Theor Appl Genet (1986) 71:765–771

THEORETICAL AND APPLIED GENETICS

The suitability of restriction fragment length polymorphisms as genetic markers in maize

S. V. Evola *, F. A. Burr and B. Burr

Department of Biology, Brookhaven National Laboratory, Upton, NY 11973, USA

Received September 20, 1985; Accepted October 31, 1985
Communicated by A. L. Kahler

Summary. Strain identification in *Zea mays* by restriction fragment length polymorphism should be feasible due to the high degree of polymorphism found at many loci. The polymorphism in maize is apparently higher than that currently known for any other organism. Five randomly selected maize inbred lines were examined by Southern filter hybridization with probes of cloned low copy sequences. Typically, several alleles could be distinguished among the inbred lines with any one probe and an appropriately selected restriction enzyme. Despite considerable polymorphism at the DNA level, 16 RFLP markers in three inbred lines of maize were examined for six to 11 generations and found be stable. Mapping of RFLP markers in maize can be accelerated by the use of B-A translocation stocks, which enable localization of a marker to chromosome arm in one generation. The use of recombinant inbred lines in further refinement of the map is discussed.

Key words: *Zea mays* L. – Restriction fragment length polymorphism (RFLP) – Genetic mapping – B-A translocations – Recombinant inbreds

Introduction

Comparative examination of gene loci at the DNA level has revealed differences between individuals, or groups of individuals, that were previously unsuspected. In part, these differences are manifested as variations in the size of DNA fragments complementary to a given probe in a Southern filter hybridization. Briefly, the methodology entails digesting purified DNA with restriction endonucleases that recognize and cleave at specific four to seven base pair sequences in DNA. The digests are electrophoresed in agarose gels that separate the DNA fragments according to size. The pattern of the DNA fragments is transferred, commonly to nitrocellulose sheets, by the Southern blotting procedure (Southern 1975), then hybridized with a radioactively labeled DNA probe. The nonhybridized counts are washed off and the filter is exposed to X-ray film. Only fragments complementary to the labeled probe will show up as bands on the film. The sensitivity of the technique is such that single copy genes, which typically comprise less than 10^6 of the genome of a higher eukaryote, can routinely be detected.

The variation observed in the size of restriction fragments homologous to a specific probe has been called "restriction fragment length polymorphism", or RFLP. These differences are the consequence of heritable changes in the DNA: point mutations create or abolish restriction endonuclease sites while DNA rearrangements, insertions or deletions, alter their relative positions. By analysis of the sizes of the fragments produced by single and double restriction enzyme digests, the linear order of restriction sites, or "map" of a DNA segment, can be determined.

Restriction fragment length variants were first used as genetic markers to map temperature sensitive mutants of adenovirus (Grodzicker et al. 1974). More recently, RFLPs have been described in several eukaryotes, including *Drosophila melanogaster* (Langley et al. 1982) and man (Jeffreys 1979; Tuan et al. 1979). There is ample evidence in the mammalian literature that single copy sequences from nuclear DNA behave as co-dominant Mendelian factors and can be mapped to chromosomal location (Wyman and White 1980; Ruddle 1981). Botstein and White (Botstein et al. 1980; White

* Current address: CIBA-GEIGY Corp., Biotechnology Research, P.O. Box 12257, Research Triangle Park, NC 27709, USA

et al. 1985) have discussed the advantages and mechanics of constructing a genetic map of the human genome based on RFLPs to aid in the mapping of inherited traits. Linkage between an abnormal allele and an RFLP marker has provided a useful tool for the detection of human diseases and for genetic mapping (Kan and Dozy 1978; Gusella et al. 1983; Bhattacharya et al. 1984; Gitscher et al. 1985).

RFLPs supplement existing data from morphological traits and from biochemical markers, such as isozymes. Eventually, RFLPs should provide more genetic information than either type of marker because DNA sequence changes in and around genes do not always alter the phenotype or the charge of amino acid side chains. In one of the best studied examples, 11 *Adh* alleles from wild populations of *Drosophila melanogaster* were compared (Kreitman 1983). Among 43 changes in nucleotide sequence that were found, only one resulted in an amino acid substitution.

In maize, a high degree of RFLP was discovered among wild-type *Shrunken1* (*Sh1*) alleles of various genetic stocks and inbred lines (Burr and Burr 1981; Burr et al. 1983). RFLPs are also prevalent at the *Alcohol dehydrogenase1* (*Adh1*) locus (Johns et al. 1983). The differences observed in the *Sh1* and *Adh1* maps appear to be largely, if not entirely, due to point mutations. However, insertion/deletion polymorphisms have been reported in maize at the *Waxy* (*Wx*) locus (Schwarz-Sommer et al. 1984; Wessler and Varagona 1985).

Burr et al. (1983) had examined the possibility that the *Sh1* and *Adh1* loci were unusually polymorphic in maize. The probes employed in that study were clones from a maize endosperm cDNA library that hybridized to only one, or up to three, DNA fragment(s). DNAs from three to six inbreds were examined with three restriction enzymes and 18 probes. Sixteen of the 18 probes detected polymorphisms, suggesting that RFLP was a general property of single copy sequences in maize.

The theoretical considerations and potential applicability of RFLPs in plant breeding – for strain identification, mapping and monitoring alleles associated with qualitative and quantitative traits, and assessing genetic heterogeneity – have previously been addressed (Beckmann and Soller 1983; Burr et al. 1983; Soller and Beckmann 1983).

The present paper seeks to demonstrate that, in maize, the considerable degree of nucleotide polymorphisms and their genetic stability makes the use of RFLP markers a viable and efficient method of strain identification. In addition, we discuss strategies for mapping these markers to chromosomal location by the use of B-A translocation stocks and recombinant inbred lines.

Materials and methods

DNA methodology

DNA was isolated from shoots of three to five day old dark-germinated seedlings or, in the case of the B-A translocations, from young leaves of immature plants that had been phenotypically classified. The preparation of DNA was as previously described (Burr and Burr 1981) except that the concentration of EDTA in the extraction buffer was increased to 50 mM. Restriction enzymes *Eco* RI and *Hind* III were used according to the recommendations of the supplier (Bethesda Research Laboratory). All DNA samples were alcohol precipitated following digestion. Gel electrophoresis in 0.5% ultrapure agarose (Bethesda Research Laboratory) was carried out according to McDonnell et al. (1977). The gels were blotted to nitrocellulose filters (Schleicher and Schuell) by the Southern procedure (Southern 1975); hybridization and washing conditions were those of Klessig and Berry (1983). Cloned DNAs used as hybridization probes were nick-translated according to Rigby et al. (1977) to 10^8 cpm/μg DNA. Generally 10^6 cpm of probe were used per 13.2 × 14.5 cm filter. Filters were exposed to Kodak X-Omat XAR-5 X-ray film with a Dupont Cronex Lightning Plus intensifying screen for one to three days at −85 °C. Radioactivity was removed from filters to be reprobed by washing them two to three times for 15 min each in 0.1 × SSC, 0.1% SDS at 95 °C (Gatti et al. 1984).

DNA probes

1 Sh1. The Pst38 subgenomic clone of the *Sh1* gene has been described (Burr and Burr 1982).

2 Wx. The *Waxy* (*Wx*) subgenomic clone pBF224 is a 4.7 kb *Bam* HI fragment covering the 5′ end of the gene; pBF225 contains a 3.4 kb *Pvu* I fragment spanning the transcribed region. Both fragments, which were cloned into pBR327 (Soberon et al. 1980), were obtained from an 11 kb *Eco* RI genomic clone, designated pWx5, that was provided by S. Wessler. A description of the Wx gene has been published (Shure et al. 1983).

3 Random maize genomic clones. a) Subgenomic fragments from two *Mutator*-induced *bronze1* (*bz1*)-mutable lines (Robertson 1978) were cloned into pUC9 (Vierra and Messing 1982). The fragments were obtained from 15–20 kb fragments of *Sau* 3A partial digests cloned into *lambda* EMBL4 (Frischant et al. 1983). The subclones used were p6–12 : 2, p6–18 : 2, p8–9 : 1, p8–18 : 25, p782–12a : 6, and p814–14 : 36, p814–24 : 65; all contained sequences hybridizing to *Mu1* (Freeling 1984). b) A gel-purified 2 kb *Acc* I fragment from p7.6–1, a pUC13 clone carrying an unidentified maize sequence from a genetic stock containing *c1–m5* (K. C. Cone, unpublished), was also used. The relevant band was cut out of an ethidium bromide-stained gel and purified by an Elutip column (Schleicher and Schuell) according to the protocol of the manufacturer.

4 Zein. B59 is a zein cDNA clone that hybridizes to only two or three sequences in the maize genome (Burr et al. 1982).

5 Random cDNA clones. The cDNA clones 1–45, 1–67, 1–297, 1–326, 1–556, 2–323, and 2–369 were prepared from poly(A) + RNA of developing maize endosperm and have been described previously (Burr and Burr 1981).

Plant material

The strains of maize inbreds and genetic stocks used in this study are listed in Table 1 along with the names of the sources of the seed.

Results and discussion

Use in strain identification

It was mentioned in the Introduction that differences among maize wild-type *Adh1* and *Sh1* alleles are readily detectable (Johns et al. 1983; Burr and Burr 1981; Burr et al. 1983). An

Table 1. Inbred lines and genetic marker stocks of maize used for restriction enzyme analyses

Strain	Source	Location
A. Inbreds		
A188	R.L. Phillips	Univ. of Minnesota
ASK	D.E. Alexander	Univ. of Illinois
B37	D.N. Duvick	Pioneer Hi-Bred Intl.
B73	N.M. Frey	Pioneer Hi-Bred Intl.
M14	O.E. Nelson	Univ. of Wisconsin
SK2	N.M. Frey	Pioneer Hi-bred Intl.
W22	D.V. Glover	Purdue Univ.
W64A	O.E. Nelson	Univ. of Wisconsin
B. Genetic stocks		
TB-9Sb	Maize Genetics Stock Center	Univ. Illinois
yg C sh bz wx	B. McClintock	Cold Spring Harbor Lab.

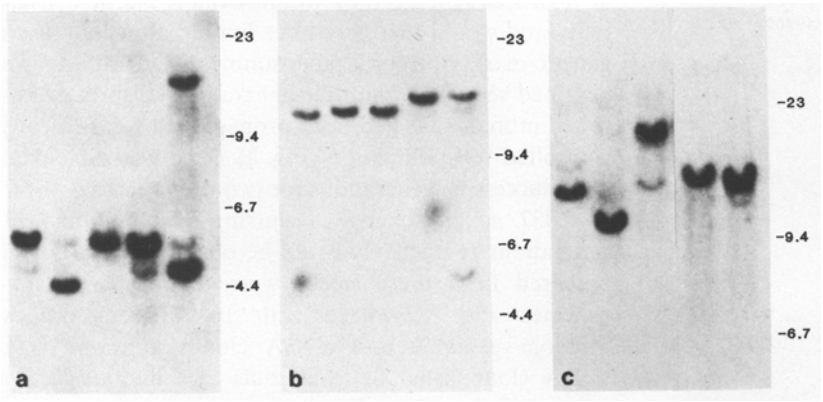

Fig. 1a–c. The extent of RFLP in maize inbreds as shown by Southern transfer hybridizations. These blots illustrate the unusually high degree of polymorphism that seems to be characteristic of maize. All five of the randomly selected inbreds shown can be distinguished from each other with a combination of two restriction enzymes and two probes. Each blot contains DNAs of the same inbreds; from *left to right;* A188, B37, M14, W22, and W64a. Four μg of DNA were used for each digest. **a** *Hind* III digest; probed with *Sh1* subclone Pst38. **b** The same blot shown in **a**, stripped of radioactivity, and reprobed with the *Wx* subclone p224. **c** *Eco* RI digest; probed with *Sh1* subclone Pst38. Bacteriophage *Hind* III molecular length markers in kilobase pairs are indicated to the right of each blot

objective estimate of the amount of polymorphism in a given population is provided by two similar statistics called "nucleotide diversity" (Nei and Li 1979) or "heterozygosity" (Engels 1981). Both measure the number of nucleotides per thousand nucleotides that distinguish any two individuals taken at random. Kazazian et al. (1983), for example, had reported a nucleotide diversity of 0.002 for the human β-globin gene cluster, based on either restriction site or sequence data. The human serum albumin locus was determined to have a heterozygosity of 0.003 by restriction analysis (Murray et al. 1984). A heterozygosity of 0.006 per nucleotide for the *Adh* locus of *Drosophila melanogaster* had been estimated by Langley et al. (1982), based on restriction analyses, and also by Kreitman (1983), who compared the nucleotide sequences of 11 *Adh* alleles. For the maize *Adh1* locus, one can calculate a heterozygosity estimate of 0.082 by using equation 11 of Engels (1981) and the six most completely mapped maize *Adh1* alleles of the seven published by Johns et al. (1983). From the restriction maps of four wild-type *Sh1* alleles – two that had been previously published (Burr et al. 1983) and two more, from Black Mexican Sweet Corn and the inbred A188, that were subsequently cloned and mapped in this laboratory – we calculate a heterozygosity of 0.048 for the *Sh1* locus. These figures, however, may be an overestimate of the magnitude of polymorphism in maize because neither the *Sh1* or the *Adh1* alleles were sampled at random. In fact, Johns et al. (1983) emphasize that the *Adh1* alleles they selected were known to be different. Nevertheless, the ease of obtaining RFLP markers in maize (Burr et al. 1983) versus humans (Helentjaris and Gesteland 1983) indicates that the apparent abundance of nucleotide diversity in maize is real.

If a heterozygosity of 0.05 can be assumed for maize, then by using three hexanucleotide recognition enzymes – each cleaving on both sides of the region being probed – 36 nucleotides would be assayed. In this number of nucleotides one would expect to find 1.8 differences between any two strains. We have tested this idea with five randomly selected North American maize inbreds. DNAs were digested with two restriction enzymes, *Hind* III and *Eco* RI, and probed with *Sh1* or *Wx* probes. At least three restriction fragments per strain were examined. As may be seen in Fig. 1, all five inbreds can be unambiguously distinguished from one another.

Soller and Beckmann (1983) estimated that twenty or more polymorphic loci would have to be examined in order to differentiate two inbred lines or self-pollinating varieties. They had conservatively assumed that there were only two alleles for any one locus, each with a frequency of 0.5 in the population. Most strains would therefore share many alleles in common, necessitating the use of many probes to find allelic combinations that differed. Fortunately, where genetic loci have been studied at the DNA level, many alleles at a locus have been found. Moreover, these polymorphs are not rare but occur at appreciable frequencies. This suggests that a comparison of any two alleles is likely to show RFLP differences. With respect to maize, therefore, the number of probes needed to distinguish strains or inbreds should be substantially less than twenty.

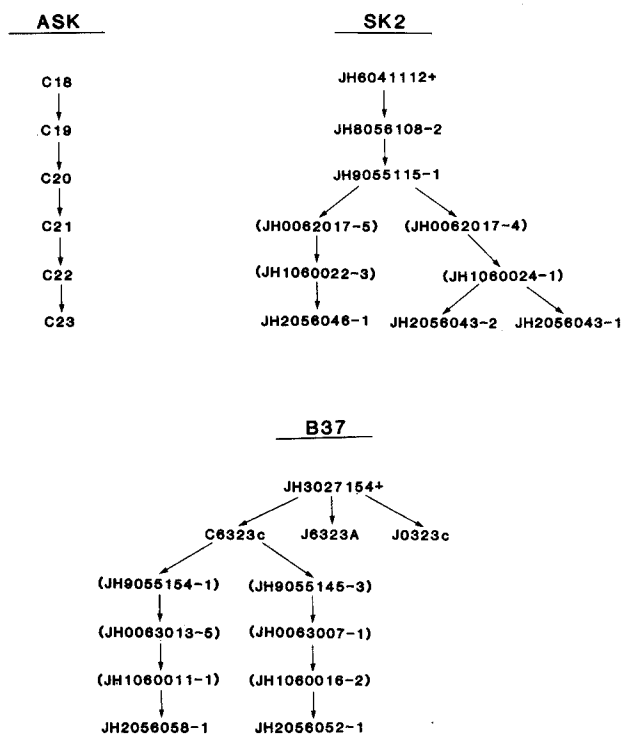

Fig. 2. Lineage of the three maize inbred lines used to examine the stability of RFLP markers in successive generations. DNA was prepared from approximately 24 seedlings of each generation except for those indicated in parentheses. The results of the examination are shown in Fig. 3

Stability of maize RFLPs

Given the very high levels of DNA polymorphism observed in maize, there was concern that the polymorphs would not be useful as genetic markers. In an equilibrium population, the extent of nucleotide diversity is proportional to the mutation rate (Nei 1983). If new RFLPs were to arise continually in maize at high frequencies, they would exhibit marked instability and would therefore have limited applicability as genetic markers. In order to examine this possibility, we obtained seed from multiple generations of three established inbreds that had been propagated by ear-to-row, controlled self-pollination (Fig. 2). Seed was not taken from successive generations for two of the inbreds, SK2 and B37, so that changes occurring over nine and 11 generations respectively could be observed. The DNAs prepared from these stocks were digested with two enzymes and hybridized with 16 different probes (random genomic and cDNA clones and one zein cDNA clone listed in "Materials and methods"). In each case the DNA fragment patterns did not vary from generation to generation; representative results are shown in Fig. 3. Although it was important to verify the stability of our RFLP markers, the mutation rate would have had to be exceptionally high, approximately 10^{-4} per nucleotide per generation, for variants to have been observed in such a limited survey.

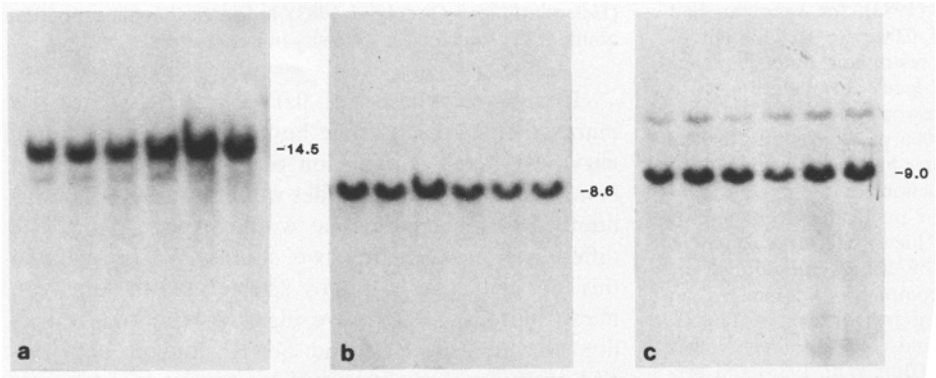

Fig. 3a–c. The stability of RFLPs in successive generations of inbred lines as examined by Southern transfer hybridization. Three μg of DNA from each of the generations of three inbreds listed in Fig. 2 were digested with *Hind* III. Following electrophoresis and Southern transfer, the blots were probed with a 2 kb *Acc* I fragment from the clone p 7.6-1. From *left to right:* (**a**) ASK, (**b**) B37, (**c**) SK2. Molecular lengths in kilobase pairs are indicated to the *right* of each figure

Mapping RFLP markers

There has been much progress recently in mapping new genes in maize (Neuffer and Sheridan 1980). The B-A translocations have greatly aided this effort, as they allow mapping of a recessive trait to chromosome arm in one generation (Beckett 1978). The method has been successfully used to map isozyme loci (Newton and Schwartz 1980) and can be adopted to map RFLP markers. The use of B-A translocations derives from the observation that B chromosome centromeres frequently undergo nondisjunction in the second postmeiotic mitosis of microsporogenesis (Roman 1947). The result of nondisjunction is that either the endosperm or the embryo fails to receive a copy of the paternal chromosome arm that has been translocated to the B centromere. If a recessive mutation carried in the female maps to this chromosome arm, it will be "uncovered" in the hypoploid tissue.

To illustrate the use of B-A translocations in mapping RFLP markers, we have used a translocation carrying *Sh1* on the short arm of chromosome 9. Plants heterozygous for the translocation TB-9Sb, which carries most of the short arm of chromosome 9 were used as pollen parents. These were crossed onto females homozygous for *yg C sh bz wx* on the short arm of chromosome 9. The male plants possessed dominant alleles at these loci and all except *Wx* are carried on the translocated segment. Kernels with the phenotype *C Sh Bz Wx* were planted in the field and classified at the five to six leaf stage. Most of the progeny were green (*Yg*) with the exception of a few *yellow-green* (*yg*) plants. These plants had apparently lost the dominant markers on the short arm of 9 through nondisjunction, uncovering the recessive *yg* trait. DNA was prepared from the immature leaves of both *Yg* (green) and *yg* plants. Blots of the restricted DNAs were probed with bot a *Sh* subgenomic probe, representing a sequence carried on the translocated segment (Fig. 4a), and a *Wx* probe, comprising a sequence linked to the normal centromere (Fig. 4b). Three restriction enzymes were tested to find one that best distinguished the parental alleles.

In Fig. 4a, the paternal translocation heterozygote shows two *Eco* RI fragments hybridizing to the *Sh1* probe. The female parent, which is homozygous for the allele *sh1-R*, has a different-sized *Eco* RI fragment. As expected, the DNAs of the *Yg* progeny show that they each received one allele from the female parent and one of the two alleles of the male parent. The *yg* plants, on the other hand, having lost the dominant paternal markers by nondisjunction of the B-A translocation, have only the maternal *sh1-R* allele. In Fig. 4b, restriction digests of the same DNAs were probed with a *Wx* clone. The paternal translocation heterozygote again has two allelic fragments, but this time the female *wx-R* allele cannot be differentiated from the larger male allele using this particular enzyme. Among the progeny of the cross, two of the three *Yg* plants have received the smaller paternal allele and one has received the larger allele. All three of the *yg* plants received the smaller paternal allele in addition to the maternal allele. This result agrees with the genetic interpretation which assumes that both the *Yg* and *yg* plants should have *Wx/wx* alleles of both parents, because the breakpoint of the translocation is distal to *Wx*.

It is estimated that 85% of the maize genome is covered by existing B-A translocations (Beckett 1978). Any marker occurring within this portion of the maize genome can be mapped to a chromosome arm by examining the DNAs of the hypoploid progeny of the 18 maize translocation stocks. Markers mapping to much of the remaining portion of the

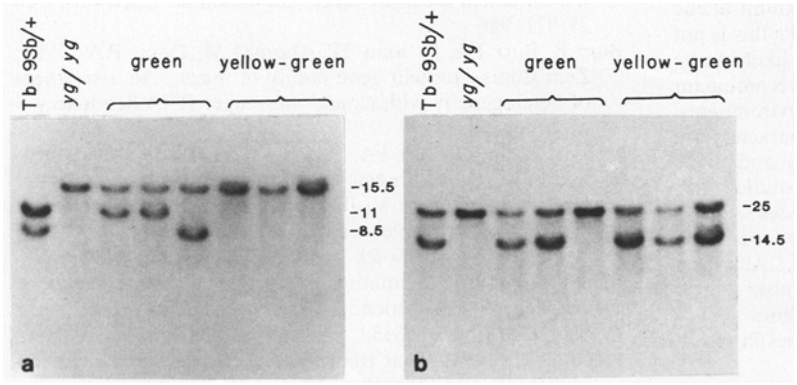

Fig. 4a, b. Demonstration of the use of B-A translocations to map RFLP markers. DNA was prepared from parents and individual progeny of a cross of a *yg C sh bz wx* tester strain pollinated by a translocation heterozygote, Tb59B, carrying dominant alleles at these loci. The breakpoint of the translocation is between *Bz* and *Wx*, very near *Wx*. Plants were classified as being green (*Yg*) or yellow green (*yg*) prior to DNA preparation. Five μg of DNA from each plant was digested with *Eco* RI. In (**a**) the probe was the *Sh1* clone Pst38. The male parent contains two distinguishable *Sh1* alleles; the female has a different *sh* allele. The green progeny receive the female *sh* allele and one or the other of the male *Sh* alleles. In contrast, the *yg* plants, which have lost the dominant male contribution by nondisjunction of the B-A translocation, have only the female *sh* allele. In (**b**) the probe was the *Wx* subgenomic clone p225. Because the chromosomal segment being lost does not involve the *Wx* locus, both the green and *yg* plants receive an allele from each parent. Molecular lengths of the fragments in kilobase pairs are indicated to the *right* of each figure

genome can be placed in the second generation after hypoploid progeny have been self-pollinated or test-crossed (Beckett 1978). Hypoploid plants carry a chromosome with a deficiency for the translocated segment. Gametes receiving this chromosome will not function, so a majority of the progeny will receive the non-deficient, maternal chromosome. Paternal alleles carried on the deficient chromosome and tightly linked to the translocation breakpoint will also be poorly transmitted. Therefore, RFLP alleles not covered by a B-A translocation, but mapping near the translocation breakpoint, will be transmitted at a low frequency from hypoploid plants.

Once a number of RFLP loci have been assigned to chromosome arms, they can be mapped relative to any other markers using a segregating population. Correlation with the existing genetic map can, in part, be achieved by measuring linkage with previously mapped isozyme loci and with RFLPs of known cloned genes. Because gene mapping has been a cooperative effort in many organisms, the choice of the segregating population in which to measure linkage is important. Recombinant inbred lines have proven useful to mouse geneticists in mapping molecular, as well as morphological, characters (Taylor 1978; Bailey 1981). Recombinant inbred lines are derived by repeated sib-mating or self-fertilization from an F_2 population of two distinct inbreds. The production of recombinant inbreds is similar to the production of random inbreds from an F_2 population by single seed descent (Brim 1966), except that selection is avoided so that all allelic combinations have an equal chance of being represented. These lines fix linked blocks of parental alleles. Linkage data is obtained by typing each recombinant inbred for every allele that distinguishes the two original parental lines. Parental combinations of alleles that occur together at high frequency are assumed to be linked. Recombinant inbreds have the advantage over backcross or F_2 populations in that the information gained from screening them is cumulative. Whereas F_2 and backcross populations have a finite lifetime and must be continually regenerated and retyped, recombinant inbred families need only be typed once for any character. When a new trait is found, the investigator uses the recombinant inbreds to type only that additional character, using all previous information to make a map assignment. The use of recombinant inbreds is, of course, limited to mapping those loci which exhibit allelic differences in the original parental lines. For RFLPs this is not a great disadvantage since unrelated inbreds are likely to be distinct at many loci. An additional advantage of recombinant inbreds is that they can be screened in different environments. This may be particularly useful if quantitative characters are being scored. Jinks and his colleagues (Perkins and Jinks 1973; Jinks et al. 1977) have constructed and studied two extensive recombinant inbred families in *Nicotiana rustica*. Two sets of maize recombinant inbred lines are currently being developed in collaboration with M. C. Albertsen (Pioneer Hi-Bred International, Inc.) and C. W. Stuber (North Carolina State University). Recombinant inbred lines should be useful for preparing linkage maps for any species in which inbred lines have been established.

These results show that maize has a very high degree of restriction fragment length polymorphism, greater than any other organism examined thus far. Yet despite the exceptional amount of polymorphism, the RFLP markers are stably maintained for several generations of inbreeding. Thus RFLPs should facilitate the construction of a detailed molecular genetic map for maize. Mapping these markers can be aided by the use of special genetic stocks, particularly the B-A translocations that can locate the marker to a chromosome arm, and by recombinant inbred lines.

Acknowledgements. We thank Jacqui Beckmann for valuable discussions at the outset of this work and Sue Wessler for the *Waxy* clone, pWx5. This work was supported by a grant from Pioneer Hi-Bred International, Inc. and by Brookhaven National Laboratory, which is administered by Associated Universities, Inc., under funding provided by the U.S. Department of Energy.

References

Bailey DW (1981) Recombinant inbred strains and bilineal congenic strains. In: Foster HL, Small JD, Fox JG (eds) The mouse in biomedical research, vol 1. Academic Press, New York, pp 223–239

Beckett JB (1978) B-A translocations of maize. J Hered 69: 27–36

Beckmann JS, Soller M (1983) Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor Appl Genet 67:35–43

Bhattacharya SS, Wright AF, Clayton JF, Price WH, Phillips CI, McKeown CME, Jay M, Bird AC, Pearson J (1984) Close genetic linkage between X-linked *Retinitis pigmentosa* and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. Nature 309: 253–255

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314–331

Brim CA (1966) A modified pedigree method of selection in soybeans. Crop Sci 6:220

Burr B, Burr FA (1981) Controlling element events at the *Shrunken* locus in maize. Genetics 98: 143–156

Burr B, Burr FA (1982) *Ds* controlling elements of maize at the *Shrunken* locus are large and dissimilar insertions. Cell 29:977–986

Burr B, Burr FA, St John TP, Thomas M, Davis RW (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, Evola SV, Burr FA, Beckmann JS (1983) The application of restriction fragment length polymorphisms to plant breeding. In: Setlow JK, Hollaender A (eds) Genetic engineering principles and methods, vol 5. Plenum, New York London, pp 45–59

Engels WR (1981) Estimating genetic divergence and genetic variability with restriction endonucleases. Proc Natl Acad Sci USA 78:6329–6333

Freeling M (1984) Plant transposable elements and insertion sequences. Annu Rev Plant Physiol 35:277–298

Frischauf A-M, Lehrach H, Poustka A, Murray N (1983) Lambda replacement vectors carrying polylinker sequences. J Mol Biol 170:827–843

Gatti RA, Concannon P, Salser W (1984) Multiple use of Southern blots. BioTechniques 2: 148–155

Gitschier J, Drayna D, Tuddenham EGD, White RL, Lawn RM (1985) Genetic mapping and diagnosis of haemophilia A achieved through a *Bcl* I polymorphism in the factor VIII gene. Nature 314:738–740

Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young AB, Shoulson I, Bonilla E, Martin JB (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306:234–238

Helentjaris T, Gesteland R (1983) Evaluation of random cDNA clones as probes for restriction fragment polymorphisms. J Mol Appl Genet 2:237–247

Jeffreys AJ (1979) DNA sequence variants in the Gγ-, Aγ-, δ- and β-globin genes of man. Cell 18:1–10

Jinks JL, Jayasekara NEM, Boughy H (1977) Joint selection for both extremes of mean performance and of sensitivity to a macroenvironmental variable. 2. Single seed descent. Heredity 39:345–355

Johns MA, Stromer JN, Freeling M (1983) Exceptionally high levels of restriction site polymorphism in DNA near the maize *Adh1* gene. Genetics 105:733–743

Kan YW, Dozy AM (1978) Polymorphism of DNA sequence adjacent to human β-globin structural gene: relationship to sickle mutation. Proc Natl Acad Sci USA 75:5631–5635

Kazazian HH, Chakravarti A, Orkin SH, Antonarakis SE (1983) DNA polymorphisms in the human β-globin gene cluster. In: Nei M, Koehn RK (eds) Evolution of genes and proteins. Sinauer, Sunderland, pp 137–146

Klessig DF, Berry JO (1983) Improved filter hybridization method for detection of single copy sequences in large eukaryotic genomes. Plant Mol Biol Rep 1:12–18

Kreitman M (1983) Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature 304:412–417

Langley CH, Montgomery EA, Quattlebaum WF (1982) Restriction map variation in the *Adh* region of Drosophila second chromosome. Proc Natl Acad Sci USA 79: 5631–5635

McDonell MW, Simon MN, Studier FW (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

Murray JC, Mills KA, Demopulos CM, Hornung S, Motulsky AG (1984) Linkage disequilibrium and evolutionary relationships of DNA variants (restriction enzyme fragment length polymorphisms) at the serum albumin locus. Proc Natl Acad Sci USA 81:3486–3490

Nei M (1983) Genetic polymorphism and the role of mutation in evolution. In: Nei M, Koehn, RK (eds) Evolution of genes and proteins. Sinauer, Sunderland, pp 165–190

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Neuffer MG, Sheridan WF (1980) Defective kernel mutants of maize. 1. Genetic and lethality studies. Genetics 95: 929–944

Newton KJ, Schwartz D (1980) Genetic basis of the major malate dehydrogenase isozymes in maize. Genetics 95: 424–442

Perkins JM, Jinks JL (1973) The assessment and specificity of environmental and genotype-environmental components of variability. Heredity 30:111–126

Rigby PW, Dieckmann M, Rhodes C, Berg P (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113: 237–251

Robertson DS (1978) Characterization of a mutator system in maize. Mutat Res 51:21–28

Roman H (1947) Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics 32:391–409

Ruddle FH (1981) A new era in mammalian gene mapping: somatic cell genetics and recombinant DNA methodologies. Nature 294:115–120

Schwarz-Sommer Z, Gierl A, Klosgen RB, Wienand U, Peterson PA, Saedler H (1984) The *Spm* (*En*) transposable element controls the excision of a 2-kb DNA insert at the *wx-m8* allele of *Zea mays*. EMBO J 3:1021–1028

Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the *Waxy* locus in maize. Cell 35: 225–233

Soberon X, Covarrubias L, Bolivar F (1980) Construction and characterization of new cloning vehicles. 4. Deletion derivatives of pBR322 and pBR325. Gene 9:287–305

Soller M, Beckmann JS (1983) Genetic polymorphism in varietal identification and genetic improvement. Theor Appl Genet 67:25–33

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Tuan D, Biro PA, deRiel JK, Lazarus H, and Forget BG (1979) Restriction endonuclease mapping of the human β-globulin gene loci. Nucleic Acids Res 6:2519–2544

Taylor BA (1978) Recombinant inbred strains: use in gene mapping. In: Morse HC (ed) Origins of inbred mice. Academic Press, New York, pp 423–438

Viera J, Messing J (1982) The pUC plasmids, an M13-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259–268

Wessler SR, Vargona MJ (1985) Molecular basis of mutations at the waxy locus of maize: Correlation with the fine structure map. Proc Natl Acad Sci USA 82:4177–4181

White R, Leppert M, Bishop DT, Barker D, Berkowitz J, Brown C, Callahan P, Holm T, Jerominski L (1985) Construction of linkage maps with DNA markers for human chromosomes. Nature 313:101–105

Wyman AR, White R (1980) A highly polymorphic locus in human DNA. Proc Natl Acad Sci USA 77:6754–6758