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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND GENETIC IMPROVEMENT OF AGRICULTURAL SPECIES*

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ABSTRACT

Evidence is accumulating demonstrating the ubiquity and abundance of a new class of genetic markers, restriction fragment length polymorphisms (RFLPs). These markers should allow the genetic map of agricultural species to be saturated in the near future. This holds great promise for useful applications, including: protection of breeder's rights, and more effective means for the characterization and manipulation of individual genetic loci affecting traits of economic importance.

INTRODUCTION

The study of segregational patterns of Mendelian genetic markers remains the most powerful route to the understanding of hereditary transmission. Application of this method to the detailed analysis of the genomes of many important agricultural species, as well as of man, has been limited by the paucity of available markers. Molecular biology has recently provided methodologies that enable the list of useful markers to be extended considerably. These methodologies are based upon the availability of molecularly cloned sequences that can be utilized to probe specific genomes for the presence of variation at the DNA level. Specifically, these variations are monitored as changes in the length of defined DNA fragments produced by digestion of the DNA sample with restriction endonucleases, and have therefore been termed 'Restriction Fragment Length Polymorphisms' (RFLP's) (GRODZICKER et al., 1974; BOTSTEIN et al., 1980).

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THE PROMISE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

RFLP markers have generated considerable excitement in human genetics by holding out the promise of saturating the human genome with polymorphic markers (JEFFREYS, 1979; MURRAY et al., 1984), by providing a means for prenatal diagnosis of genetic diseases (KAN & DOZY, 1978; KURNIT et al., 1982), and by allowing human genes to be more readily mapped (BOTSTEIN et al., 1980; SOLOMON & BODMER, 1978).

The purpose of this communication is to point out that RFLPs also hold great promise for useful application in agricultural plants and animals. This application is not in the area of prenatal diagnosis, which is of only marginal interest in farm animals, but rather in the areas of varietal identification, genetic analysis of economic traits and breeding methodologies (BECKMANN & SOLLER, 1983; BURR et al., 1983; SOLLER & BECKMANN, 1982 and 1983). This promise derives from the expected frequency of RFLPs (BECKMANN & SOLLER, 1983; BLATT, personnal communication; BURR et al., 1983; JEFFREYS, 1979; KIMURA, 1980; MURRAY et al., 1984), which should allow the genetic map of agricultural organisms to be saturated with polymorphic genetic markers. This promise also derives from the convenience of RFLPs for genetic analysis.

It should be stressed that the impact of molecular genetic engineering on genetic improvement is usally considered in unconventional terms of the molecular cloning of valuable genes and their subsequent reinsertion in the genome of a given cultivar, in this way generating superior breeds or varieties. In contrast, RFLPs are not concerned with restructuring genomes, but are service oriented, basically intended to provide additional, more sophisticated and discriminatory tools to the breeder, enabling him to proceed with increased efficiency via the conventional procedures.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

The demonstration of an RFLP involves extraction and purification of the DNA from an individual, and digestion of the DNA with a restriction endonuclease to form a mixture of restriction fragments, differing in length according to the specific distribution of cleavage sites along the DNA molecule (ZABEAU & ROBERTS, 1979). Genetic variation in DNA nucleotide sequence between individuals (i.e., polymorphism at the DNA level) means that the specific distribution of cleavage sites along their respective DNA molecules will also differ, resulting in a somewhat different mix of restriction products. The restriction fragments can be separated according to their length by gel electrophoresis (Fig. 1). It is not possible, however, to detect differences in the fragment length distribution pattern of eukaryotic DNA directly, since the great number of fragments formed produce a continous smear when separated along the gel. Instead, the fragments are transferred from the gel to a solid support, such as a nitrocellulose filter (SOUTHERN, 1975). Individual fragments can then be picked out from the mass by hybridizing them to an appropriately labelled DNA probe, homologous to the entire fragment, or some part of it (Fig. 1). Since DNA probes including a highly repetitive DNA sequence can be expected to hybridize with a large number of DNA fragments on the gel, giving a continuous smear on autoradiography, unique DNA sequences are generally used as probes in determining RFLPs. These can be obtained

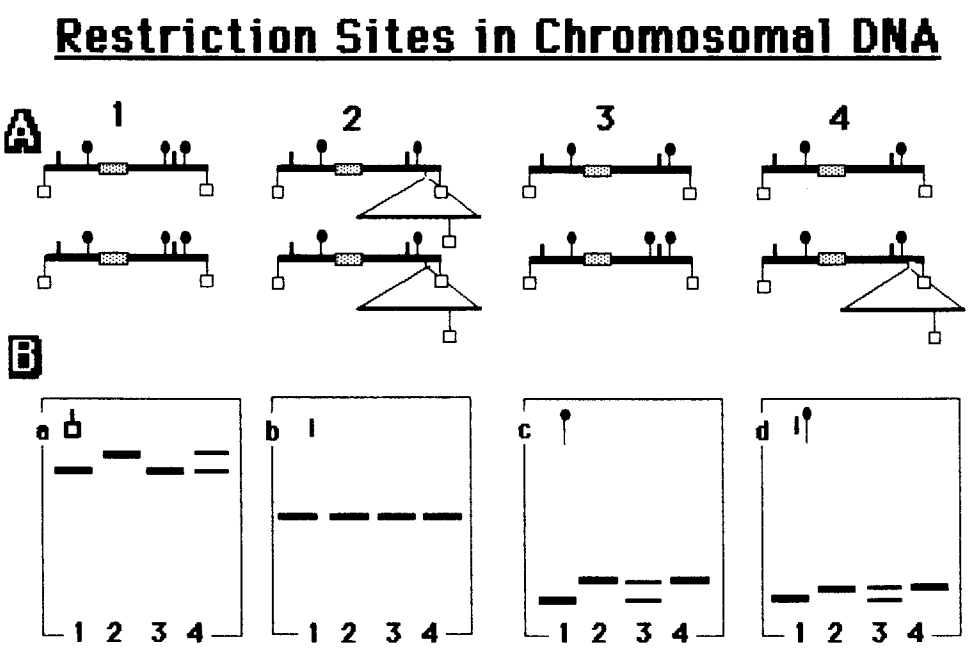

Fig. 1. The distribution of putative restriction sites (, &) in the vicinity of a specific probe DNA sequence (dotted box) in the case of four hypothetical diploid individuals (numbered 1 through 4, respectively). The individuals differ with respect to presence or absence of a particular polymorphic restriction site (indicated by) or insertion (indicated by). Individuals 1 and 2 are homozygous for the presence of the polymorphic restriction site and insertion, respectively. Individuals 3 and 4 are heterozygous for one of these events.

B. The results that would be obtained by autoradiography of genomic DNAs from these four individuals, after digestion with the indicated endonucleases (, &) and hybridization with the probe specific for the sequence in the dotted box.

by using cloned DNA of specific genes, cloned reverse transcribed DNA, by testing clones from a random DNA library for repetitive sequences retaining those that prove to be single copy DNA (WYMAN & WHITE, 1980), or by synthesis of specific oligonucleotides. The DNA fragment that hybridizes with the probe can be visualized by autoradiography, appearing as a band on the film (Fig. 1). If two individuals differ in a restriction site that affects the length of a particular DNA fragment homologous to the probe, the band will appear at a different location in their respective autoradiographs. In this way, a restriction site polymorphism at the DNA level is detected as a restriction fragment length polymorphism at the phenotypic level. Fig. 1 illustrates a hypothetical situation in which four diploid individuals are screened for RFLPs with a specific probe. The individuals are assumed to carry among themselves – in homozygous as well as heterozygous condition – two types of DNA variation, a point mutation abolishing an existing restriction site and an insertion. In both cases the mutation events lie outside of the sequence homologous to the probe. Banding patterns generated by these events are shown in the Figure. It can also be seen that DNA phenotypic variation caused by large rearrangement events shows up as RFLPs for all endonucleases that flank the probed sequence and the site of rearrangement, illustrating

Table 1. Properties of RFLPs as genetic markers.

Useful
- Ubiquitous
- Mendelian inheritance (Genomic RFLPs)
- Maternal inheritance (Organelle RFLPs)
- Stably inherited
- Codominant expression
- Multiple alleles for each RFLP
- Devoid of pleiotropic effects on economic characters

Convenient
- Detectable in all tissues
- Detectable at all ages (enabling early detection)
- Long shelf life of the DNA samples
- Informative about the nature of the variation

Numerous
- Virtually unlimited number of probe × enzyme combinations available
- Probes not restricted to coding sequences (random clones)
- Probes detect silent variations (introns, spacers, ...)
- Probes detect variations within hybridizing and flanking sequences

that RFLPs can be informative as to the nature of the genetic variation uncovered. It should be clear from the above that an RFLP heterozygote will show two discrete allelic bands, i.e., an RFLP will show a codominant mode of inheritance (BOTSTEIN et al., 1980; BURR et al., 1983; PETES & BOTSTEIN, 1977). In addition, RFLPs are expected to be relatively free of secondary effects on economic characters, abundant in multiple alleles (ANTONARAKIS et al., 1985; BECKMANN & SOLLER, 1983; BURR et al., 1983), and expressed at an early age and, with few exceptions, in all tissues (BECKMANN & SOLLER, 1983). Also, the longevity of the DNA samples and of filters prepared therefrom by far exceeds the lifetime expectancy of the individuals sampled, allowing retrospective and post-mortem studies to be carried out. Table 1 summarizes the main properties that can be expected to make RFLPs convenient and numerous genetic markers, useful at all levels of biological organization (e.g. from retroviruses and DNA viruses through higher eukaryotes).

Although the search for RFLPs is a relatively new field of investigation, these have now been documented for a variety of loci in numerous organisms, including man (25 RFLPs were documented in 1981, 159 in 1983 – SKOLNICK et al., 1984), various experimental organisms [mouse – (BENNETT et al., 1982; BLATT et al., 1983 and 1984), *Drosophila* (DAVIS & DAVIDSON, 1984), *C. elegans* (AQUADRO et al., 1984; ROSE et al., 1982), *X. laevis* (MARTENS & HERBERT, 1984)], a natural plant population *Calycadenia ciliosa* (SHEPHERD & EMERSON, 1984), and a variety of agricultural species including maize (BURR et al., 1983; RIVIN et al., 1983), tomatoes (BERNATZKY et al., 1984), peas (POLANS et al., 1984), soybean (LADIN et al., 1984), swine (CHARDON et al., 1985), chicken (CRITTENDEN, 1981; LAI et al., 1978) and cattle (BECKMANN et al., 1986). Studies by JEFFREYS (1979) and by MURRAY et al., (1984) suggest that there may be as many as 20000 detectable RFLPs in the human genome. An extensive analysis of the human beta-globin region, excluding thallassemic patients, have so far uncovered 17 different RFLPs (reviewed by ANTONARAKIS et al., 1985), indicative of the richness

of RFLPs in multiple allelic variants. In maize, a search for RFLPs in six inbred strains (BURR et al., 1983), using 18 random single copy cDNA probes and three restriction endonucleases, showed polymorphism with 16 of the probes, often including multiple allelic variants. Although these strains were chosen on the basis of maximal isozyme differences between them, the degree of RFLP polymorphism detected, exceeded by a considerable margin, that shown by the isozyme variants. In dairy cattle (BECKMANN et al., 1986) 68 bulls were examined, in an as yet incomplete survey for RFLPs, with 3 probes (specific for growth hormone, chymosin and muscle β-actin, respectively) and up to 4 enzymes. Despite the high degree of selection in the tested bull population, 8 variant individuals were recognized that exhibited one of three polymorphisms that were most probably expressed in a codominant heterozygous state. If these preliminary data on the Israeli Holstein-Friesian dairy cattle population can be considered as an indication of the extent of DNA variation over their entire genome, they would suggest that at least one in ten screened DNA bands is likely to behave as an RFLP. Thus, these initial studies support the notion that in most species, a comprehensive search will have good chances of uncovering numerous RFLPs. Indeed, in the mouse, BLATT et al. (pers. commun.) found 65 polymorphic sites using a battery of 100 cloned human genes and 10 restriction endonucleases.

VARIETAL IDENTIFICATION AND SCREENING OF GERMPLASM RESOURCES

As the number of identified RFLPs increases, they will add to the repertoire of genetic markers available to discriminate between strains and varieties of agricultural importance. Based on a priori calculations, for example, it appears likely that 20–30 RFLPs would be sufficient to differentiate all tomato or maize inbred strains (SOLLER & BECKMANN, 1983). If multiple allelic forms – as expected a priori (BECKMANN & SOLLER, 1983), and as found in preliminary studies (ANTONARAKIS et al., 1985; BURR et al., 1983; WYMAN & WHITE, 1980) – are common, even fewer RFLPs would suffice. Such RFLP profiles could become a powerful means for protecting and ensuring breeder's rights to novel varieties as they are produced (BARTON, 1982). In some cases probes hybridizing to repetitive DNA and giving multiple discrete bands on autoradiography, might be of particular usefullness (HELENTJARIS & GESTELAND, 1983; JEFFREYS et al., 1985), inasmuch as they can generate highly specific fingerprints for strain identification (or, in general, enable a number of polymorphisms to be monitored by a single probe). A special class of repetitive DNA, transposons (CALOS & MILLER, 1982), are of special interest in this context, since, in addition to serving as multiple probes, they may also serve themselves as a source of DNA polymorphisms (WYMAN & WHITE, 1980) and be a direct source of genetic variation in traits of economic importance. These repetitive RFLP probes might also prove to be a useful and sensitive tool in monitoring somaclonal variation, occurring in tissue-culture propagated cells (ORTON, 1983).

RFLPs may also provide a convenient means of assessing heterogeneity within and between stocks retained as germplasm resources. The assumption would be that heterogeneity for RFLPs is indicative of heterogeneity for other characters as well. This kind of screening might be one way to ensure that germplasm resources do indeed represent a wide range of genetic variability.

GENETIC MAPPING

As additional RFLPs are identified, it can be expected that mapping studies will be carried out, assigning them to linkage groups and to specific chromosomal positions and eventually saturating the entire chromosomal map (BOTSTEIN et al., 1980; SOLLER & BECKMANN, 1983; SOLOMON & BODMER, 1978). As evidenced by mouse (BLATT, pers. commun.) and human studies (SKOLNICK et al., 1984) this technique may be the fastest and most convenient way to generate genetic markers for mapping purposes, particularly for species that are relatively unexplored genetically. Even for well characterized agricultural species, such as maize (NEUFFER & COE, 1974), barley (MILAN, 1974) or tomato (RICK, 1974), RFLPs – because of their potentially large numbers and convenience – may become the genetic marker of choice whenever fine and precise mapping is required.

Assigning chromosomal map positions to a large number of markers is at present a long and tedious endeavor. In many agricultural species, however, this can be simplified by the use of specific tester strains harboring A/B translocations – as in maize (BECKETT, 1978; ROMAN & ULLSTRUP, 1951), the complete series of substitution lines or any series of aneuploids – as in wheat (GARCIA-OLMEDO et al., 1982; LAW & WORLAND, 1973; LONGWELL & SEARS, 1963; SEARS, 1953, 1954 and 1962), the appropriate interspecific somatic hybrid cell-lines (BENNETT et al., 1982; D'EUSTACHIO & RUDDLE, 1982), or a suitable set of recombinant inbred lines. Indeed, using the latter technique, considerable progress has been made in saturating the genetic map of the mouse (BLATT et al., 1983, 1984 and pers. commun.).

GENETIC ANALYSES AND IMPROVEMENT OF TRAITS OF ECONOMIC IMPORTANCE IN SELF-FERTILISING AGRICULTURAL SPECIES (SELFERS)

With the exception of specific disease resistances and morphological and color pattern traits which are often determined by allelic differences at one or two loci, genetic variation in most traits of agricultural importance (e.g., egg production, milk production, growth-rate, grain yield, general disease resistance) is attributed to allelic differences at a 'large' (> 5), but generally unknown number of loci having relatively small individual effects. These loci are termed quantitative trait loci. Genetic analysis and improvement of such traits is generally based on biometrical approaches that treat the overall global effect of all genetic loci and environmental factors affecting the traits in question. These biometrical approaches are, however, unable to identify, characterize or manipulate the specific loci involved on an individual basis (SIMMONDS, 1979).

THODAY and colleagues (SPICKETT & THODAY, 1966; THODAY, 1961), working with *Drosophila* and using special tester strains, have shown how polymorphic genetic markers can be used to locate and evaluate linked loci affecting a quantitative trait (in their case, bristle number). The basic design involves looking for differences in value of a quantitative trait associated with parental marker genotypes in the F_2 of a cross between two inbred lines differing in one or more marker loci. Such differences are indicative of linkage between the marker locus and one or more nearby loci affecting the quantitative trait. By use of maximum likelihood or other statistical techniques, the map distance and quantitative effect of the quantitative locus can be estimated

(SPICKETT & THODAY, 1966; TANKSLEY et al., 1982; WELLER, 1983). In this way, the overall difference between two lines in a quantitative trait can be analysed into components attributable to the effect of specific marker-linked chromosomal regions. Theoretical studies show that under most circumstances the majority of marker-linked quantitative effects should be due to no more than one or two quantitative loci in the near vicinity of the marker locus (SOLLER et al., 1979). Thus this approach provides a means for the genetic analysis of a quantitative genetic difference, at the level of specific quantitative trait loci. A similar approach can be utilised for genetic analysis of quantitative trait differences between inbred or semi-inbred lines within agricultural species; this would include those cultivated plants that reproduce by self-fertilization, e.g. wheat, tomato, coffee and cotton, but would not include species that reproduce by cross-fertilization, e.g. livestock, poultry and most fruit species. Theoretical (SOLLER et al., 1976) and field studies (GELDERMANN et al., 1985; PATTERSON et al., 1968; TANKSLEY et al., 1982; WELLER, 1983; ZHUSCHENKO et al., 1979) with agricultural species, have shown that relatively modest experiments, involving 1000–2000 individuals, can enable differences in the quantitative value of chromosomal regions adjoining a pair of alternative allelic markers differentiating two inbred lines to be determined. It is important to note that if two lines differ in many markers, well spaced along the genome, a single F_2 generation will allow a total analysis of the entire genome for all quantitative traits scored (SOLLER & BECKMANN, 1982 and 1983; TANKSLEY et al., 1982). Thus, this approach has great elegance and power when sufficient differentiating markers are available.

Crosses of this type can be used to screen germplasm resources (in the form of wild populations, land varieties or cultivars) and identify high-frequency chromosomal segments that can be potentially useful for the improvement of quantitative-economic traits in commercial varieties (WELLER, 1983). Furthermore, when commercial variety and 'resource' strain differ at a quantitative locus and at the adjoining marker locus, repeated backcrosses to the commercial strain, retaining only backcross progeny carrying the exotic marker allele, will allow the effective transfer (introgression) of the adjoining quantitative allele from 'resource' to commercial strain, particularly when quantitative trait locus and marker are closely linked (SOLLER & PLOTKIN-HAZAN, 1979).

Clearly, the more markers available to differentiate commercial and resource strains, the greater the proportion of the genome that can be compared, and the greater the likelihood of identifying a useful chromosome region and of precisely locating the quantitative locus within it. Similarly, it can be shown that the subsequent introgression procedure will be particularly effective when commercial and 'resource' strain differ at two nearby marker loci with the quantitative trait locus bracketed between them (SOLLER & PLOTKIN-HAZAN, 1979), or when unwanted 'resource' strain chromosomes also carry markers allowing marker-assisted selection against them in the backcross generations (TANKSLEY et al., 1981). Maximum benefit from these procedures will become possible, therefore, as large numbers of differentiating markers become available.

GENETIC ANALYSIS AND IMPROVEMENT OF HYBRID VIGOR IN CROSSES BETWEEN INBRED LINES

The genetic analysis described above can also be used to identify specific marker-linked segments or brackets showing dominance or overdominance (i.e., superiority of the heterozygote) in the F_2 of a cross between two inbred lines (SOLLER & BECKMANN, 1982 and 1983), as in hybrid corn. This would enable an existing hybrid to be analyzed in terms of the number and location of chromosomal regions involved in producing the observed hybrid vigor (heterosis). 'Resource' inbred lines could then be crossed to the commercial inbreds and the F_2 progeny analyzed for additional regions showing dominance or overdominance. These could then be accumulated in the alternative commercial inbred by marker-assisted introgression methods (SOLLER & PLOTKIN-HAZAN, 1979; TANKSLEY et al., 1981) as described above. In this way, increasing degrees of heterosis could be built up in an existing hybrid. Similarly, marker analysis of quantitative trait loci differences between two improved cultivars could enable the planned construction of a superior recombinant cultivar, including effects dependent on interactions between loci, through marker-assisted identification and manipulation of the desired chromosome regions (SOLLER & BECKMANN, 1982 and 1983; WELLER & SOLLER, 1981). This may prove more effective than current methods based on production and testing of many hundreds of recombinant inbred lines produced by selfing or by diploidization of haploid gametes (ALLARD, 1960; SIMMONDS, 1979).

GENETIC ANALYSIS AND IMPROVEMENT IN CROSS-FERTILIZING SPECIES (OUTCROSSERS)

The use of polymorphic genetic markers for genetic analysis and genetic improvement in outcrossers is more problematic than in selfers or inbred lines (BECKMANN & SOLLER, 1983; SOLLER, 1978; SOLLER & BECKMANN, 1983). The reason for this is that in outcrossers, alternative alleles for the marker and quantitative locus will be in linkage equilibrium, so that the specific quantitative allele associated with any particular marker allele will be different in different chromosomes (SOLLER & GENIZI, 1978). That is, in some cases a quantitative allele of positive effect will be associated with a specific marker allele, while in other cases the opposite may be true. Even in this case, however, it can be shown that if sufficient markers become available, it would become possible to utilize them for analysis of genetic 'resource' populations, for identification and introgression of useful chromosome regions, and as an important auxillary for within-strain selection (BECKMANN & SOLLER, 1983; SOLLER & BECKMANN, 1983). This is derived from the fact that a well marked chromosomal region (e.g. a 20 centimorgan segment, delimited by specific marker alleles at each end, and containing one or more internal specific marker alleles) can be followed from generation to generation almost as readily as a single rare allele in a multiple allelic series. In this way, information on the quantitative economic value of the marked segment can be accumulated and rendered progressively more precise. Such an evaluated and marker-defined segment can then be treated in breeding programs as a single complex 'allele', allowing for selection for the quantitative economic trait early in the life cycle, when large numbers of offspring can be raised and selection can be correspondingly intense. This can make a significant contribution to genetic improvement (SOLLER, 1978). For

example, under some circumstances, marker-assisted evaluation and selection of young sires prior to their entry into progeny testing programs, could virtually double the rate of genetic progress for milk production in dairy cattle populations (BECKMANN & SOLLER, 1983; SOLLER & BECKMANN, 1983). Similar increases might also be obtained in fruit tree breeding, by marker-assisted evaluation and selection of seedlings prior to growing them to maturity.

CONCLUSIONS

It is our belief that the advent of RFLPs, by greatly increasing the total number of polymorphic genetic markers available to the agricultural community, may signal the advent of a new and promising era for the understanding and genetic improvement of quantitative economic traits through the use of the marker-assisted breeding methodologies described above. Based on findings with enzyme polymorphisms (BROWN, 1979; NEVO et al., 1979; RICK & FOBES, 1975) and on the initial RFLP studies cited above (e.g. BECKMANN et al., 1986; BERNATZKY et al., 1984; BURR et al., 1983; CHARDON et al., 1985; CRITTENDEN, 1981; LADIN et al., 1984; LAI et al., 1979; POLANS et al., 1984; RIVIN et al., 1983), it can be anticipated that numerous RFLPs will be found segregating in wild populations of the major agricultural species, and that numerous RFLPs differentiating such populations from typical cultivars will be found. This will allow investigation of wild populations as a source of useful quantitative trait alleles and introgression of such alleles from wild populations to cultivars. For full usefulness, however, RFLPs differentiating improved cultivar strains from one another will have to be numberous as well. Only thorough investigation of the major agricultural species for the presence of RFLPs can establish this point.

However, even if, in some instances, naturally occuring RFLPs are not detected, it may be possible in the near future to produce novel RFLPs artificially. Recent advances in recombinant DNA technology, allow the random, or quasi-random insertion of defined DNA sequences into the eukaryotic genome. These techniques have already allowed the production of numerous 'transgenic' individuals in mice (e.g. GORDON & RUDDLE, 1981; PALMITER et al., 1983 and 1984), *Drosophila* (e.g. SPRADLING & RUBIN, 1983) and a variety of dicotyledenous plants (e.g. BEVAN & CHILTON, 1982; HOOYKAAS & SCHILPEROORT, 1984; URSIC et al., 1983). Each such insertion will result in a change in the local distribution of restriction sites at the point of insertion, thereby creating novel RFLPs. Thus, a randomly distributed set of insertions would produce a corresponding set of randomly distributed RFLPs. These would be detectable by virtually any restriction endonuclease, using the inserted sequence itself, or the corresponding uninterrupted homologue at the site of insertion, as a probe. An immediately useful application of such inserts could lie in the area of designed protection of breeder's rights. If the inserts were also designed to contain an easily scorable gene (e.g. a resistance trait), the latter trait could serve as a convenient screen for the presence of the insert. In some instances, insertions in the vicinity or within defined genes, will affect the normal expression of these genes. Such insertional mutagenesis events (SCHNIEKE et al., 1983) affecting economically important traits, may allow the molecular cloning of the affected loci and of their normal homologues. Appropriately designed experiments of this sort, carried out in a systematic manner, could play a major

role in the cloning of QTL (BECKMANN & SOLLER, 1985; SOLLER & BECKMANN, 1985).

If numerous (natural or artificial) RFLPs distinguishing strains within cultivars are found, we foresee no serious problems in mapping them, using the standard techniques referred to above, or in their use in varietal identification. There are a number of additional requirements that must be met, however, if RFLPs (or any other class of markers) are to make a positive contribution to more advanced breeding programs. These include the requirement that (i) quantitative traits loci having effects large enough to be detected by linkage analysis, are present and are not closely linked to deleterious alleles for the same or some other trait; (ii) RFLPs differentiating strains of agricultural interest are located near the quantitative trait loci differentiating these strains; and (iii) effects of quantitative trait loci are basically additive, i.e., relatively unaffected by genotype × environment interactions and relatively independent of the genetic background in which they are expressed. This last condition is necessary if quantitative trait alleles identified as being potentially useful on the basis of tests in a small number of years and locations, and in the highly heterogeneous and heterozygous F_2 populations generated for analytical purposes, are to retain their effects in highly homozygous and uniform cultivar populations grown over many years and locations.

If these basic biological conditions are present, initial estimates for a variety of RFLP applications, based on current methodologies, indicate that costs are commensurate with expected returns (BECKMANN & SOLLER, 1983). Furthermore, given the very wide application of RFLPs to biological sciences and medicine, it can be expected that methods will be simplified and costs reduced in the near future. An exciting development along these lines has been the recent discovery of hypervariable multiply-dispersed regions in the human genome: JEFFREYS et al., (1985) have reported a probe of this nature in man, homologous to the generalized recombinational signal of bacteria. This probe was able to simultaneously detect many highly variable loci. JEFFREYS et al., (1985) indicate that the same probe might exhibit similar characteristics across a wide variety of species and suggest that additional probes of this nature remain to be uncovered. Similarly, transposons can be expected to generate polymorphisms at multiple loci within the genome. These could be detected by use of transposon sequences as probes. Indeed, endogeneous viruses exhibit multiple highly polymorphic bands in chicken (CRITTENDEN, 1981) and mouse (COHEN & VARMUS, 1979). Transposons also show multiple and highly polymorphic patterns in corn (FEDEROFF, 1983; MCCLINTOCK, 1984). Conceivably, a small number of probes, each detecting polymorphisms at a large number of highly polymorphic sites, could allow complete genome coverage to be obtained with a small number of probe × enzyme combinations. Mapping by means of such probes could reduce costs by an order of magnitude or more. Further developments are the finding that filters can be routinely reused for as many as 10 hybridizations, and progress is being made towards the development of filters that may be reused virtually indefinitely. Work is also actively in progress on the development of highly sensitive nonradioactive probes [biotin- (FORSTER et al., 1985) or enzyme- (RENZ & KURZ, 1984) labelling of DNA sequences] for the detection of unique sequences in total genomic blots. All of these suggest that an appreciable reduction in the costs of large scale screening of DNA samples for RFLPs can be expected in the near future. Detailed comparative cost/benefit analyses are now needed

over the entire gamut of potential marker-assisted breeding methodologies, in order to define those circumstances under which these methodologies may prove superior to alternative means of achieving particular breeding goals.

Thus, the challenge is now for the agricultural geneticist to identify and map these marker polymorphisms in agricultural species; for the biometrical community to develop experimental designs and data analyses for the efficient utilization of marker polymorphisms to characterize and map quantitative trait loci affecting traits of economic interest, and to design breeding methods for the effective manipulation of quantitative loci by way of associated markers; and for the geneticist and biometrician together to carry out the initial analytical studies utilizing these methods to map and characterize quantitative trait loci in agricultural species, and to carry out the detailed simulations of the costs and gains expected from marker-assisted breeding methodologies as compared with current breeding procedures. An initial attempt in this direction will be published elsewhere (HALLERMAN et al., 1985). Depending on the results of these studies, the way will be open to the breeder to implement the marker-assisted breeding methodologies described here in genetic improvement programs.

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