait. When considered in an epistatic model, these three loci could now account for over 50% of the variance exhibited by this trait (Slocum et al. 1985). Clearly, RFLP analyses of this type may also be productive in future studies of even more complex traits, like salt tolerance or heterosis, which are affected significantly by environmental factors.

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