Copyright © 1988 by the Genetics Society for America

Gene Mapping with Recombinant Inbreds in Maize

Benjamin Burr,* Frances A. Burr,* Keith H. Thompson,* Marc C. Albertson† and Charles W. Stuber‡

**Biology Department, Brookhaven National Laboratory, Upton, New York 11973, †Corn Breeding Department, Pioneer Hi-Bred International, Inc., Johnston, Iowa 50131, and ‡Department of Genetics, North Carolina State University Raleigh, North Carolina 27695*

Manuscript received September 18, 1987
Accepted December 3, 1987

ABSTRACT

Recombinant inbred lines of maize have been developed for the rapid mapping of molecular probes to chromosomal location. Two recombinant inbred families have been constructed from F_2 populations of T232 × CM37 and CO159 × Tx303. A genetic map based largely on isozymes and restriction fragment length polymorphisms has been produced that covers virtually the entire maize genome. In order to map a new gene, an investigator has only to determine its allelic distribution among the recombinant inbred lines and then compare it by computer with the distributions of all previously mapped loci. The availability of the recombinant inbreds and the associated data base constitute an efficient means of mapping new molecular markers in maize.

RECOMBINANT inbred (RI) lines are produced by inbreeding the progeny of an F_2 derived from two well-established progenitor inbreds. After sufficient generations of inbreeding to achieve homozygosity, the RIs become fixed for short linkage blocks of progenitor alleles. Mapping is done by typing each RI for allele composition at a number of loci. A characteristic "strain distribution pattern" among the RIs is obtained for each locus (BAILEY 1971). Linkage is indicated when two loci have similar strain distribution patterns.

RI lines have long been employed by mouse geneticists for linkage determination (BAILEY 1981). Among the many applications of RIs to linkage studies in mice is the demonstration that the *dilute* mutation for mouse coat color is the result of the integration of an ectopic virus (JENKINS *et al.* 1981). In plants, RI lines have also been constructed and used for estimations of the components of variance (JINKS 1981), for plant breeding (BRIM 1966), and for the mapping of legumin genes in *Pisum* (DOMONEY, ELLIS and DAVIES 1986).

BAILEY (1981) has listed the advantages that RI families offer over segregating populations derived from a single meiosis. Their primary advantage is that they constitute a permanent population in which segregation is complete, or nearly complete, that can be used indefinitely for mapping. As such, they can be readily disseminated and all information obtained for the allelic distribution of different traits is accretive, that is, new data is continuously added to the preexisting map. In addition, RI families can be evaluated in many different environments. Since a genotype is represented by an inbred line, rather than by an individual, a more accurate assessment of the genetic component of variance can be made in studying quantitative traits.

A particular property of RIs is that they must undergo several rounds of meiosis before homozygosity is reached. In considering the problem of inbreeding in a heterozygous population, HALDANE and WADDINGTON (1931) described the mathematical relationship between recombination proceeding to fixation in RIs with recombination in a single meiosis. They found that the proportion of recombination among self-pollinated inbreds is twice the conventional rate when linkage distances were small and rose nonlinearly to 50% for unlinked markers. This result presents some difficulties in the initial stages of mapping because it is more difficult to establish linkage. Ultimately, however, the twofold expansion of the map for linked markers gives RIs an advantage over a conventional segregating population because recombination between closely linked markers is more readily detected (TAYLOR 1978).

For the investigator not intent on working with particular maize inbred lines, the RIs provide an efficient means of mapping a new molecular marker. Only two Southern blots are required: The first determines which restriction enzymes best distinguish the parental alleles using the new probe. Selecting the most appropriate restriction enzyme, the second blot types all members of the RI family for the distribution of these alleles. This distribution is then compared with the database containing the distribution of all previously mapped markers to determine linkage. The laborious alternative would be to request previously mapped probes, map them in a new

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

segregating population, and then determine linkage of the marker of interest. Even if only 50 well-placed markers were chosen, this latter procedure would require a minimum of 100 Southern blotting experiments.

An extensive restriction fragment length polymorphism (RFLP) map for maize has already been published by HELENTJARIS, WEBER and WRIGHT (1986, 1988) and HELENTJARIS *et al.* (1986a), and alternative RFLP maize maps are being constructed by other groups. Probes provided by these investigators were helpful in completing the map described in this paper.

MATERIALS AND METHODS

Strains: The F_2 populations of T232 × CM37[1] and CO159 × Tx303[1] have been described (STUBER and EDWARDS 1986; EDWARDS, STUBER and WENDEL 1987). In accordance with suggested nomenclature (BAILEY 1981), the resultant RI families are designated TXCM and COXTx, respectively. Seed from approximately 50 self-pollinated plants in each F_2 population were inbred for six generations by ear-to-row propagation. To minimize selection, the first ear in a row was generally chosen for propagation in the next generation. Forty-six inbreds reached the sixth generation in the TXCM family and 38 inbreds were self-pollinated for six generations in the COXTx family. Data presented in this paper include two additional inbreds from the first family and three from the second family that were selfed for only four or five generations. Except for the cases just mentioned, all of the characters scored in this report were based on fifth generation plants. For isozyme and DNA analyses, at least three plants were sampled from each inbred. Extracts were mixed for DNA analyses, but individual plant extracts were used for isozyme analyses.

B-A translocations for 19 of the 20 chromosome arms of maize were obtained from the Maize Genetics Cooperation Stock Center and from J. Beckett. These were maintained as described (BECKETT 1978) using a full color stock as the recurrent male parent. Stocks carrying seedling and plant markers for the various chromosome arms were also obtained from the Maize Genetics Cooperation Stock Center. Translocation heterozygotes were chosen on the basis of a high frequency of pollen abortion. Pollen from these plants was put onto plants homozygous for appropriate seedling markers. Self-pollination contaminants were ruled out by scoring plant and seed color in the progeny.

Isozyme analyses: Etiolated coleoptile tissue from 5-day-old seedlings was sampled and used for starch gel electrophoresis as detailed by STUBER *et al.* (1987). Assays were made for a total of 23 isozymic loci, located on nine chromosomes, in the two populations of RI lines.

Recombinant DNA Clones: Clones designated by 1. or 2. are random cDNA clones constructed from developing maize endosperm RNA (BURR and BURR 1981); these were screened for sequences hybridizing to single copy DNA (BURR *et al.* 1983). Random genomic clones, designated by 3. to 16., were prepared from a 1.5–2.5-kb fraction of a *Pst*I digest of inbred B37 DNA, size-selected on low melting temperature agarose (Bethesda Research Laboratories). DNA was purified from the agarose using Elutip-d columns (Schleicher & Schuell) and cloned into the *Pst*I site of pUC12 (MESSING 1983). Colonies were picked and grown in 1 ml cultures from which rapid lysates were prepared (HOLMES and QUIGLEY 1981). After verifying the presence of an insert, half of the lysate was digested with *Pst*I and the digest was electrophoresed in a 1% low melting temperature agarose gel. The band containing the insert was cut out and stored in a 1.5-ml microcentrifuge tube at 4°. The inserts of the random clones were screened by nick translating and hybridizing them to Southern blots containing DNA of the four progenitor inbreds that had been digested with *Bam*HI, *Bgl*II or *Eco*RI. Only those clones that gave strong hybridization signals to a single polymorphic band were retained. The random genomic clones were also screened for mitochondrial sequences by hybridizing them with nick translated mitochondrial DNA. The few clones that gave a positive signal were discarded. None of the genomic clones generated by this method that were eventually employed showed uniparental inheritance.

Other clones used are listed in Table 1. In accordance with the nomenclatural suggestions of COE and HOISINGTON (1987), we have assigned provisional numbers rather than names to all probes that might recognize pseudogenes. Accordingly, all our numbered loci should be preceeded by the three letter institutional designation *bnl* but that prefix has been omitted in the present paper for simplicity.

In addition, we used pBF243, which detects the polymorphic spacer region of ribosomal DNA (MCMULLEN *et al.* 1986). This subclone originated from an approximately 10-kb *Eco*RI rDNA repeat from the genetic stock *sh bz-m4* which had been cloned into EMBL4 (FRISCHAUF *et al.* 1983). A 3.2-kb *Sau*3A fragment was isolated from the λ clone and inserted into the *Bam*HI site of pUC9 (VIEIRA and MESSING 1982).

Nucleic acid hybridization: Generally only the cloned inserts were used as hybridization probes. Following digestion with restriction enzymes, the inserts were purified on 1% low melting temperature agarose gels. Nick translation (RIGBY *et al.* 1977) was usually performed by melting the slice containing the cloned fragment at 65° and using a small aliquot in the reaction (final concentration of agarose = 0.31%). The reaction also included 10 mM $MgSO_4$, 50 mM Tris-HCl (pH 7.4), 50 μg/ml bovine serum albumin, 1 mM dithiothreitol, 30 μM each of dCTP, dGTP, dTTP and 0.75 μM dATP[α-^{32}P] (3000 Ci/mmol). After 2 hr at 14° the reaction was stopped by the addition of 1 mg denatured and sonicated salmon sperm DNA and desalted by ethanol precipitation from 2.5 M ammonium acetate (MANIATIS, FRITCH and SAMBROOK 1982). One μg maize DNA samples, usually digested with *Bam*HI, *Bgl*II, or *Eco*RI, were electrophoresed on 0.5% agarose gels (MCDONELL, SIMON and STUDIER 1977) in 40 mM Tris-acetate (pH 8.0), 10 mM EDTA. The gel dimensions were 13.3 cm x 14.3 cm (model 800; Aquebogue Machine and Repair Shop, Box 205, Main Road, Aquebogue, NY 11931); total volume of agarose was 100 ml. A comb for 25 sample wells was used, each well being 3 mm wide. Following electrophoresis the gel was rocked in 500 ml of 0.25 N HCl for 7.5 min, then in 500 ml 0.5 M NaOH, 1.5 M NaCl, 2 mM EDTA for at least 15 min, and finally in 0.5 M Tris-HCl (pH 7.2), 3 M NaCl, 2 mM EDTA for 45 min to 1 hr. The gel was blotted to Nytran (Schleicher & Schuell) by capillary transfer in 20 × SSC. After blotting overnight with at least one change of paper towels, the nylon membranes were rinsed in 6 × SSC, dried, and baked at 70° in a vacuum oven for 2 hr. Prehybridization, hybridization, and washing conditions were those of KLESSIG and BERRY (1983). All hybridizations were done at 42°. Filters were exposed to Kodak X-Omat

[1]Origins are as indicated: T232—Tennessee; CM37—Morden, Canada; CO159—Ontario, Canada; Tx303—Texas.

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

TABLE 1

Additional recombinant clones used in typing the RI families

Clone	Locus or Gene	Source
pAmu2	*a1*[a]	O'REILLY *et al.* (1985)
pMAcI	*act1*	SHAH, HIGHTOWER and MEAGHER (1983)
pZML841	*adh2*	DENNIS *et al.* (1985)
pUC9α-1	α-tubulin[b]	BRUNKE *et al.* (1982)
pMBzPA	*bz1*	FEDOROFF, FURTEK and NELSON (1984)
pEco1.0	*c1*	CONE, BURR and BURR (1986)
pC2-c46	*c2*[c]	WIENAND *et al.* (1986)
pXho0.9	*o2*	SCHMIDT, BURR and BURR (1987)
pC1	*rbcS*[d]	BROGLIE *et al.* (1984)
Pst38	*sh1*	BURR and BURR (1982)
pshD13	*ss2(css1)*	GUPTA *et al.* (1987)
p7.6-1	Unknown[e]	EVOLA, BURR and BURR (1986)
pPC1.2	Unknown[f]	B. LOWE and P. S. CHOMET (unpublished data)
pBF225	*wx1*	EVOLA, BURR and BURR (1986)
B36	*zpB36*	BURR *et al.* (1982)
pBF245	Unknown[g]	This paper

The following cDNA clones have been mapped (HELENTJARIS *et al.* 1986; HELENJARIS, WEBER and WRIGHT 1986, 1987) and are listed with their chromosome arm assignment: NPI113(*7L*), NPI220(*8S*), NPI223(*6L*), NPI235(*6S*), NPI239(*2S*), NPI253(*9S*), NPI264(*10L*), NPI268(*8L*), NPI269(*2S*)[h], NPI271(*2L*), NPI283(*7L*), NPI285(*10S*), NPI298(*2L*), NPI306(*10L*), NPI400(*7S*), NPI409(*5S*), NPI411(*1S*), NPI414(*8L*), NPI421(*2S*)[h], NPI425(*3L*), NPI451(*4L*), NPI456(*2L*).

The cDNA clone AASr115 has been mapped to a position 28 map units distal to *adh2* on *4S* (Agrigenetics Maize Mapping Group, unpublished data).

[a] A second locus recognized by this probe on *8L* is designated *17.01*.

[b] The locus recognized by the *Chlamydomonas* α-tubulin probe is designated *17.04*.

[c] A second locus on *2L* recognized by the *c2* probe is designated *17.03*.

[d] A locus on *4L* recognized by the ribulose bisphosphate carboxylase small subunit probe is designated *17.05*.

[e] The locus recognized by p7.6-1 is designated *17.06*.

[f] The locus recognized by pPC1.2 is designated *17.02*.

[g] The locus recognized by pBF245 is designated *17.07*.

[h] A duplicate locus is found on *10L*.

AR film with a Dupont Cronex Lightning Plus Screen at −85°.

Linkage analysis: Each locus was scored for parental alleles to generate the computer data base. In the TXCM family, the CM37 allele at any locus was arbitrarily designated 1 and the T232 allele was designated 2. In the COXTx family the Tx303 allele at any locus was 1 and the CO159 allele was 2. Heterozygotes were designated 1.5 and a 0 was assigned in cases where there were no data. The data base consisted of columns for each locus and lines for each inbred. A FORTRAN program was written that compared each column one by one. In a pair of columns the absolute difference was recorded for each inbred that differed in allele assignments. No differences were recorded when a 0 was present in a given inbred at one of the two loci. The sum of absolute differences was divided by the number of functional (nonzero) comparisons; this fraction, R, represents the proportion of recombinant lines. Only R values of 0.36 or less were recorded. R is related to the proportion of recombinants in a single meiosis, r, by the equation $r = R/(2 - 2R)$ where r is map distance in Morgans (HALDANE and WADDINGTON 1931). This value was also calculated by the FORTRAN program. Relative map position was determined by placing the loci in an order requiring the least number of recombination events. When the estimate of map distance differed between the two RI families, an average value was taken.

RESULTS

Approximately 25% of the deoxycytosine residues in maize DNA are methylated (SHAPIRO 1976) which means that most methyl-sensitive enzymes recognize few sites in maize DNA. However, we and others had empirically found that sequences in and adjacent to transcribed regions are generally cleaved by these enzymes. This occurs even if the DNA has been prepared from a tissue in which the gene is not normally expressed. It therefore seemed possible that we could enrich for transcribed sequences that might be single copy regions by cloning small pieces of DNA digested with a methyl-sensitive enzyme. *Pst*I was selected because its recognition sequence contains two CXGs possibly making it more sensitive to methylation control (GRUENBAUM *et al.* 1981). Our expectations were sustained: of the last 77 inserts of the *Pst*I random clones we examined, 50.6% gave strong hybridization signals to unique polymorphic bands.

Two operations are necessary to type an RI family for the distribution of progenitor alleles. The first is to establish that a difference for a particular trait can be measured. In the case of DNA markers, it is sufficient to probe a Southern blot containing DNAs from the progenitor strains digested with a few enzymes. For many isozymes, electrophoretic variants for these inbreds have already been determined (STUBER and GOODMAN 1983). The second step is to use the assay system which distinguishes progenitor inbreds and determine which parental allele has been fixed in each RI line. For DNA markers this means probing a Southern blot containing DNA from each line digested with the enzyme that best distinguishes the progenitor alleles. In the present study, all measurements were made after five generations of selfing. At this point there was an average of 7.5% heterozygosity for all loci which is higher than the expected 3.125% heterozygosity. This is most likely the result of unintentional selection during propagation, but pollen contamination from other lines in the same family might also be a factor. Allele frequency varied from 0.30 to 0.70 with a mean of 0.48 and a standard deviation of 0.09. The deviation of allele frequency from 0.5 for some loci might again be an indication of unintentional selection.

A total of 62 mapped loci were assayed. These included fifteen linked pairs of known loci that could be typed in one or the other of our two RI families; this allowed a comparison of the map distances we calculated from the RIs with published values. These

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

TABLE 2

Linkage estimates of known mapped markers based on recombination in two maize RI families

Chromosome	Locus	Position	TXCM	COXTx
1L	*mdh4*	97		
				-/-
	adh1	128		
			6	11
	phi1	140		
			-/-	-/-
	acp4	176		
3L	*tpi4*	61		
			19	
	pdg2	71		
	mdh3	138		
			3	8
	a1	141		
5S	*pgm2*	0		
			24	
	mdh5	17		
			22	
	amp3	32		
6	*nor*			
				18
	pdg1	10		
				5
	enp1	13		
	pl1	49		
				21
	hex2	58		
	idh2	101		
			4	4
	mdh2	103		
9	*c1*	26		
			4	
	sh1	29		
			6	3
	bz1	31		
			11	21
	wx1	56		
			14	16
	acp1	63		

Values beneath the RI families represent estimates of map distance between adjacent markers. No estimate was made where there was no polymorphism for two linked loci within a given family. -/- means that no linkage could be detected. Map positions were taken from COE, HOISINGTON and NEUFFER (1987), WENDEL *et al.* (1986) and J. F. WENDEL (unpublished results).

TABLE 3

Distribution of marker loci

Marker loci	TXCM	COXTx	Total
Previously mapped	50	51	62
Previously unmapped	84	85	99

data are presented in Table 2. Inspection of Table 2 indicates that linkage frequently could not be detected beyond 20 map units. Furthermore, linkage estimates varied between the two RI families and were not always in agreement with map distances calculated from single meiotic populations. These differences were not great, however, and rarely deviated by a factor of two. In any event, they never interfered with the determinations of gene order.

The RI strains were typed with an additional 99 unmapped probes (Table 3). Linkage with known markers allowed us to map all of these (Figure 1). All loci were not mapped in both families because in some instances we failed to detect polymorphism in one set of inbred parents.

In addition to linkage with known markers, B-A

TABLE 4

Loci whose assignment to chromosome arm has been confirmed by B-A translocations

Chromosome	Locus	Translocation	Marker
1S	*8.05*	Tb-1Sb	*sr1*
2S	*npi239* *npi421*	Tb-3La-2S	*lg2*
2L	*npi298* *npi456*	Tb-1Sb-2L	*v4*
4L	*c2* *npi451* *5.67* *7.65* *10.05*	Tb-9Sb-4L6504	*j2*
5L	*5.24*	Tb-5La	*v2*
7L	*8.21* *15.21* *15.40*	Tb-7Lb	*gl1*
9L	*ss2(css1)* *14.28*	Tb-9Lc	*bm4*
10L	*17.07*	Tb-10L-19	*gl1*

translocations were sometimes used to locate markers to chromosome arm (BECKETT 1978, EVOLA, BURR and BURR 1986). For instance, *o2* is our only known marker on chromosome *7*. To determine whether the linked markers *15.40*, *zpB36*, and *15.21* were distributed toward the distal end of *7S* or were oriented in the direction of *7L*, we used *15.21* as a hybridization probe on DNA from plants homozygous for *gl1*, a *7L* marker, and from progeny of *gl1* pollinated by a plant heterozygous for the B-A translocation Tb-7Lb (Figure 2). B-A translocations undergo a high frequency of nondisjunction at the second postmeiotic division during microsporogenesis. If both B^A centromeres go to one nucleus and the second hypoploid nucleus fertilizes the embryo, then recessive markers covered by the chromosome arm that has been translocated to the B centromere will be uncovered in the progeny. Linked markers carried on the same translocated segment will also not be transmitted. The probe recognizes only one band in both the homozygous *gl1* plants and in the hemizygous *gl1* progeny. However, an additional band is seen in the wild-type progeny that received a paternal marker. This test suggests that 15.21 maps distal to the breakpoint of Tb-7Lb on *7L*. Table 4 summarizes confirmation of chromosome arm assignment of other loci using B-A translocations.

Only one locus did not map as expected in these populations. WENDEL, STUBER and GOODMAN (1985) placed *dia1* on *2S*. We observed that it was 4 map units distal to *npi456* in TXCM. We were further able to confirm that *npi456* was on *2L* (Table 4).

At present, we estimate that all of the maize genome

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

FIGURE 1.—Chromosome map of maize showing distribution of previously mapped and newly mapped loci in TXCM and COXTx RI families. Known markers are shown with their map positions (COE, HOISINGTON and NEUFFER 1987; EDWARDS, STUBER and WENDEL 1987; J. F. WENDEL, personal communication). Newly mapped loci are placed relative to these and to each other based on linkage estimates derived from recombinant inbred analyses. None of the new loci have been mapped with respect to centromeres; the location of the centromeres is approximate.

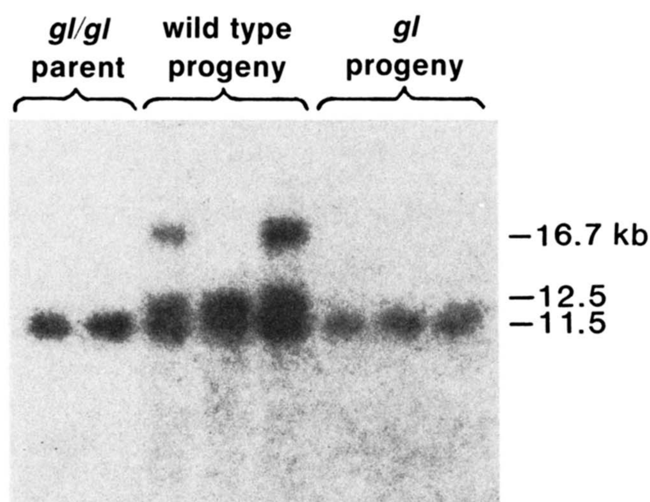

FIGURE 2.—Southern blot showing the use of a B-A translocation to demonstrate that clone 15.21 maps to *7L*. DNAs were prepared from individual plants: lanes 1 and 2 = *gl1/gl1*; lanes 3 to 8 = *gl1/gl1* × Tb-7Lb/+ (lanes 3 to 5 were phenotypically wild-type, lanes 6 to 8 had glossy phenotypes). DNA samples were digested with *Eco*RI and probed with nick translated 15.21.

is within 20 map units of loci mapped in these two families. The fact that we have succeeded in mapping 100% of previously unmapped markers is consistent with this estimate.

DISCUSSION

RIs present several advantages over F_2 and backcross populations that are traditionally used for gene mapping. First of all, RIs are perpetual populations that can be propagated by many investigators and in a variety of environments. In contrast, F_2 and backcross populations, unless they can be asexually propagated, can be grown only once and material derived from them will eventually be depleted. When this happens, the distribution of marker alleles among the segregants will have to be redetermined for a new population. Another advantage of RIs is that all information obtained from mapping in these populations is cumulative. A novel gene or sequence is readily mapped by comparing data obtained using it as a probe with information obtained from all previously mapped loci. The placement of another

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

marker in turn adds to the data base and refines the genetic map. Using this strategy, it is the RIs and the associated data base that are disseminated rather than the clones initially used to make the map.

TAYLOR (1978) has pointed out that because of the expansion of the map for short map distances a RI population is more efficient for estimating recombination than a backcross population when map distances are relatively small ($r < 12.5$ cM). The same breakeven point pertains whether RIs are obtained by selfing or sib mating. When molecular markers are used, the population size cannot be too large or the task becomes burdensome. In the present work we used populations of 41 and 48 lines. HELENTJARIS *et al.* (1986) used an F_2 population of 46 individuals. It seems unlikely that molecular geneticists will use significantly larger populations. SILVER (1985) has drawn attention to the fact that confidence intervals for estimates of linkage are large for small populations of RIs. However, comparison of his table with the table of confidence intervals for the binomial distribution (SNEDECOR 1956) that would pertain to a conventional segregating population confirms that even small populations of RIs have smaller confidence limits than F_2 or backcross families of the same size when the proportion of recombinants is low. Thus the problem of estimating map distance is the necessity of working with relatively small populations rather than an inherent problem associated with RIs. What benefit would be obtained by going to larger populations? Extrapolating from SNEDECOR's table, we see that the upper 95% confidence limit for distance separating two markers when no recombinants are detected drops from 3.8 to 2.1 cM when the RI population is expanded from 50 to 100. The benefit in the decrease of difference between upper and lower confidence limits obtained with doubling of population size decreases with increasing map distance.

Two large F_2 populations were available to us at the outset of this work. We purposely used both of them to construct two RI families in the event a parental chromosomal region was unrepresented, *e.g.*, if a gametophyte factor was present or if there was strong selection in favor of a particular parental gene combination. Although this was not observed, typing concurrently in two families has important advantages over working with only a single family. The first advantage is that it is less difficult to find polymorphisms and linkage. The second is that indications of linkage and determination of gene order derived with one family can be checked against results obtained in the second family. In fact, TAYLOR *et al.* (1975) have shown that data from a number of RI sets can be combined to obtain an accurate estimate of linkage between immunoglobulin heavy chain and serum prealbumin loci. Although the differences in linkage estimates varied somewhat for each locus in the two families (Table 2), no serious discrepancies were discovered.

The major limitation of RIs is that they cannot be used for mapping a trait that is not differentiated in the parental lines. This is seldom a problem for molecular probes in maize. We have encountered only one exception to this, however, which proves instructive. One of our probes was derived from the *c1* locus (CONE, BURR and BURR 1986). Most commercial inbreds lack anthocyanin pigmentation of the seed because breeders have incorporated recessive alleles at both the *c1* and *r1* loci. From restriction enzyme analyses, we discovered that three of the four progenitor strains used in this study were indistinguishable with the *c1* probe suggesting that they probably carried the same *c1* allele.

Several factors make the construction of a molecular map using RIs less difficult in maize than in mammals. First, maize is a remarkably polymorphic species. At the DNA level we had earlier estimated that maize is twenty times as polymorphic as humans and eight times as polymorphic as *Drosophila melanogaster* (EVOLA, BURR and BURR 1986). Consequently, hybridization probes for detecting RFLPs in this species are readily found. Second, the size of the maize genome, in terms of genetic recombination, is about one-third the size of a mammalian genome; hence fewer markers are required to span the map. Finally, inbreeding can be achieved by self-pollination rather than by sib mating. In practice this means that homozygosity can be achieved in about half the number of generations and that inbreds undergo fewer rounds of meiosis while they are still heterozygous. For closely linked markers, the genetic map is thereby expanded only two times, instead of four (TAYLOR 1978), making it easier to establish linkage.

It can be noted that with the availability of segregating populations mapped for a large percentage of the genome, Southern blotting replaces *in situ* hybridization to diploid chromosomes as the method of choice for locating cloned sequences. Not only does this approach offer greater ease and rapidity in mapping, but also better accuracy in the placement of a probe relative to preexisting markers.

We are especially grateful to TIM HELENTJARIS of NPI for providing us with many of the probes used to complete the construction of our RFLP map and to YU MA of Agrigenetics for probing our blots with the AASr115 clone. We would like to thank other scientists who have shared clones with us, JONATHAN WENDEL for communicating unpublished mapping data, and BEN TAYLOR for helpful discussions. We would also like to acknowledge the technical assistance of KAREN ARCHER. This work was supported by National Institutes of Health grant GM 31093 and by the Office of Basic Energy Sciences of the U.S. Department of Energy.

LITERATURE CITED

BAILEY, D. W., 1971 Recombinant inbred strains. Transplantation **11:** 325–327.

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

BAILEY, D. W., 1981 Recombinant inbred strains and bilineal congenic strains. pp. 223–239. In: *The Mouse in Biomedical Research*, Vol. 1, Edited by H. J. FOSTER, J. D. SMALL and J. G. FOX. Academic Press, New York.

BECKETT, J. B., 1978 B-A translocations in maize. I. Use in locating genes by chromosome arms. J. Hered. **69:** 27–36.

BRIM, C. A., 1966 A modified pedigree method of selection in soybeans. Crop Sci. **6:** 220.

BROGLIE, R. G., G. CORUZZI, B. KEITH and N.-H. CHUA, 1984 Molecular biology of C_4 photosynthesis in *Zea mays:* differential localization of proteins and mRNAs in the two leaf cell types. Plant Mol. Biol. **3:** 431–444.

BRUNKE, K. J., E. E. YOUNG, B. U. BUCHBINDER and D. P. WEEKS, 1982 Coordinate regulation of the four tubulin genes of *Chlamydomomas reinhardii*. Nucleic Acids Res. **10:** 1295–1310.

BURR, B., and F. A. BURR, 1981 Controlling element events at the *Shrunken* locus in maize. Genetics **98:** 143–156.

BURR, B., and F. A. BURR, 1982 *Ds* controlling elements of maize at the *Shrunken* locus are large and dissimilar insertions. Cell **29:** 977–986.

BURR, B., F. A. BURR, T. P. ST. JOHN, M. THOMAS and R. W. DAVIS, 1982 Zein storage protein gene family of maize. An assessment of heterogeneity with cloned messenger RNA sequences. J. Mol. Biol. **154:** 33–49.

BURR, B., S. V. EVOLA, F. A. BURR and J. S. BECKMANN, 1983 The application of restriction fragment length polymorphism to plant breeding. pp. 45–59. In: *Genetic Engineering*, Vol. 5, Edited by J. K. SETLOW and A. HOLLAENDER. Plenum Press, New York.

COE, E., and D. HOISINGTON, 1987 Toward unambiguous designations for loci defined by restriction fragment polymorphisms. Maize Genet. Coop. Newslett. **61:** 49.

COE, E. H., D. A. HOISINGTON and M. G. NEUFFER, 1987 Linkage map of corn (maize) (*Zea mays* L.). Maize Genet. Coop. Newslett. **61:** 116–147.

CONE, K. C., F. A. BURR and B. BURR, 1986 Molecular analysis of the maize anthocyanin regulatory locus *C1*. Proc. Natl. Acad. Sci. USA **83:** 9631–9635.

DENNIS, E. S., M. M. SACHS, W. L. GERLACH, E. J. FINNEGAN and W. J. PEACOCK, 1985 Molecular analysis of the *Alcohol dehydrogenase 2* (*Adh2*) gene of maize. Nucleic Acids Res. **13:** 727–743.

DOMONEY, C., T. H. N. ELLIS, and D. R. DAVIES, 1986 Organization and mapping of legumin genes in *Pisum*. Mol. Gen. Genet. **202:** 280–285.

EDWARDS, M. D., C. W. STUBER and J. F. WENDEL, 1987 Molecular marker facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116:** 113–125.

EVOLA, S. V., F. A. BURR and B. BURR, 1986 The suitability of restriction fragment length polymorphisms as genetic markers in maize. Theor. Appl. Genet. **71:** 765–771.

FEDOROFF, N. W., D. B. FURTEK and O. E. NELSON, JR., 1984 Cloning of the *bronze* locus in maize by a simple and generalizable procedure using the transposable controlling element *Activator* (*Ac*). Proc. Natl. Acad. Sci. USA **81:** 3825–3829.

FRISCHAUF, A.-M., H. LEHRACH, A. POUTSKA and N. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:** 827–843.

GRUENBAUM, Y., T. NAVEH-MANY, H. CEDAR and A. RAZIN, 1981 Sequence specificity of methylation in higher plant DNA. Nature **292:** 860–862.

GUPTA, M., P. S. CHOUREY, B. BURR and P. E. STILL, 1987 cDNAa of two non-allelic sucrose synthase genes in maize: cloning, expression and molecular mapping of the *sucrose synthase 2* gene. Plant Mol. Biol. In press.

HALDANE, J. B. S., and C. H. WADDINGTON, 1931 Inbreeding and linkage. Genetics **16:** 357–374.

HELENTJARIS, T., D. F. WEBER and S. WRIGHT, 1986 Use of monosomics to map cloned DNA fragments in maize. Proc. Natl. Acad. Sci. USA **83:** 6035–6039.

HELENTJARIS, T., D. WEBER and S. WRIGHT, 1988 Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics **118:** 353–363.

HELENTJARIS, T., M. SLOCUM, S. WRIGHT, A. SCHAEFER and J. NIENHUIS, 1986 Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. **72:** 761–769.

HOLMES, D. S., and M. QUIGLEY, 1981 A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:** 193–197.

JENKINS, N. A., N. G. COPELAND, B. A. TAYLOR and B. K. LEE, 1981 *Dilute* (*d*) color mutation of DBA/2J mice is associated with the site of integration of an ectopic MuLV genome. Nature **292:** 370–374.

JINKS, J. L., 1981 The genetic framework of plant breeding. Philos. Trans. Roy. Soc. Lond. **B 292:** 407–419.

KLESSIG, D. F., and J. O. BERRY, 1983 Improved filter hybridization method for detection of single copy sequences in large eukaryotic genomes. Plant Mol. Biol. Rep. **1:** 12–18.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MCDONELL, M. W., M. N. SIMON and F. W. STUDIER, 1977 Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. J. Mol. Biol. **110:** 119–146.

MCMULLEN, M. D., B. HUNTER, R. L. PHILLIPS and I. RUBENSTEIN, 1986 The structure of the maize ribosomal DNA spacer region. Nucleic Acids Res. **14:** 4953–4969.

MESSING, J., 1983 New M13 vectors for cloning. Methods Enzymol. **101:** 20–78.

O'REILLY, C., N. S. SHEPHERD, A. PEREIRA, Z. SCHWARZ-SOMMER, I. BERTRAM, D. S. ROBERTSON, P. A. PETERSON and H. SAEDLER, 1985 Molecular cloning of the *a1* locus of *Zea mays* using the transposable elements *En* and *Mu1*. EMBO J. **4:** 877–882.

RIGBY, P. W., M. DIECKMANN, C. RHODES and P. BERG, 1977 Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113:** 237–251.

SCHMIDT, R. J., F. A. BURR and B. BURR, 1987 Transposon tagging and molecular analyses of the maize regulatory locus *opaque-2*. Science **238:** 960–963.

SHAH, D. M., R. HIGHTOWER and R. B. MEAGHER, 1983 Genes encoding actin in higher plants: Intron positions are highly conserved but the coding sequences are not. J. Mol. Appl. Genet. **2:** 111–126.

SHAPIRO, H. S., 1976 Distribution of purines and pyrimidines in deoxynucleic acid. p. 259. In: *Handbook of Biochemistry and Molecular Biology*, Ed. 3, *Nucleic Acids*, Vol. 2, Edited by G. D. FASMAN. CRC Press, Cleveland.

SILVER, J., 1985 Confidence limits for estimates of gene linkage based on analysis of recombinant inbred strains. J. Hered. **76:** 436–440.

SNEDECOR, G. W., 1956 *Statistical Methods*, Ed. 5. Iowa State University Press, Ames, Iowa.

STUBER, C. W., and M. D. EDWARDS, 1986 Genotypic selection for improvement of quantitative traits in corn using molecular marker loci (Proceedings of the 41st Annual Corn and Sorghum Research Conference). Am. Seed Trade Assoc. **41:** 70–83.

STUBER, C. W., and M. M. GOODMAN, 1983 Allozyme genotypes for popular and historically important inbred lines of corn, *Zea mays* L. USDA Agric. Res. Results, Southern Ser., No. 16.

STUBER, C. W., J. F. WENDEL, M. M. GOODMAN and J. S. C. SMITH, 1987 Techniques and scoring procedures for starch gel

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024

electrophoresis of enzymes from maize (*Zea mays* L.). N.C. State Expt. Station Res. Bull. In press.

TAYLOR, B. A., 1978 Recombinant inbred strains: use in gene mapping. pp. 423–438. In: *Origins of Inbred Mice*, Edited by H. C. MORSE. Academic Press, New York.

TAYLOR, B. A., D. W. BAILEY, M. CHERRY, R. RIBLET and M. WEIGERT, 1975 Genes for immunoglobulin heavy chain and serum prealbumin protein are linked in mouse. Nature **256:** 644–646.

VIEIRA, J., and J. MESSING, 1982 The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:** 259–268.

WENDEL, J. F., C. W. STUBER, and M. M. GOODMAN, 1985 Twelve new isozyme loci in maize: Progress report on chromosomal locations, the subunit composition, and subcellular localization of their products. Maize Genet. Coop. Newslett. **59:** 87–88.

WENDEL, J. F., C. W. STUBER, M. D. EDWARDS and M. M. GOODMAN, 1986 Duplicated chromosome segments in maize (*Zea mays* L.): further evidence from hexokinase isozymes. Theor. Appl. Genet. **72:** 178–185.

WIENAND, U., U. WEYDEMANN, U. NIESBACH-KLOSGEN, P. A. PETERSON and H. SAEDLER, 1986 Molecular cloning of the *c2* locus of *Zea mays*, the gene coding for chalcone synthase. Mol. Gen. Genet. **203:** 202–207.

Communicating editor: S. D. TANKSLEY

Downloaded from https://academic.oup.com/genetics/article/118/3/519/5997562 by guest on 18 April 2024