

CHARACTERIZATION OF D-GENOME REPEATED DNA
CLONES FROM AEGILOPS SQUARROSA

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LITERATURE REVIEW

Common wheat or Triticum aestivum L. cv. Chinese Spring is an allohexaploid (AABBDD) derived from the hybridization of T. turgidum (AABB) and Aegilops squarrosa (DD). A. squarrosa is a very important contributor as it carries many desirable qualities such as resistance to leaf rust, powdery mildew, greenbug, and Hessian fly (Gill et al., 1986), increased salt tolerance (Shah et al., 1987), and cold hardiness (Limin and Fowler, 1981). Although A, B, and D chromosome groups are homoeologous, there is a large amount of variation in the location and organization of repeated DNA sequences between the genomes. Even though nulli-tetrasomic chromosome complements will compensate, compensation is not always complete and reduced vigor and fertility can occur (Sears, 1966). Gerlach and Peacock (1980) have proposed that although these differences may be due in part to differences in homoeologous genes, variations in repeated DNA may also be a factor. Appels and Moran (1984) suggested that the 'micro-environment' of a gene can change its expression. Repeated DNA sequences flanking genes could be one way to alter this environment.

Analysis of the wheat genome by Smith and Flavell (1975) with renaturation kinetics has estimated 4-10% of the DNA is highly repetitive, 80% is intermediate, and 12% is single copy. However, the 12% figure is thought to be an overestimate as part of this fraction may represent

fragments of highly diverged repeats. Large amounts of repeated DNA are found in higher plants which possess a high DNA content. This is suggested to have occurred by sequence amplification, polyploidy, and duplication of chromosomes (Flavell et al., 1974).

The formation of a repeated sequence family as theorized by Britten and Kohne (1968) occurs by the replication of the sequence which is then integrated into a chromosome and associates with a favorable genetic element. The sequence is then dispersed into other species through natural selection. The sequence can amplify at any time and then diverge through mutations, translocations, and/or deletions. Amplifications and deletions can be a result of unequal crossing over or intrastrand recombination (Jones and Flavell, 1982b). Repeats can vary in size from 2 to 5,000 base pairs with the size of the repeated family ranging from few to several million, and there is 6-10% heterogeneity within a family sequence. A sequence family usually diverges slowly and independently of other families. Results of renaturation studies show that short repeats are highly mismatched alluding to a large amount of divergence with an organization similar to multigene families, whereas long repeats show more homology and are probably of more recent origin or more conserved.

Although most repetitive DNA functions are unknown, known functions of these sequences include coding for gene

sequences of ribosomal DNA and histones. In addition, centromeres and heterochromatin are largely composed of repeated DNA suggesting a structural function (John and Miklos, 1979). However, most repeated DNA roles remain undefined and are proposed to be involved in evolutionary mechanisms and speciation or regulation (Hake and Walbot, 1980). Britten and Davidson (1969) proposed a regulatory function for repeated DNA, as single copy DNA is usually interspersed with repeated sequences.

In previous years studies of repeated DNA or DNA sequences in general used satellite DNA or mechanically sheared DNA. With the advent of cloning techniques to incorporate sequences into phage or bacteria, more sequences have been purified for use in research. This advancement allows for greater resolution using techniques like Southern blot and in situ hybridization analysis.

Differences in wheat heterochromatin can be detected with N- and C-banding. Based on this, the heterochromatin of wheat chromosomes can be described as C^+N^+ and C^+N^- . Repeated DNA sequences have been used for further characterization of heterochromatin of wheat chromosomes. It has been found that a simple satellite sequence DNA is associated with C^+N^+ type of heterochromatin (Gill, 1987).

Teoh et al. (1983) isolated a probe (TC22b) which results in situ hybridization patterns which resemble C-banding. Centromeric heterochromatin appeared to be an

exception as the amount of heterochromatin was not correlated with labeling of TC22b. TC22b was used to determine the variation between different ploidy levels of Aegilops species. Comparing C-banding and in situ, they discovered species with reduced amounts of heterochromatin. These results suggest the C-banded heterochromatin is probably composed of several repeated families and the TC22b sequence has been amplified and deleted during Aegilops evolution.

Increasing the number of chromosome segments which can be identified independently allows for greater use of techniques to trace the introgression of DNA segments when transferring desirable characteristics into agronomically useful varieties. Lapitan et al. (1986) detected wheat-rye translocations using in situ hybridization with biotin labeling of a rye dispersed, repeated DNA (pSC119). This DNA is present at a few localized sites in wheat chromosomes. These differential patterns of hybridization were used to identify rye chromosomes in wheat-rye hybrids and wheat-rye translocations. Moreover, breakage points in chromosomes could also be determined. Thus, these types of probes are useful for tracing the introgression of alien chromatin in wheat.

Koebner et al. (1986) found a recombinant phenotype in which homoeologous pairing was suppressed in wheat-rye crosses. The plant showed protein markers for 1RL yet

lacked some terminal C-banded heterochromatin. When analyzed with probes from the 350 bp family of rye, it was realized that part of the sequence was still present but in reduced amounts. Due to the presence of rye protein markers and other DNA sequences associated with telomeric heterochromatin it appeared that no recombination had taken place, only a loss of heterochromatin. Since this family is thought to be of recent evolution and in tandem repeats (Jones and Flavell, 1982a), Koebner et al. suggest that the deletion has occurred through unequal sister chromatid exchange.

Flavell et al. (1979) reported from renaturation studies that a significant amount of highly repeated DNA in Chinese Spring appears to be from the B genome with the A and D genomes contributing lesser amounts. Moreover, 11% of the Chinese Spring genome contains repeated sequences not found in T. monococcum. Flavell and Smith (1976) also reported the discovery of repeated sequences in T. monococcum which were not found in A. speltoides or A. squarrosa, and sequences present in A. speltoides that were not found in A. squarrosa or T. monococcum. These results verify the existence of genome-specific sequences.

Gerlach and Peacock (1980) isolated a repeated probe from Chinese Spring which hybridizes strongly to T. dicoccoides and Chinese Spring but very lightly to A. squarrosa and T. monococcum showing it is present

predominantly in the B genome. Gerlach et al. (1978) also isolated satellite DNA from Chinese spring which labels all B genome chromosomes in addition to two A genome chromosomes.

A D-genome clone isolated by Rayburn and Gill (1986) was recorded as D-genome specific at the in situ level since it labeled the telomeres of 14 chromosomes in Chinese Spring heavily. However, further studies indicate it does not hybridize exclusively to the D-genome because sites of less intense hybridization are seen on some of the remaining 28 chromosomes (Henry, unpublished results; Gill and Sears, 1988).

Due to the many desirable genes in the D genome and an insufficient number of markers for that genome, we set out to characterize D-genome derived clones to elucidate new chromosome and molecular markers through the implementation of molecular and cytological techniques.

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INTRODUCTION

Common wheat or Triticum aestivum L. cv. Chinese Spring is an allohexaploid (AABBDD) derived from the hybridization of T. turgidum (AABB) and Aegilops squarrosa (DD). A. squarrosa is a very important contributor as it carries many desirable qualities such as resistance to leaf rust, powdery mildew, greenbug, and Hessian fly (Gill et al., 1986b), increased salt tolerance (Shah et al., 1987), and cold hardiness (Limin and Fowler, 1981). Although A, B, and D chromosome groups are homoeologous, there is a large amount of variation in the location and organization of repeated DNA sequences between the genomes.

Analysis of the wheat genome by Smith and Flavell (1975) with renaturation kinetics has estimated 4-10% of the DNA is highly repetitive, 80% is intermediate, and 12% is single copy. Large amounts of repeated DNA are found in higher plants which possess a high DNA content. This is suggested to have occurred by sequence amplification, polyploidy, and duplication of chromosomes (Flavell et al., 1974).

Repeated DNA sequences which predominantly lie in the heterochromatic regions have been used as cytological markers in many cytogenetic studies (for review see Appels, 1982; Rayburn and Gill, 1987a). These sequences have been used to trace the introgression of DNA segments during the transfer of desirable characteristics into agronomically

useful varieties. Lapitan et al. (1986) detected wheat-rye translocations and their breakage points using in situ hybridization with biotin labeling of a rye dispersed repeated DNA (pSC119).

A D-genome clone (pAS1) isolated by Rayburn and Gill (1986) was recorded as D-genome specific at the in situ level since it heavily labeled the telomeres of 14 D-genome chromosomes in common wheat. Further studies indicate pAS1 does not hybridize exclusively to the D-genome because sites of less intense hybridization are seen on some of the remaining 28 chromosomes (Henry, unpublished results; Gill and Sears, 1988). Nevertheless, pAS1 is a useful probe for cytological labeling of D-genome chromosomes in the wheat nucleus.

Genome-specific repeated DNA sequences have been used to develop chromosome-specific unique DNA probes for detailed genetic mapping in mammalian somatic hybrids (Kasahara et al., 1987). A similar strategy should be applicable in the Triticeae hybrids where a large number of wheat-alien addition and translocation lines are available. Thus, a repeated DNA sequence present in the alien genome that is absent in wheat can be used to identify clones specific to alien chromosomes in a genomic library of a wheat-alien addition line.

In our laboratory, we have identified and transferred a large number of disease and insect resistance genes from

A. squarrosa into wheat (Gill et al., 1986a; Gill and Sears, 1988). The genes for resistance to Hessian fly, greenbug and leaf rust have been mapped on specific D-genome chromosomes (Gill and Raupp, 1987; and unpublished results). It will be of great interest to undertake saturation mapping of specific chromosome arms for molecular tagging of resistance genes. For this purpose, D-genome specific repeated DNA sequences will be useful for construction of chromosome-specific probes of individual D-genome chromosomes (for example, in a genomic library of single D-genome chromosome addition lines in durum wheat). Therefore, we undertook the characterization of a number of D-genome repeated DNA clones in search of new cytological and molecular markers for describing the D-genome of wheat. The results of this study are reported in this paper.

MATERIALS AND METHODS

Plant material was obtained from Kansas State University Wheat Genetics Resource Center. The species used and their designated genomes are listed in Table 1.

Repeated DNA clones of A. squarrosa were from the library prepared by Rayburn and Gill (1986). The library consists of 150 clones in the plasmid pUC8 with inserts ranging from 7 kb to 0.2 kb. Plasmid DNA was isolated according to Maniatis et al. (1982) by an alkaline lysis mini prep.

Plant genomic DNAs were isolated using a method similar to Blin and Stafford (1976) by digestion of leaf material in N-lauryl sarcosine lysis buffer containing protease. After dialysis, two chloroform/isoamyl extractions were performed. DNA was purified on two CsCl gradients followed by ethidium bromide elution with butanol. DNA samples were dialyzed to eliminate CsCl.

The dot blot procedure of Cullis et al. (1984) was followed making two identical dot blots. Each blot had one dot (1 ug of insert) representing each of the 150 clones. The blots were probed separately with nick translated, ³²P labeled genomic A. squarrosa (D genome) or T. turgidum L. cv. Langdon (AB genomes). Autoradiograms were made and compared (D vs. AB) to select ten clones which displayed hybridization to A. squarrosa in conjunction with little or no hybridization to Langdon.

Southern blots of genomic DNAs were prepared (10-20 ug DNA per lane) by depurination in 0.25 N HCl for 30 minutes (Wahl et al., 1979), denatured for 15 minutes with 1.5 M NaCl/0.5 M NaOH, and neutralized for 30 minutes with 3 M NaCl/1 M Tris pH 7.4. DNA was blotted to nitrocellulose paper with 20X SSC overnight. Blots to check cross hybridization between the ten selected clones were performed as in Rayburn and Gill (1986) utilizing biotin incorporation and detection by alkaline phosphatase except hybridization time was reduced to 16 hours.

Southern blots of genomic DNAs were probed with ³²P-labeled clones. Hybridizations were carried out in 5X SSC, 10X Denhardt's, 0.02 M sodium phosphate buffer, 0.25 mg/ml salmon sperm, and 10% dextran sulfate at 65°C for 16 hours. Blots were then rinsed in 0.1X SSC and 1% SDS at the same temperature for 3 hours with three solution changes. Blots were placed in x-ray film cassettes with two intensifying screens for 24 hours before developing.

In situ was performed using biotin labeled clones (Rayburn and Gill, 1985). Slides were pretreated in 70% formamide followed by an alcohol dehydration series (70%, 95%, 100%). Hybridizations were at 37°C for 6.5 hours in 50% formamide, 10% dextran sulfate, 2X SSC, and 0.4 mg/ml salmon sperm. Hybridization was visualized as brown precipitate after a reaction of peroxidase with diaminobenzidine tetrahydrochloride. Two modifications to

the original procedure were incorporated: 1) the probe was denatured at 100°C instead of 85°C and 2) the slides were incubated for 5 minutes at 80°C immediately after the probe was applied.

RESULTS

Dot blot screening

In order to analyze approximately 150 clones quickly and as a preliminary screen for D-genome specific clones the dot blot procedure was used. After comparing identical dot blots probed with ^{32}P labeled A. squarrosa or Langdon, ten clones were selected which appeared to be present predominantly in the D-genome (Figure 1). The clone designations with insert size are listed in Table 2.

Each of the ten clones was hybridized against the others to check for cross hybridization and sequence uniqueness. The clones were also checked against pAS1, 2 and 12 which also originated from the library. Clones pAS26 and pAS24 and clones pAS23 and pAS27 have partial sequence homology. No further tests were done to determine the extent or the exact portions of the clones involved in cross hybridization.

In situ hybridization

Each of the ten selected probes labeled with biotin was hybridized to a chromosome squash of Chinese Spring to view the sequence pattern and genome dispersion at the chromosomal level.

A very dispersed hybridization pattern over all the chromosomes of each genome was shown with pAS21, 23, 27, 29 and 30. Figure 2a shows this dispersed pattern with pAS21

hybridized to Chinese Spring. In addition to the above hybridization pattern, pAS23 and pAS27 show more intense hybridization at several sites (fig. 2b). Appels et al. (1986) isolated a 350 bp family in rye which labels the heterochromatin of all chromosomes. They suggested this sequence underwent several amplifications and distributions as Secale evolved. The sequences in pAS21, 23, 27, 29 and 30 are probably similar to the sequence in the 350 bp of rye and are not of recent origin. Therefore, they have undergone extensive distribution through previously mentioned mechanisms.

More intense hybridization was seen with pAS28 in more localized areas than the previously mentioned clones (fig. 3a). This sequence results in hybridization dots rather than turning the whole chromosome brown. Therefore, this sequence has probably been more amplified and maintained in smaller regions of the chromosome and has not been extensively dispersed over the whole chromosome area.

Hybridization of pAS22 and pAS25 revealed a restricted site dispersion similar to pAS28 but with reduced intensity. Figure 3b shows an example of this type of hybridization pattern with pAS25. This sequence is present in lower copy number than pAS28 at many regions of the chromosomes.

The distinct hybridization patterns of pAS24 and pAS26 primarily labeled the telomeres. Figure 3c shows

pAS26 hybridization as an example of the distinct pattern. Jones and Flavell (1982b) proposed that a repeat which starts at the telomere, through breakage, translocation, inversion and fusion or excision and reintegration, may move to interstitial positions or perhaps to other telomeres. In another paper, Jones and Flavell (1982a) discussed the possibility that repeated families at telomeric regions may be better tolerated or perform some function in this position. As the sequence amplifies, translocates and deletes, heteromorphisms are produced which provide variation within a population.

Southern blots

Each clone was hybridized to an array of digested genomic DNAs representing the various genomes listed in Table 1. These results represent the distribution of the cloned sequence at the molecular level.

TaqI digests of genomic DNAs hybridized with pAS23 resulted in a similar pattern in *A. squarrosa*, Chinese Spring, Langdon, *T. monococcum* and rye with reduced hybridization to *E. ciliaris*, barley and *D. villosum* (fig. 4a). This sequence shows little to no change in the more closely related genomes and may have amplified since wheat diverged during cereal evolution.

The least variable hybridization patterns were obtained from pAS22 and pAS25 (data not shown). Figure 4c

depicts this characteristic in pAS22. The lack of variability is distinguished by few bands, little smearing, and little difference in hybridization between the various genomes. Upon longer exposures of the autoradiogram another band is visible at approximately 2.3 kb with some smearing in the D. villosum lane.

More hybridization to all the genomes by pAS24 was denoted by smearing and the presence of many bands (fig. 4d). A slight hybridization increase is observed in the more closely related grasses A. squarrosa, Chinese Spring, Langdon and T. monococcum.

Intense hybridization was exhibited by pAS26 to A. squarrosa, Chinese Spring, Langdon, T. monococcum, moderate to rye, and lighter to E. ciliaris, barley and D. villosum (fig. 4b). Therefore, pAS26 seemed to show an evolution similar to pAS 22.

Figure 5a indicated pAS28 was present predominantly in E. ciliaris. Slight hybridization was seen with A. squarrosa, Chinese Spring, Langdon, D. villosum and rye. No hybridization was seen in barley. Apparently, the pAS28 sequence amplified after E. ciliaris diverged from the main evolutionary stem. This same sequence has been deleted in barley.

A band present in A. squarrosa and Chinese Spring after hybridization with pAS29 was absent in T. monococcum and Langdon (fig. 5b). This band is also present in

reduced amounts in D. villosum and therefore is not present only in the D genome. However, the band is not in the A or B genomes.

While pAS30 hybridized strongly with rye and weakly with D. villosum and barley, the other grasses showed intermediate hybridization (fig. 5c). This sequence appears to support the evolutionary scheme proposed by Flavell et al. (1977) where barley diverged from common cereal progenitors with wheat and rye diverging simultaneously in an evolutionary fork at a later period. This sequence may have amplified only slightly during early evolution and underwent a large scale amplification after wheat and rye diverged.

Upon hybridization of pAS21 with HindIII restricted genomic DNA, two possible D-genome specific bands were visualized as denoted by the arrows in figure 6a. When pAS21 was probed to TaqI digestions of all the genomes (fig. 6b), A. squarrosa, Chinese Spring and Langdon all appeared the same suggesting the HindIII restriction sites which separated the above mentioned bands were probably inside TaqI sites. However if the fragments were split by TaqI sites, they may have migrated to the end where hybridization was too intense to distinguish differences. This sequence showed no hybridization to barley and little to E. ciliaris.

Restriction patterns of pAS27 (which was partially

homologous with pAS21) gave patterns very similar to pAS21 (fig. 6). The HindIII blot for pAS27, unlike the blot for pAS21, consisted of the entire array of genomes. Figure 6c shows that the data from this autoradiogram made it possible to discern that the lower presumed D-genome specific band was also present in T. monococcum. The larger band was also in T. monococcum but in very reduced amounts. Therefore, these bands were not D-genome specific.

Partial sequence homology was exhibited between pAS27 and pAS21 in the cross hybridization check. By comparing the southern blots, it is seen that pAS21 must contain a sequence not present in pAS27 which is specific for E. ciliaris, D. villosum and rye.

Bedbrook et al. (1980) found 120 bp and 2.2 kb repeats in rye. By comparing hybridizations of these sequences to rye and wheat, they concluded that this sequence was amplified before divergence due to increased amounts of the 120 bp sequence in both cereals. However, the 2.2 kb sequence was present in wheat in reduced amounts alluding to divergence followed by amplification in rye. The sequences of pAS21 and pAS27 appear to be evolutionarily related resembling the 120 bp and 2.2 kb sequences.

DISCUSSION

The data collected through in situ and southern blot hybridization analysis showed that no D genome specific sequences were found despite the preliminary results from the dot blot comparison. Discrepancies between the screening and further analyses are probably due in part to the crudeness of the DNA mini-preparations used. Overall this method was not sensitive enough for the resolution desired.

Jones and Flavell (1982b) compared accessions of rye on ethidium bromide stained gels of digested genomic DNA. They suggested this may be a good way to visualize differences as they were able to detect differences between species at this level. Therefore, comparison of digested and stained DNA gels with subsequent cloning of bands which appear to be specific to the level desired may be a better approach for identifying species specific sequences.

The clones used in this study were dispersed extensively throughout the various genomes. Appels et al. (1986) located a repeated, non-heterochromatin rye sequence. Their conclusions are based on hybridization to many fragments in digestions, dispersed in situ patterns on all chromosomes, and the lack of repeated arrays detected after sequencing. This description is appropriate for the clones used in this study.

Although D-genome specific clones were not isolated,

several clones were identified that were widespread in several genomes but were virtually absent in others. Related clones pAS21 and pAS27 are dispersed in wheat but are absent in barley. In addition pAS27 is virtually absent in E. ciliaris, D. villosum and rye genomes. Because of the contrasting labeling patterns, these clones may be used to monitor alien chromatin transfers from these genomes into wheat. In such materials the wheat chromosomes will be completely labelled and unlabeled segments will be identified as alien chromatin. Similarly, these clones can be used in identifying wheat chromosomes in the rye-wheat addition lines developed by Schlegel (personal communication).

Harris et al. (1986) reported that sequencing of several wheat repeats revealed direct terminal repeats resembling those found in transposable elements of Drosophila, Ty-1 of yeast and retroviruses. They also documented sequence duplication at the insertion site. These observations led them to propose an evolutionary mechanism by reverse transcriptase. If this was a method of amplification for some repeats, they no longer retain the ability to transpose due to mutations or deletions of recognition sequences or important transposition sequences needed. Therefore they conclude many dispersed repeats are the result of short segments transposed during evolution.

Further evidence for a large number of short

rearrangements is provided by Flavell (1982) who reported that 50% of a genome is family repeated DNA between short non-repeated and/or unrelated repeats. Most of the clones that we analyzed were of dispersed type and may have originated by mechanisms proposed by Harris et al. (1986).

Two of the clones (pAS24 and pAS26) appear to be tandem repeats. There are many hypotheses on the origin of tandem repeats which are amplified sequences that have not undergone much rearrangement or divergence. Unequal crossing over of sister chromatids is one proposed mechanism (Brown and Blackler, 1972). This would be a very gradual process assuming little change in the sequences over a long period of time.

Amplification due to replication errors (Alt et al., 1978) has also been proposed. This event would produce sequences more rapidly than unequal crossing over. Replication forks would terminate at different sites giving sequences with the same origin but of different lengths.

The rolling circle model entails the excision of a DNA sequence which circularizes and is then replicated and reintegrated (Wells et al., 1967). The template model is similar in that the previous repeat is used as a template but without being excised and reintegrated (Lohe and Brutlag, 1987). These proposed mechanisms of amplification are a few of the ways tandem repeated sequences may have originated.

Distribution of different repeated DNA sequences varied widely among Triticeae genomes analyzed. Some such as pAS22 were equally distributed among all genomes. Others were widespread in a few genomes and were present in reduced amounts in others. Overall most of the differences in genome hybridization can be attributed to the three possibilities mentioned by Jones and Flavell (1982a): 1) separate amplification in diverged species, 2) deletion of the repeat in evolving species and 3) introgression from one species to another.

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Figure 1

Dot blot screen for D-genome specific clones.

Dot blots representing the collection of clones with A. squarrosa inserts.

a. Probed with A. squarrosa (D genome).

b. Probed with Langdon (AB genomes).

Selected clones are circled with designation to the left.

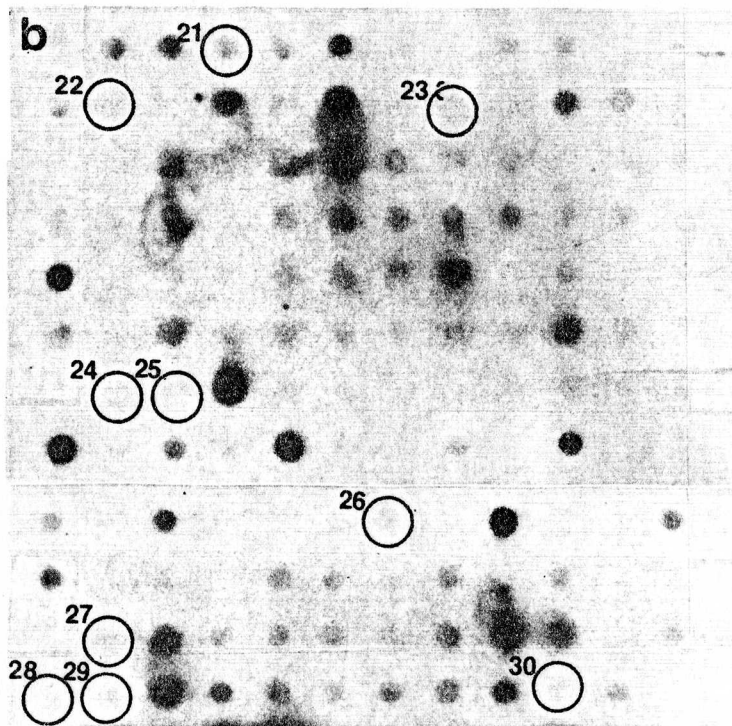
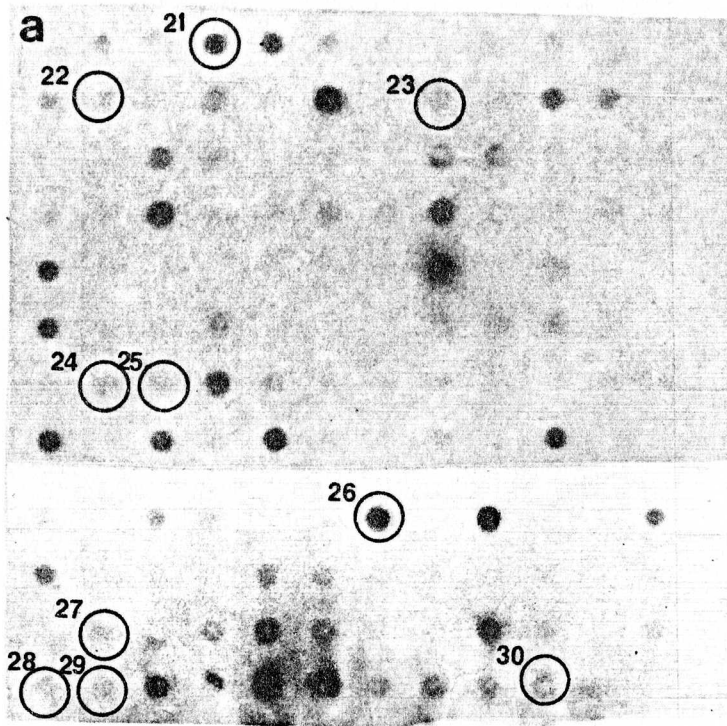


Figure 2

Analysis of clones by in situ hybridization.

Biotin labeled clones hybridized to chromosome squashes of Chinese Spring.

- a. pAS21 with a dispersed hybridization pattern.
- b. pAS23 with a dispersed hybridization pattern in addition to more specific sites of hybridization.

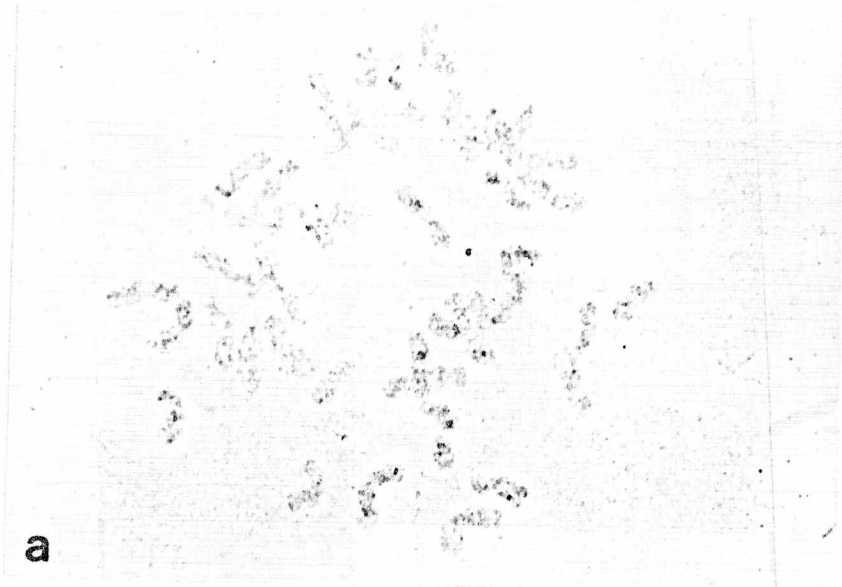


Figure 3

Analysis of clones by in situ hybridization.

Biotin labeled clones hybridized to chromosome squashes of Chinese Spring.

- a. pAS28 showed an intense hybridization at many specific sites.
- b. pAS25 showed less intense hybridization but was still highly distributed over localized sites.
- c. pAS26 showed heavy labeling predominantly at the telomeres.

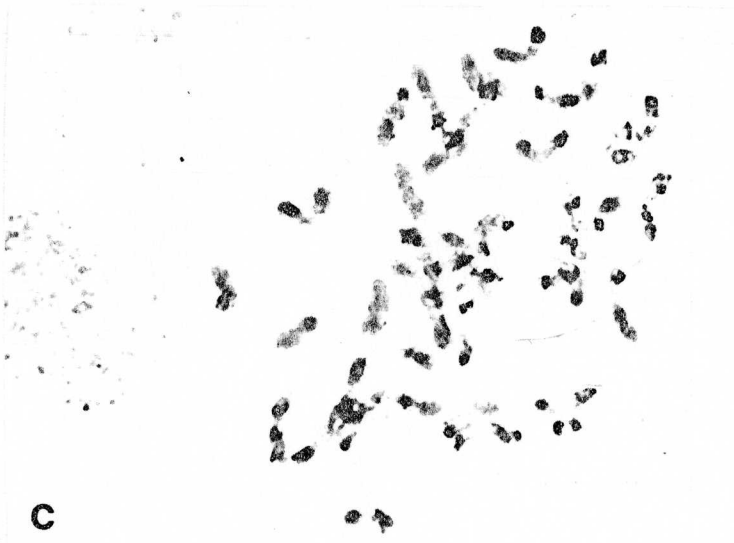
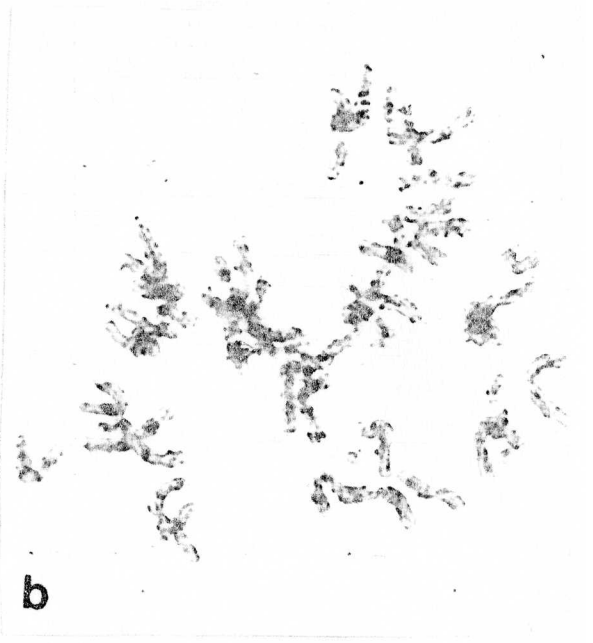
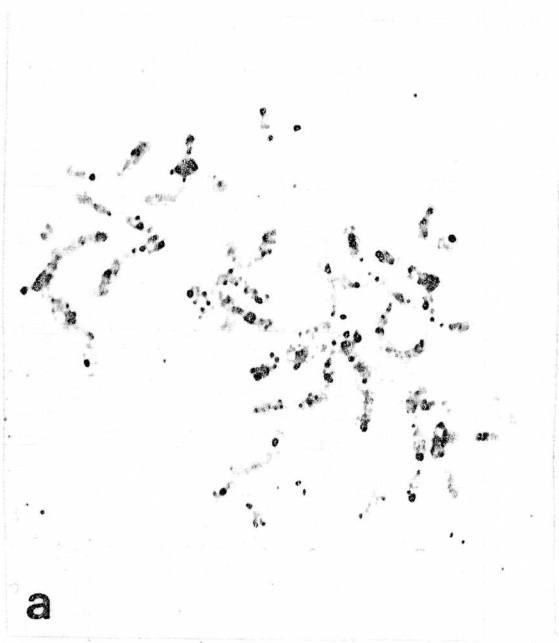


Figure 4

Southern blot hybridization analysis of clones.

a. HindIII digest, probed with pAS23.

b. TaqI digest, probed with pAS26.

c. TaqI digest, probed with pAS22.

d. TaqI digest, probed with pAS24.

Lanes for a and b: 1 - E. ciliaris, 2 - H. vulgare, 3 - A. squarrosa, 4 - Chinese Spring, 5 - Langdon, 6 - T. monococcum, 7 - D. villosum, 8 - S. cereale.

Lanes for c and d: 1 - E. ciliaris, 2 - H. vulgare, 3 - A. squarrosa, 4 - Chinese Spring, 5 - Langdon, 6 - D. villosum, 7 - T. monococcum, 8 - S. cereale.

Lambda DNA restricted with HindIII size marker: 23.1 kb, 9.4 kb, 6.7 kb, 4.3 kb, 2.3 kb, 2.0 kb and 0.56 kb.

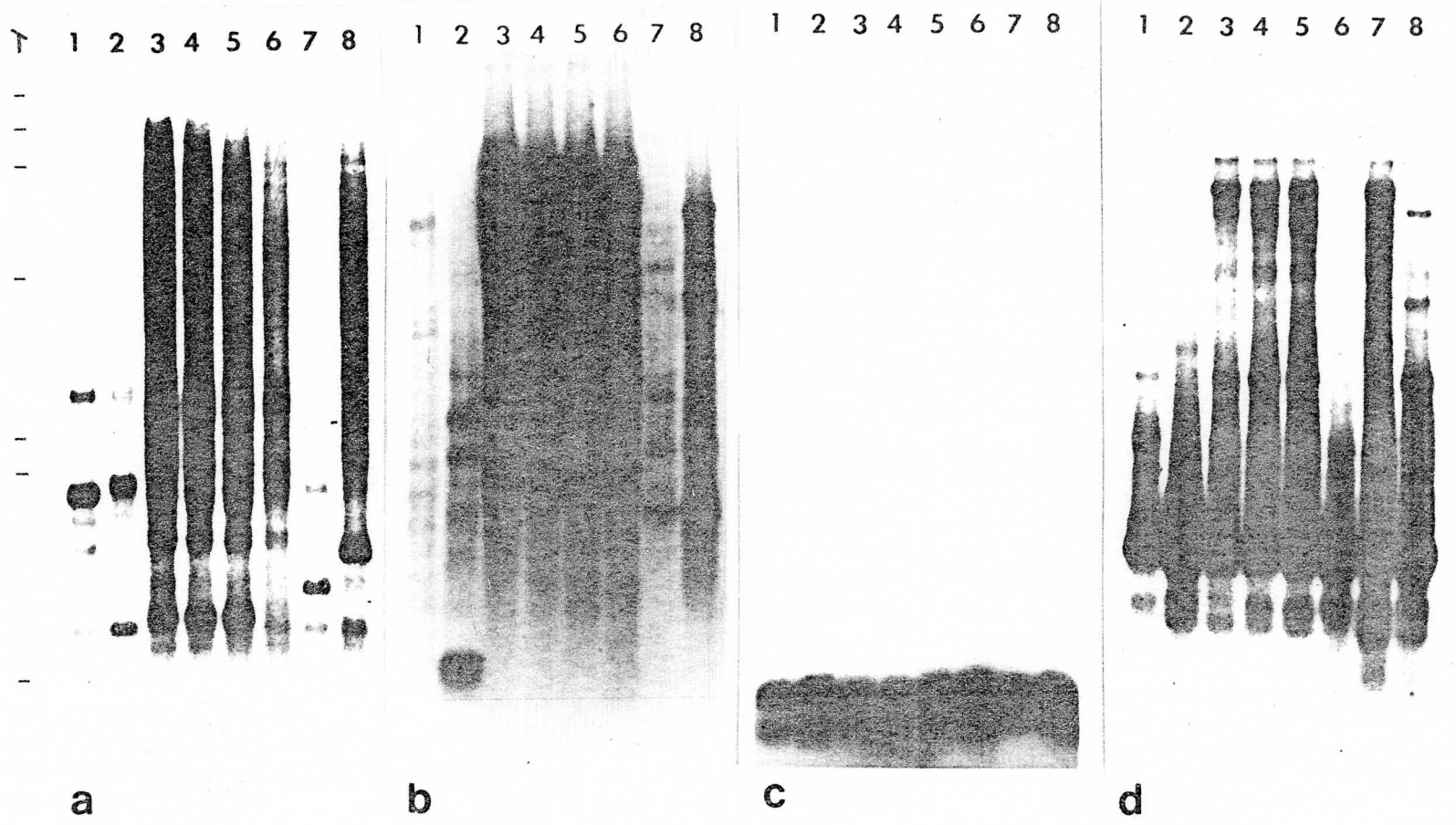


Figure 5

Southern blot hybridization analysis of clones.

a. TaqI digest, probed with pAS28.

b. TaqI digest, probed with pAS29.

c. TaqI digest, probed with pAS30.

Arrow in 5b indicates band in D and V genomes.

Lanes: 1 - E. ciliaris, 2 - H. vulgare, 3 - A. squarrosa,
4 - Chinese Spring, 5 - Langdon, 6 - T. monococcum, 7 - D.
villosum, 8 - S. cereale.

Lambda DNA restricted with HindIII size marker: 23.1 kb,
9.4 kb, 6.7 kb, 4.3 kb, 2.3 kb, 2.0 kb and 0.56 kb.

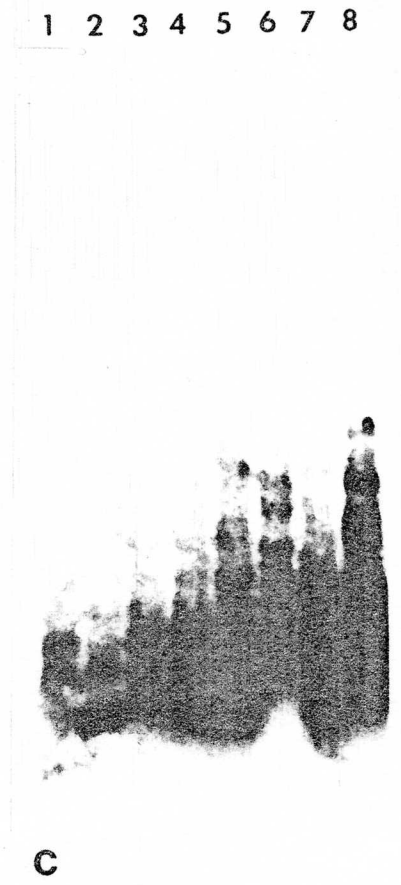
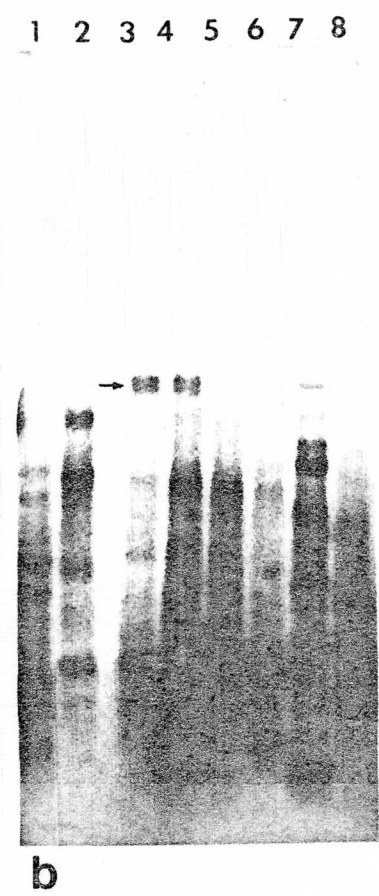
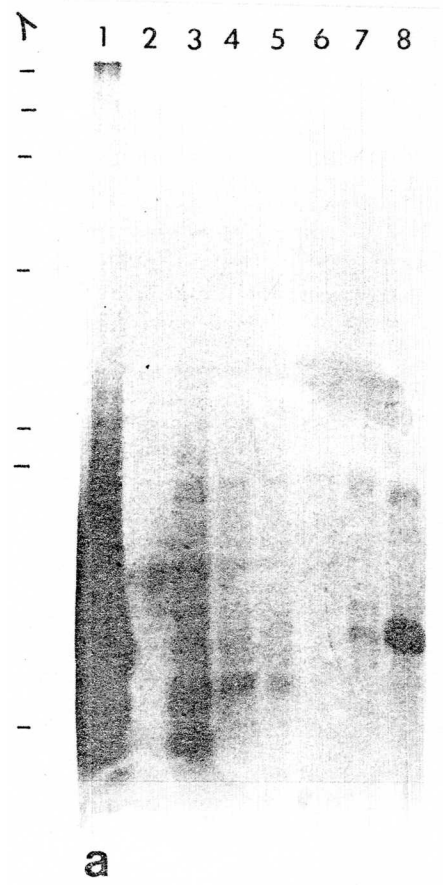


Figure 6

Southern blot hybridization analysis of clones.

- a. HindIII digest, probed with pAS21.
- b. TaqI digest, probed with pAS21.
- c. HindIII digest, probed with pAS27.
- d. TaqI digest, probed with pAS27.

Top arrow indicates D-genome specific band. Lower arrow indicates band previously thought to be D-genome specific.

Lanes for a: 1 - A. squarrosa, 2 - Chinese Spring, 3 - Langdon.

Lanes for b-d: 1 - E. ciliaris, 2 - H. vulgare, 3 - A. squarrosa, 4 - Chinese Spring, 5 - Langdon, 6 - T. monococcum, 7 - D. villosum, 8 - S. cereale.

Lambda DNA restricted with HindIII size marker: 23.1 kb, 9.4 kb, 6.7 kb, 4.3 kb, 2.3 kb, 2.0 kb and 0.56 kb.

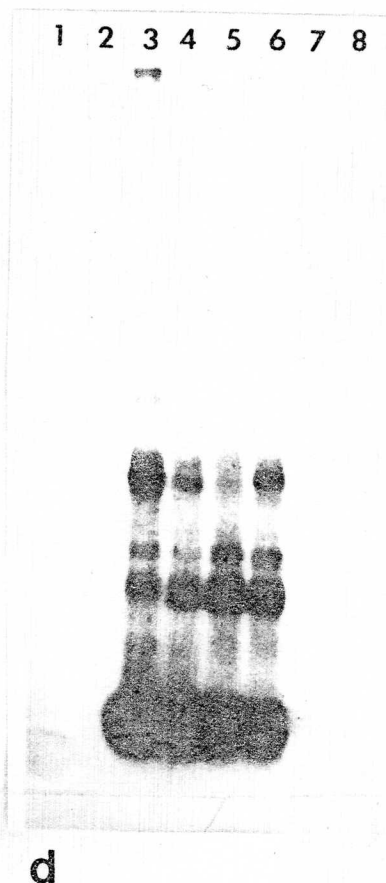
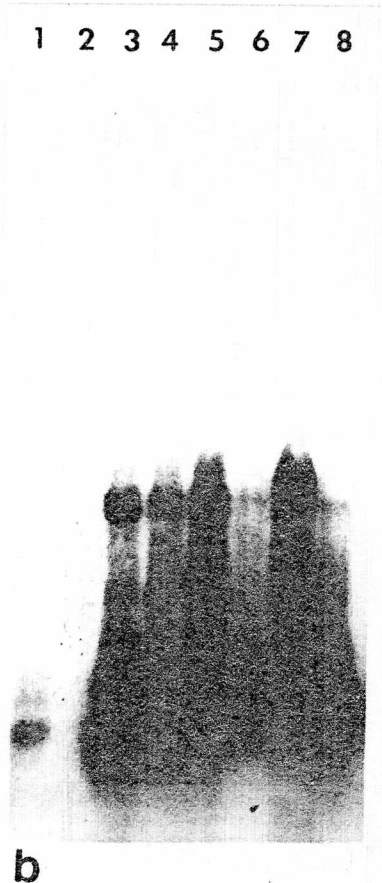
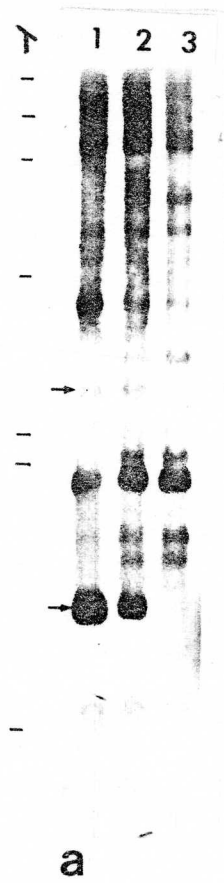


Table 1. Plant material used with genome designation.

<u>Species</u>	Accession ¹	
	<u>Number</u>	<u>Genome</u>
<u>Elymus ciliaris</u> L.	TA2006	SY
<u>Hordeum vulgare</u> L. cv. Betzes	TA9001	I
<u>Aegilops squarrosa</u> L.	TA1649	D
<u>T. aestivum</u> L. cv. Chinese Spring	TA3008	ABD
<u>T. turgidum</u> L. cv. Langdon	TA1168	AB
<u>T. monococcum</u> L.	TA138	A
<u>Dasypyrum villosum</u> L.	TA2138	V
<u>Secale cereale</u> L. cv. Chaupon	TA9002	R

¹Kansas State University accession number

Table 2. Selected clone designations and size of *A. squarrosa* insert with summary of Southern blot and in situ hybridization analysis.

Clone	Insert size	In situ	<u>Genomic distribution</u> ¹						
			SY	I	D	AB	A	V	R
pAS21	0.70	dispersed over entire chromosome	+	-	+++	+++	+++	++	++
pAS22	0.98	intermediate labeling at many sites ++	+++	+	+				
pAS23	0.57	dispersed with some specific sites	+	+	+++	+++	++	+	+++
pAS24	0.74	heavy labeling at telomeres	++	++	+++	+++	+++	++	++
pAS25	0.36	intermediate labeling at many sites	+	+	+	+	+	+	+
pAS26	0.32	heavy labeling at telomeres	+	+	+++	+++	+++	+	++
pAS27	0.34	dispersed over entire chromosome	+	+	++	++	++	+	+
pAS28	0.38	intense labeling at many specific sites	+++	-	++	+	+	+	++
pAS29	1.40	dispersed over most chromosomes	++	++	++	+++	++	++	++
pAS30	0.52	dispersed over most chromosomes	+	+	++	++	++	++	+++

¹- no, + slight, ++ intermediate, +++ intense hybridization

CLONES FROM AEGILOPS SQUARROSA

by

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B.S., Kansas State University, 1963

AN ABSTRACT OF A THESIS

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ABSTRACT

Molecular and cytological techniques were used for the characterization of repeated DNA clones from the D-genome of Aegilops squarrosa. For preliminary genome designation and estimation of copy number, identical dot blots of the A. squarrosa genomic clone library in pUC8 were prepared and probed with nick translated, ^{32}P labeled DNA of A. squarrosa (D genome) or Triticum turgidum L. cv. Langdon (AB genomes). Autoradiograms of the two dot blots were compared, and ten clones hybridizing only to the A. squarrosa blot were selected for further analysis by in situ and Southern blot hybridizations.

The Southern blots included an array of genomic DNAs from the ancestors of wheat. There were large differences in the organization and amount of repeated DNA sequences among different genomes. Hybridization patterns varied widely between the clones and among genomes. A few clones showed no hybridization to barley. Other clones were distributed equally among all genomes or exhibited varied intensities of hybridization between the genomes. A few D-genome specific bands were discovered but none of the clones were totally D-genome specific.

In situ hybridization with each clone to Chinese Spring was also done. Hybridization patterns varied from dispersed to intense localized hybridization. As expected from the Southern blot analysis none of the clones were

predominantly in the D genome.

Despite early screening results no D-genome specific clones were found. Therefore the screening method used was not sensitive enough for this purpose. However, several clones that were dispersed and labelled all wheat chromosomes were virtually absent in E. ciliaris, barley, D. villosum and rye as determined by southern blot analysis. Therefore, because of these contrasting patterns, some of these clones may be used to monitor alien chromatin transfers from these genera into wheat.