



"Near-isogenic line" revisited by DNA markers in maize

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Abstract. Restriction fragment length polymorphism (RFLP) markers were used to estimate recurrent genome recovery in backcross conversions of corn inbred A632 to two dominant disease resistance factors, *Ht1* and *Rp1*. Neither conversion was found to reach the theoretical value (99.2%) for recovery of the recurrent genome at the BC₆ generation. Observed recurrent genome recoveries, based on analysis of 156 DNA markers, were 89 and 87% respectively, for the *Ht1* and *Rp1* conversions. Substantial chromosomal segments which have no known effect on either trait were retained in both conversions, and several of these segments were shared by the two converted lines. RFLP DNA markers can provide a valuable adjunct to the backcross procedure by enabling accurate assessment of individual's genomic constitutions.

Key words: Backcross; Breeding; Corn; DNA; Linkage; RFLP.

Introduction

Backcross conversion is a routine plant breeding procedure used to move individual traits into established inbreds. After sufficient cycles of backcrossing the converted lines are considered to be near-isogenic lines (NILs) with respect to the recurrent parent. The theoretical rate of recovery of the recurrent genome during backcrossing is $1 - [(0.5)/n + 1]$ (Fisher, 1949), where n equals the number of backcross generations, for regions not linked to the desired non-recurrent genes. Theoretically, six backcrosses followed by selfing should produce a progeny containing about 99.2% of the recurrent genome. Bartlett and Haldane (1935) give the mean length of a chromosome segment introduced by backcrossing as $(2/n) \times 100$ centimorgans (cM) after n cycles. However, non-recurrent parental DNA segments surrounding selected loci are eliminated more slowly than unlinked chromosomal regions. Fisher (1949) calculated that the average length of a heterozygous segment surrounding a

selected dominant locus decays asymptotically to $2/n$ cM. Muehlbauer *et al.* (1988) calculated that about 4 out of 100 molecular markers from a donor parent would be retained in a BC₅S₁ NIL model species with 20 chromosomes of equivalent 50 cM lengths, the majority being on the chromosome bearing the introgressed locus. Chromosomal segments thus "dragged" into the genotype of the recurrent parent may undermine the recovery of the line's potential (Brinkman and Frey, 1977).

The fidelity of recurrent genome recovery in backcross breeding programs has customarily been judged by the plant's morphological appearance, performance, and combining ability patterns. Accurate determination of the actual genetic recovery has been limited due to the insufficiency of "conventional" genetic markers. RFLP-based linkage maps now permit assessment of the genetic composition of individuals at a level of detail not previously possible. For example, using RFLP markers, Young and Tanksley (1989) found introgressed segments flanking the *Tm-2* gene varying in size from 4 to 51 cM in various NILs of tomato. Similarly, in a study of maize *Ht1* NILs, Gardiner *et al.* (1989) found a particular form of the chro-

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mosome 2L RFLP marker, UMC122, to be correlated with the presence of *Ht1* in five of the six converted lines. And in line B37*Ht1*, all of chromosome 2 originated from the *Ht1* donor. I herein report the comparisons made among maize NILs A632, A632*Ht1*, and A632*Rp1*, using RFLP DNA markers to assess their isogenicity and the possible implications on plant breeding theory and gene mapping.

Materials and Methods

Plant Material

Seeds of corn inbreds A632, A632*Rp1*, and A632*Ht1* were obtained from Jacques Seed Co. (Prescott, WIS). A highly inbred Iowa version of A632*Rp1* designated as A632*Rp1*-Iowa was obtained from the Committee for Agriculture Development, Iowa State University. A632*Rp1* was selected through six backcrosses from a cross between A632 and Cuzco variety (donor of *Rp1d*, resistance to *Puccinia sorghi*, race 1). A632*Ht1* was selected similarly from a cross between A632 and GE440 (donor of *Ht1*, resistance to *Helminthosporium turcicum* Pass. race 1) (A. L. Hooker, personal communication).

RFLP Analysis

DNA was extracted from bulked husk tissues obtained from greenhouse-grown plant. Total genomic DNA from each corn line was digested with three restriction enzymes (*EcoRI*, *EcoRV*, and *DraI*) and the genomic Southern blots were made with nylon base membrane (Southern, 1975). Probes were cloned into transcriptional vectors, pGEM-2 or pGEM-3, and the genomic Southern bands were detected with high specific activity single stranded RNA transcripts using the riboprobe system (Promega).

Helminthosporium turcicum Inoculation

Helminthosporium turcicum race 1 and 2 were assayed for virulence on inbreds carrying the *Ht1* resistance gene. Lesioned leaf tissues infected with defined

races of the pathogen were cultured on Difco potato-dextrose-agar (PDA) to produce sufficient inoculum. Seedling were inoculated by pipetting 10 μ l of spore suspension (10^6 /ml) on fourth and fifth leaf blades. Disease symptoms were scored 10 to 14 days after inoculation. Susceptible lesions were characterized by wilting and grey discoloration of leaf tissue at the inoculation site. Resistance (presence of *Ht1*) manifest as a chlorotic response to the inoculum and lacked the grey necrotic wilt. Field inoculation was performed by spraying the spore suspension into the whorl twice at one week intervals in the middle of growing season. Disease symptoms were rated three times at two week intervals after the last inoculation.

Puccinia sorghi Inoculation

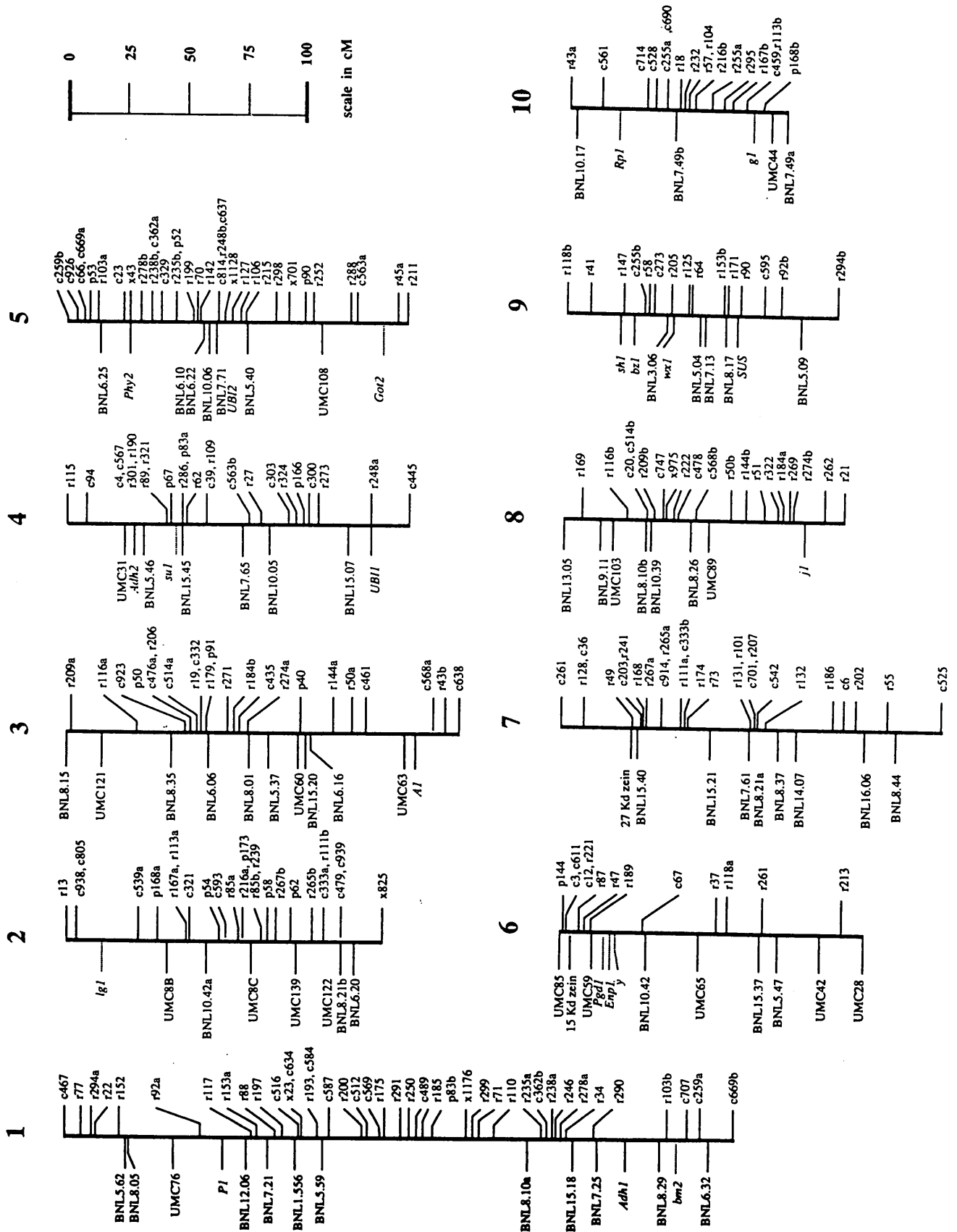
Corn rust spores (*Puccinia sorghi* race 1D; courtesy of A. Ellingboe) were scraped from infected, greenhouse-grown sweet corn leaves (cv. Florida sweet), suspended into mineral oil and brushed on leaves in the field. Rust symptoms were evident on inoculated areas within two weeks. Presence of *Rp1* was clear and unambiguous from the reaction to the inoculation.

Results and Discussions

The RFLP Linkage Map

The current Agrigenetics RFLP linkage map is an updated version of 1988 (Murray *et al.*, 1988) and consists of 327 independent loci (Fig. 1). Twenty-three of these are classical morphological markers, isozyme, or specific cloned genes obtained from other laboratories. Most of the DNA markers (231) are Agrigenetics clones obtained from two different cDNA and two genomic libraries. Fifty-six markers from Brookhaven National Laboratory (Burr *et al.*, 1988) and 17 from the University of Missouri-Columbia (courtesy of Ed Coe and D. Hoisington) have been placed on this linkage map. Table 1 summarizes the total number of mapped DNA markers on each chromosome and those used in NIL comparisons.

Fig. 1. The Agrigenetics RFLP linkage map 1989 version. Agrigenetics clones are shown on the right side of each chromosome. Established genetics markers and clones obtained from other researchers are shown on the left. Codes are: BNL=Brookhaven National Laboratory; UMC=University of Missouri-Columbia; c=coleoptile cDNA clones; r=root cDNA clones; p=*PstI* genomic clones; x=*XhoI* genomic clones; a or b=loci recognized by duplicated sequences. Genes of known identity obtained from other researchers are: *A1*=S. Schwarz-Sommer; *Adh1* and *Adh2*=E. Dennis; *bz1*=O. E. Nelson; *PI*=T. Peterson; *sh1* and *SUS*=L. C. Hannah; *Ubl1* and *UBI2*=A. Christensen; 15 Kd zein=B. Larkins; and 27 Kd zein=A. Esen.



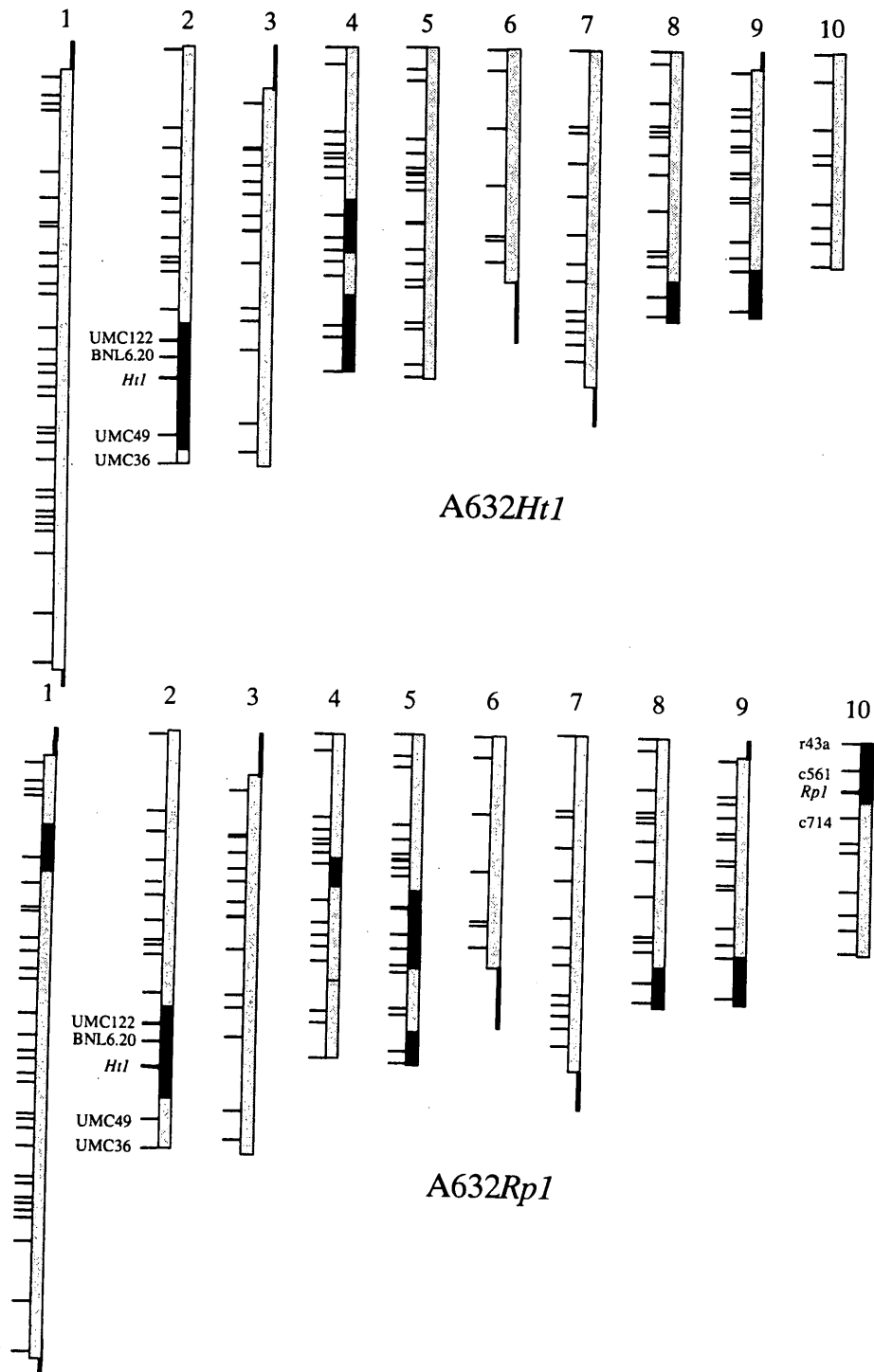


Fig. 2. The genome mosaic patterns of two A632 NILs. The scales of all chromosomes in this figure are proportioned to the correspondent chromosomal length of figure 1. The genomes of two inbreds, A632Ht1 and A632Rp1, together with their normal counterpart, A632, checked by 156 DNA markers were based on markers' location from Fig. 1. Recurrent parent (A632) chromosomal segments are shown by shadowed bars and the non-A632 segments by solid bars. Thin bars indicate chromosome segments for which RFLP type has not yet been determined. Horizontal lines indicate the positions of DNA markers used. The RFLP linkage mapping of two dominant disease resistant genes, *Ht1* and *Rp1*, are indicated by their flanking clones.

Table 1. Distribution of DNA markers on the Agrigenetics RFLP linkage map

Chromosome number	Classical map units*	RFLP units**	DNA markers mapped	NIL vs A632
1	176	281	54	31
2	155	134	31	17
3	167	172	34	15
4	137	147	31	16
5	107	147	41	20
6	78	104	21	7
7	112	163	34	13
8	42	136	26	14
9	141	117	24	14
10	95	91	21	9
Total	1202	1492	317 ^a	156

*Maize Genetics Cooperation Newsletter No. 64 (1990), p. 154-163. Edited by Ed. Coe.

**Agrigenetics RFLP linkage map, 1989 version.

^aTen morphological and isozyme loci are not included in this figure.

Ht1 Locus RFLP Mapping

Hooker (1963) mapped corn *Ht1* gene on chromosome 2L-121. Using six corn *Ht1* conversion lines, Gardiner *et al.* (1989) located *Ht1* on a segment of chromosome 2L flanked by UMC122. To obtain a more precise map location, 58 F₂ individuals from a cross of A632 × A632*Ht1* were analyzed with several chromosome 2L RFLP DNA markers. Susceptibility of each F₂ individual to *H. turcicum* race 1 was determined by F₃ progeny tests. Detailed linkage analysis placed the *Ht1* locus 20.9 ± 5.1 cM distal to BNL6.20, and 23.5 ± 4.6 cM proximal to UMC49 (Fig. 2). Clone x825 did not show polymorphism between the parents with six different restriction enzymes (*EcoRI*, *EcoRV*, *DraI*, *BamHI*, *HinDIII*, and *BglII*) and therefore was unable to mapped relative to *Ht1* locus in this cross.

Rp1 Locus RFLP Mapping

The *Rp1* locus has been mapped to the tip of chromosome 10S (Rhoades, 1935). Two populations which were segregating for *Rp1* were examined to establish linkage distance to mapped RFLP markers (43 F₂ individuals derived from A635*Rp1* × A641*Ht1*, and 104 BC₁ individuals derived from ND246 × A635*Rp1*). Plants were field-grown and second ear husk tissue harvested for DNA analysis prior to disease inoculation and

screening. Linkage analysis placed *Rp1* 11.5 ± 4.7 cM proximal to probe c561 and 20.2 ± 5.2 cM proximal to probe r43a and more than 20 cM distal to probe c714 (Fig. 1).

RFLP Analysis of A632*Ht1* and A632*Rp1* NILs

DNA samples from the inbred lines were digested separately with *DraI*, *EcoRI*, and *EcoRV* prior to Southern blotting. Since DNA samples from the original trait donor lines (i.e. GE440 and Cuzco) were not available, only the A632 type Southern blot bands can be defined. Blots were hybridized separately with 156 different RFLP markers for both the *Ht1* and *Rp1* conversions (Table 1). Loci were considered identical if a polymorphism was not detected with any of the three restriction endonucleases. When polymorphisms were revealed, they were detected by all three enzymes in 98% of the comparisons made. Consequently, it unlikely that analysis of DNA digested with additional restriction enzymes would have provided more information. Boundaries between the recurrent and nonrecurrent segments were arbitrarily placed midway between recombined markers (see Fig. 2).

Based on data resulting from 156 probes, it has found that the A632*Ht1* conversion has a 50 cM non-A632 segment of chromosome 2L which representing approximately 3.5% of the total genome (based on

approximate length of this segment divided by total map units in Fig. 1). Additional non-A632 segments on chromosomes 4L, 8L, and 9L account for approximately 7.5% of the genome (Fig. 2). The estimated recurrent genome recovery is therefore 89% in this conversion.

The A632*Rp1* conversion shows a 30 cM (approximately 2% of genome) non-A632 segment on the tip of 10S (Fig. 2). Additional non-A632 segments account for approximately 11% of the genome, were found on chromosomes 1S, 2L, 4S, 5L, 8L, and 9L. The recurrent genome recovery is estimated at 87% in this line. Non-recurrent segments on chromosomes 1, 4, and 5 of A632*Rp1* were not the same as seen in A632*Ht1*, however it is notable that both A632*Ht1* and A632*Rp1* showed the same RFLP patterns in the shared non-recurrent regions of 2L, 8L, and 9L. The non-recurrent segments of chromosome 2L from both lines varied in size but both covered the region believed to carry *Ht1*, suggesting that this particular version of A632*Rp1* may also carry *Ht1*. Indeed, inoculation with *H. turcicum* races 1 and 2 showed that this A632*Rp1* (from Jacques Seeds Co.) was resistant whereas the A632*Rp1*-Iowa version was not resistant. When the genomic DNA from the A632*Rp1*-Iowa line was probed with RFLP markers BNL6.20, UMC122, and UMC49, a non-recurrent segment was found only around UMC122 (Fig. 3).

Theoretical Value of Recurrent Genome Recovery and Breeding Considerations

In both of the *Ht1* and *Rp1* converted lines, RFLP analysis showed more donor genome retained than predicted by theory and no introgressed fragment was determined to be shorter than 10 cM. Although the average proportion of recurrent genome in a population of BC₆ individuals is expected to approximately 99.2%, sampling variance in an applied program tends to override theoretical expectations. Moreover, breeders have previously not had the means to select individuals in which auspicious recombination events have occurred that minimize linkage drag (i.e. the tendency of chromosomal regions flanking a selected locus to persist through backcross generations, Brinkman and Frey, 1977). Conversion of A632 to A632*Ht1* dragged 50 cM of nonrecurrent genome around *Ht1* on 2L, and conversion to A632*Rp1* dragged at least 30 cM around *Rp1* on 10S. In areas unlinked to the desired donor locus, retention of the non-recurrent genome may be a chance event or may indicate a selective con-

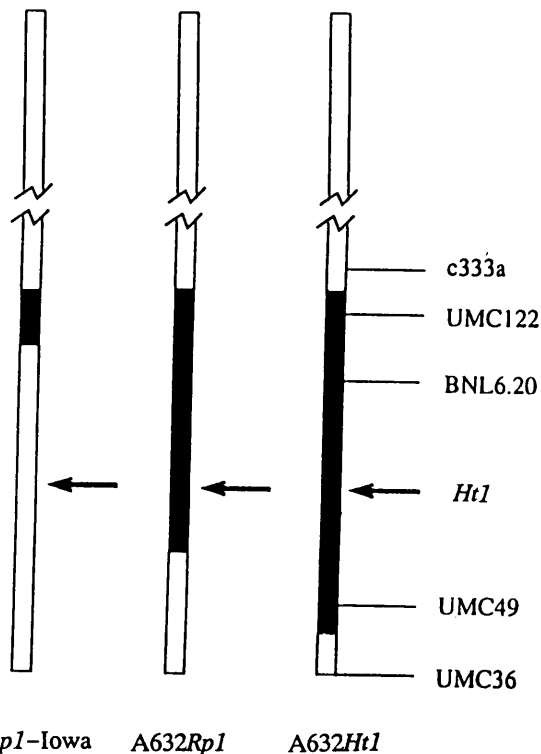


Fig. 3. RFLP-profiling of chromosome 2L of three A632 NILs. Recurrent parent (A632) chromosomal segments are shown by white bars and non-A632 segments by solid bars. Arrows indicate *Ht1* locus.

tribution of genes in these regions which caused the breeder to prefer these particular genotypes in the segregating generations. Using RFLP technology to analyze progeny, breeders can now readily assess the fidelity of recurrent genome recovery and select those individuals having minimal linkage drag in regions surrounding the desired genes. Precision will depend on the density of marker saturation in the vicinity of the donated loci. RFLP-profiling to select the optimum individual(s) can be initiated at BC₁ and repeated at each ensuing backcross until the desired genome constitution is achieved.

Implications for Gene Mapping

Muehlbauer *et al.* (1988) suggested that RFLP comparisons of many isogenic lines in soybean could be an effective means for gene mapping. In theory, only the target gene region should be retained due to selection pressure. Using the same approach Gardiner *et al.* (1989) have localized *Ht1* relative to several RFLP markers on chromosome 2L, and they observed sub-

stantial variation in the size of introgressed segments among 6 lines examined. Similarly, Young and Tanksley (1989) found introgressed segments surrounding the *Tm-2* locus in tomato ranging in size from 4 to 51 cM. Both studies involved genes where there was substantial prior knowledge as to chromosomal location, and neither considered what (if any) donor genome was retained elsewhere in the genome. The data presented here show that uncovered chromosomal segments can persist in presumptive near isogenic lines of corn. All of these observations suggest that gene mapping via examination of NILs could be misleading and should therefore be only the first step in mapping. To be reasonably effective, many independent NILs for the given trait would have to be examined to rule out spurious results due to incomplete conversion as illustrated here. In the practical sense large numbers of isogenic conversions are generally available only for traits controlled by single genes of known location. Thus, determination of linkage between a given gene or genes and closely linked RFLP markers is best accomplished by the use of an appropriately-sized, segregating population.

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以 DNA 標記再探討玉米之回交育種

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本文重點是以限制酶切開的玉米 DNA 片段做為探針，重新探討遺傳及育種上一個假說——即所謂的 Near-Isogenic Line (NIL) 基因組的實際還原率是否合乎理論值。本實驗用 156 個 DNA 探針，以南氏印 (Southern blotting) 方法，觀察一個玉米自交系及其二個含抗病基因的回交六代後選得的回交品系。發現此二回交品系僅有原先品系的 87 到 89% 的基因組，而非理論值的 99.2%。此點發現也同時印證育種者的多年經驗，即以回交法引入抗病基到一優良品系後，雖能增加抗某一病害的能力，但其他不良性狀亦隨之而來且很難在育種過程中除去，即所謂的連鎖拖累 (Linkage Drag)。本文討論其他類似的研究工作及用 NIL 做為快速鑑定重要基因與某些 DNA 片段連鎖關係的問題。