Ribosomal RNA Multigene Loci: Nomads of the Triticeae Genomes

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ABSTRACT

The nucleolus organizing regions (NORs) on the short arms of chromosomes *1A*^{m} and *5A*^{m} of diploid wheat, *Triticum monococcum* L., are at the most distal loci in the linkage maps of these two chromosome arms. This distal location differs from the interstitial location of the *Nor* loci on chromosome arms *1BS* of tetraploid *Triticum turgidum* L. and hexaploid *T. aestivum* L., *5DS* of *T. aestivum* and diploid *Ae. tauschii* Coss., and *5HS* of barley. Moreover, the barley *5HS* locus is at a different location than the *5DS* locus. However, other markers, including the centromeres, are colinear. These findings showed that the major *Nor* loci have repeatedly changed position in the chromosome arms during the radiation of species in the tribe Triticeae without rearrangements of the linkage groups. It is suggested that *Nor* loci may change position via dispersion of minor loci, that are shown here to exist in the *T. monococcum* genome, magnification of gene copy numbers in these minor loci, and subsequent deletion of the original major loci. Implications of these findings for the use of rRNA nucleotide sequences in phylogenetic reconstructions are pointed out.

THE active loci encoding 18S-5.8S-26S rRNA were among the first that have been assigned to specific chromosome synteny groups in the genomes of the tribe Triticeae because of their nucleolus organizing activity. In bread wheat, *Triticum aestivum* L. ($2n = 6x = 42$, genomes *AABBDD*), nucleolus organizing regions (NORs) were found to be on chromosomes *1A, 1B, 6B*, and *5D* (CROSBY 1957; LONGWELL and SVIHLA 1960; FLAVELL and SMITH 1974; FLAVELL and O'DELL 1976; APPELS *et al.* 1980; MILLER *et al.* 1980; MUKAI *et al.* 1991) (Table 1). These *Nor* loci contain from hundreds to thousands of rRNA repeated gene units (FLAVELL and O'DELL 1979). In other wheat (Triticum) species and those of the related genus Aegilops, or species of other genera in the tribe Triticeae, NORs have consistently been located on chromosomes homoeologous to those on which the wheat NORs are present (major loci in Table 1). In most species, however, only one or two NORs are present per genome.

In addition to the loci that function as NORs, and are clearly expressed, additional minor loci have recently been detected in Triticum, Aegilops and Hordeum genomes by *in situ* DNA hybridization (minor loci in Table 1). Minor chromosomal loci hybridizing with cloned rRNA repeated gene units (rDNA) are on wheat chromosome arms *1BL, 3DS* (JIANG and GILL 1994) and *7DL* (MUKAI *et al.* 1991), on barley chromosome arm *2HS* (LEITCH and HESLOP-HARRISON 1992), at the terminus of *T. monococcum* L. chromosome arm *5AL* (JIANG and GILL 1994) and on another, unidentified, *T. monococcum* chromosome, possibly *7A*^{m} (JIANG and GILL 1994).

The major and minor loci on the short arms of chromosomes of homoeologous group 1 in the tribe Triticeae have been assumed to be orthologous to the *Nor1* locus on the short arm of wheat chromosome *1B* (Table 1). However, the assumed orthology has been scrutinized by comparative linkage mapping only between *Nor* loci on *1BS* (*Nor-B1*) and rye chromosome arm *1RS* (*Nor-R1*) (VAN DEYNZE *et al.* 1995). The loci on the short arms of chromosomes of homoeologous group 5 are assumed to be orthologous to the *Nor3* locus on wheat chromosome *5D* (Table 1). No comparative mapping has, however, been done to substantiate this assumption.

In diploid wheats, *T. urartu* Thum. (genome *A*) and *T. monococcum* (genome *A*^{m}), the NORs are on the short arms of chromosomes *1* and *5* (MILLER *et al.* 1983) and in the *A* genome of polyploid wheats on the short arm of chromosome *1A* (CROSBY 1957; FLAVELL and SMITH 1974; MUKAI *et al.* 1991; JIANG and GILL 1994). Because the *Nor* loci on *1AS* and *1A*^{m}*S* have been assumed to be orthologous to *Nor1* and that on *5A*^{m}*S* to *Nor3*, they were respectively designated *Nor-A1* and *Nor-A3* (MUKAI *et al.* 1991; JIANG and GILL 1994). However, the satellite created by the secondary constriction on chromosome *1A*^{m} is small (GERLACH *et al.* 1980) in comparison to the satellites created by the secondary constrictions at the *Nor-B1* and *Nor-R1* loci on wheat and rye chromosomes *1B* and *1R*, respectively. A similar situation is observed in chromosome *5A*^{m}, which has a

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TABLE 1

Chromosome or chromosome arm assignments of the rDNA loci in representative species of the tribe Triticeae and their designations

Species	Ploidy	Genomes	Chromosome arm or chromosome	rDNA locus and its current designation		Proposed designation
				Major	Minor	
T. urartu	2x	*AA* (the source of the *A* genome of wheats)	*1AS*	unnamed[a]		
			5AS	unnamed[a]		
T. monococcum	2x	A^mA^m	$1A^mS$	*Nor1*[b]		*Nor9*
			$5A^mS$	*Nor3*[b]		*Nor10*
			$5A^mL$		*Nor7*[b]	
			$?7A^m$		*Norx*[b]	
T. turgidum	4x	*AABB*	*1AS*	*Nor1*[b]		
			1BS	unnamed[c]		
			6BS	unnamed[c]		
T. aestivum	6x	*AABBDD*	*1AS*	unnamed[d]		
				Nor1[e]		
			1BS	unnamed[d]		
				Nor1[f]		
			1BL		*Nor6*[b]	
			6BS	unnamed[d]		
				Nor2[g]		
			5DS	unnamed[d]		
			5DS	*Nor3*[h]		
			3DS		*Nor-D8*[b]	
			7DL		*Nor-D4*[e]	
Ae. speltoides	2x	*SS* (the closest relative of the *B* genome of wheat	*1SS*	unnamed[i]		
				Nor1[b]		
			6SS	unnamed[i]		
				Nor2[b]		
Ae. longissima	2x	S^lS^l	$1S^lS$		unnamed[j]	
			$5S^lS$	unnamed[j]		
			$6S^lS$	unnamed[j]		
Ae. tauschii	2x	*DD* (the source of the D genome of wheat)	*5DS*	*Nor3*[h]		
			7DL		*Nor4*[e]	
Ae. umbellulata Zhuk.	2x	*UU*	*1US*	unnamed[k]		
			5US	unnamed[l]		
Lophopyrum elongatum (Host) Love	2x	*EE*	*5ES*	unnamed[i]		
			6ES	unnamed[i]		
L. ponticum (Podp.) Love	10x	*EEEEEEEEE*	*5ES*	unnamed[m]		
Hordeum vulgare L.	2x	*HH* (also designated *II*)[n]	*5HS*	*Rrn2*[o]		*Rrn2*
				Nor3[p]		or *Nor11*
			6HS	*Rrn1*[o]		
				Nor2[p]		
			1HS		*Nor1*[p]	
			2H		*Nor5*[p]	
			7H		*Nor4*[p]	
Secale cereale	2x	*RR*	*1RS*	*Nor1*[q]		

[a] MILLER *et al.* (1983).
[b] JIANG and GILL (1994).
[c] HUTCHINSON and MILLER (1982).
[d] CROSBY (1957).
[e] MUKAI *et al.* (1991).
[f] SNAPE *et al.* (1985).
[g] DVOŘÁK and CHEN (1984).
[h] LASSNER *et al.* (1987).
[i] DVOŘÁK *et al.* (1984).
[j] FRIEBE *et al.* (1993).
[k] FLAVELL and O'DELL (1976).
[l] MILLER *et al.* (1983).
[m] ZHONG *et al.* (1994).
[n] LÖVE (1984).
[o] SAGHAI-MAROOF *et al.* (1984).
[p] LEITCH and HESLOP-HARRISON (1992).
[q] BAUM and APPELS (1991).

smaller satellite than the *T. aestivum* and *Ae. tauschii* Coss. (genomes *DD*) chromosomes *5D* and, particularly, barley chromosome *5H*. A possible explanation of this variation is that the orthologous loci are in different positions due to inversions that have been fixed during phylogeny of these genomes. Alternatively, the satellite size variation may simply reflect variation in the amounts of heterochromatic sequences or different amounts of spacer DNA between the genes in the satellites in different genomes.

We employed comparative linkage mapping with molecular markers to investigate the reasons for this satellite size variation and to scrutinize the relationships between the *Nor1* loci in the A^m genome of *T. monococcum* and the *B* genome of wheat and among the *Nor3* loci in the A^m genome of *T. monococcum*, the *D* genome of wheat and *Ae. tauschii* and the *H* genome of barley, *Hordeum vulgare* L.

MATERIALS AND METHODS

Mapping populations: Two F_2 populations of *T. monococcum* were used for mapping chromosome arms *1A*^{m}*S* and *5A*^{m}*S*. The first one included 76 F_3 families from a cross between wild *T. monococcum* ssp. *aegilopoides* (Link.) Thell. accessions from Turkey (G1777) and Iran (G2528). The second mapping population included 74 F_2 individuals from a cross between a cultivated *T. monococcum* DV92 and *T. monococcum* ssp. *aegilopoides* from Lebanon (G3116). Chromosome arm *1BS* was also mapped twice. One map was constructed using 91 F_2 individuals from a cross between *T. aestivum* cultivars Chinese Spring and Cheyenne, and the other was constructed using 92 recombinant substitution lines (RSLs) obtained from a cross between *T. turgidum* L. ssp. *durum* (Desf.) Husnot cultivar Langdon and disomic substitution line of chromosome *1B* of *T. turgidum* L. ssp. *dicoccoides* (Korn.) Thell. in Langdon. The population of RSLs was produced and supplied by L. R. JOPPA, ARS-USDA, Fargo, ND. Chromosome arm *5DS* was mapped using a population of 45 F_2 individuals from a cross between Chinese Spring and synthetic wheat RL5406. The synthetic wheat was produced from a cross of experimental line Tetracanthach ($2n = 4x = 28$, genomes *AABB*) with *Ae. tauschii* (KERBER and DYCK 1969). The approximate positions of the centromeres were inferred by telocentric analysis. For comparison of the marker order on chromosome arms *5A*^{m}*S* and *5DS* with that on chromosome arm *5HS* of barley, the linkage map of the barley genome (KLEINHOFS *et al.* 1993; KLEINHOFS *et al.* in MATTHEWS and ANDERSON 1994) was employed. The information about the unpublished position of *X Gsp* in *5HS* was kindly provided by A. KILLIAN, Washington State University, Pullman. Barley chromosomes are designated according to their homoeology with the wheat homoeologous chromosome groups throughout the paper.

Mapping technique: Nuclear DNAs were isolated from leaves of single plants following the procedure of DVOŘÁK *et al.* (1988). Southern hybridization was performed as described earlier (DUBCOVSKY *et al.* 1994). *Nor* loci were mapped using wheat clone pTa250.15, which contains a 900-bp *Hha*I fragment from the spacer region containing a substantial part of the promoter (APPELS and DVOŘÁK 1982). Maps were constructed using KOSAMBI (1943) function and the computer program Mapmaker/EXP 3.0 and JoinMap 1.4 (LANDER *et al.* 1987; LINCOLN *et al.* 1992; STAM 1993).

***In situ* rDNA hybridization:** The 9-Kb *Eco*RI fragment from a wheat *Nor* locus containing both the wheat coding and spacer nucleotide sequences inserted in pTa71 (GERLACH and BEDBROOK 1979) was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation. The hybridization solution contained 5 ng/ml of labeled probe, 50% (v/v) deionized formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) sodium dodecyl sulphate (SDS), and 2× SSC (0.03 M sodium citrate and 0.3 M sodium chloride). The solution was heated to 70° for 10 min. Forty milliliters of the solution was then placed on squashed cells on a microscope slide and covered with a plastic 22 × 22-mm cover glass. Slides were then placed in a 90° humid chamber for 10 min and incubated in a 37° humid chamber overnight. Slides were washed in 2× SSC, 40° for 5 min, twice in 20% formamide and 0.1× SSC, 40° for 5 min and then twice in 2× SSC, 40° for 5 min. Slides were transferred into detection buffer consisting of 4× SSC, 0.2% Tween 20, 5% (w/v) bovine serum albumin (BSA) for 5 min and then incubated in 20 ng/ml sheep anti-digoxigenin-fluorescein (Boehringer Mannheim) in detection buffer for 1 hr at 37°. For amplification of the signal, slides were washed briefly in detection buffer, transferred to rabbit serum block (Boehringer Mannheim) for 5 min at room temperature and incubated in 25 ng/ml of rabbit anti-sheep Ig FITC (Boehringer Mannheim) for 1 hr at 37°. Slides were rinsed in detection buffer and counterstained with 2 ng/ml 4′-6-diamidino-2-phenylindole (DAPI in McIlvane's citrate buffer), pH 7.0, and mounted in antifade solution Vectashield H-1000 (Vector Laboratories Inc., Burlingame, CA). Chromosomes were photographed with filters for fluorescein using a confocal microscope.

RESULTS

Homoeologous group 1: The rDNA probe hybridized with four restriction fragments in the *Hph*I restriction profiles of *T. monococcum* accessions DV92 and G3116 (Figure 1). These fragments were major rDNA variants in the *T. monococcum* genome (Figure 1). One fragment was shared by the hybridization profiles and could not be mapped whereas the remaining three were polymorphic and were mapped either to chromosome *1A*^{m} or chromosome *5A*^{m}, as indicated in Figure 1. The position of the centromere in chromosome *1A*^{m} was determined by telosome mapping, using the *1A*^{m}*S* and *1A*^{m}*L*^{rec} telosomes substituted for Chinese Spring chromosome *1A* (DUBCOVSKY *et al.* 1995). The *X Nor* locus was 58.8 cM from the centromere and was distal to the gliadin locus *X Gli1* and the 5S rRNA locus *X 5SDna1* in the linkage map of chromosome arm *1A*^{m}*S* based on this mapping population (Figure 2). A similar result was obtained with the *T. monococcum* mapping population from the cross G1777 × G2528 (Figure 2). In this map, the *X Nor* locus was 46.3 cM from the centromere and also was distal to *X Gli1* and *X 5SDna1* (Figure 2).

The *Nor* locus on chromosome *1B* was also mapped in two mapping populations. One was a *T. aestivum* mapping population and the other was a *T. turgidum* mapping population (Figure 2). The position of the centromere in *T. aestivum* chromosome *1B* (Figure 2) was determined by telosomic mapping using Chinese Spring ditelosomic lines *1BS* and *1BL*. In the map based

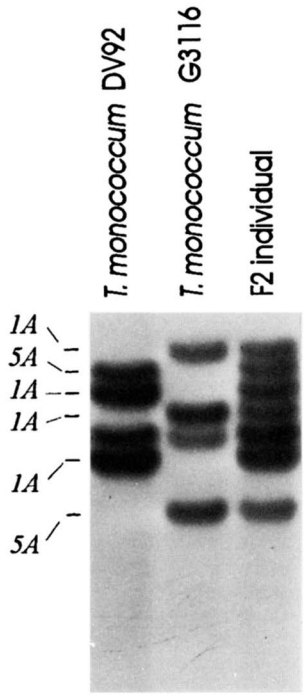

FIGURE 1.—Nuclear DNAs digested with *Hph*I and hybridized with rDNA clone pTa250.15. From the left, the parents and an F_2 individual heterozygous at both *Nor* loci. Note that major rDNA variants were used in the mapping and that all except one could be mapped.

on the *T. aestivum* population, the *Nor* locus was ~5 cM from the centromere, was completely linked to *X5SDna1*, and was 46.6 cM proximal to *XGli1*. In the map based on the *T. turgidum* mapping population, *XNor* was completely linked to the centromere and was 6.5 cM proximal to *X5SDna1*. Low levels of polymorphism precluded mapping of a sufficient number of common proximal markers in the *T. aestivum* map. This and the clustering of those that were mapped made it impossible to determine conclusively whether the differences in the linkages of the *Nor* loci in the two *1B* maps were due to sampling error, to differences in recombination frequencies or to actually different location of the *Nor* loci.

In spite of the completely different position of the *Nor* loci in the $1A^m$ and *1B* linkage maps, the other 17 common markers in the short arm showed the same order in the linkage maps of the chromosomes (Figure 2).

Homoeologous group 5: In the *T. monococcum* genome, *XNor* was the most distal $5A^m$ locus in both mapping populations (Figure 3). It was 50 and 56.1 cM from the centromere and mapped distal to *XGsp*.

Hybridization of the pTa250.15 rDNA fragment with *Taq*I-digested nuclear DNA of Chinese Spring produced a short (0.7 kb) DNA fragment and a long (1.6 kb) DNA fragment that by telosomic analysis had been shown to represent the major rDNA variant of the Chinese Spring *Nor-D3* locus on chromosome *5D* (LASSNER *et al.* 1987). The 0.7-kb fragment, shared by the Chinese Spring profile with the profile of RL5406, is from the promoter region of the rDNA units at the wheat *Nor-D3* locus (LASSNER *et al.* 1987). The 1.6-kb fragment contains a part of the promoter region and an array of six 120-bp repeats of the nontranscribed spacer (LASSNER *et al.* 1987). In RL5406, the 1.6-kb fragment was replaced by a 1.8-kb fragment that was allelic to the 1.6-kb fragment at the *XNor-D3* locus of Chinese Spring. The segregation of the fragments in the mapping population placed the *Nor-D3* locus 14.8 cM proximal to *XGsp* (Figure 3). Moreover, *XNor-D3* was completely linked to *Xmwg920* in *5DS* whereas the *XNor* locus on $5A^m$ was distal to *Xmwg920* in both *T. monococcum* mapping populations (Figure 3).

In the barley genome, the *5HS XNor* locus has also an interstitial position (KLEINHOFS *et al.* 1993; KLEINHOFS *et al.* in MATTHEWS and ANDERSON 1994). However, the position of the barley locus differs from the position of *XNor-D3*. The *Nor* locus is 5.4 cM proximal to *XDor5* on chromosome arm *5HS* but 24.6 cM distal to *XDor5* on chromosome arm *5DS* (Figure 3). The position of the barley locus also differs from the position of the *Nor* locus on the $5A^mS$ chromosome arm (Figure 3).

All except one common RFLP markers among chromosome arms *5HS*, $5A^mS$ and *5DS* were colinear. The only exception was the position of *Xabg497* between barley and *T. monococcum* (Figure 3). Flanking markers at both sides of *Xabg497* were in the same orientation relative to each other and the centromere in barley and *T. monococcum* (Figure 3) suggesting that the *Xabg497* markers that were mapped in the Steptoe × Morex barley mapping population and in both *T. monococcum* mapping populations were paralogous. Genomic clone ABG497 hybridizes with a number of restriction fragments and a *Xabg497* locus has been mapped on chromosome arm *7HS*, in addition to chromosome arm *5HS* (GRANER *et al.* 1991; GRANER *et al.* in MATTHEWS and ANDERSON 1994). The order of markers on chromosome *5D*, centromere-(*XNor, Xmwg920*)-*XGsp* (Figure 3), was only 24 times more likely (LOD 1.38) than the alternative order centromere-*XGsp*-(*XNor, Xmwg920*). This low LOD score was due to rather limited number of F_2 individuals and the large gap between *Xtam53* and *XNor-Xmwg920*. The order, centromere-*XNor-XGsp* was, however, also found on chromosome *5D* of *Ae. tauschii* (LAGUDAH *et al.* 1991). Because the Chinese Spring 1.6-kb fragment and the *Ae. tauschii* 1.8-kb fragment are allelic, the position of the *XNor-D3* locus in wheat and *Ae. tauschii* appears to be the same, and is proximal to *XGsp*.

***In situ* DNA hybridization:** Clone pTa71 hybridized *in situ* with two major and several minor sites in the *T. monococcum* metaphase chromosomes (Figure 4). Both major sites were terminally located on the short arms of submetacentric chromosomes, which must be $1A^m$ and $5A^m$. One of the minor sites was consistently ob-

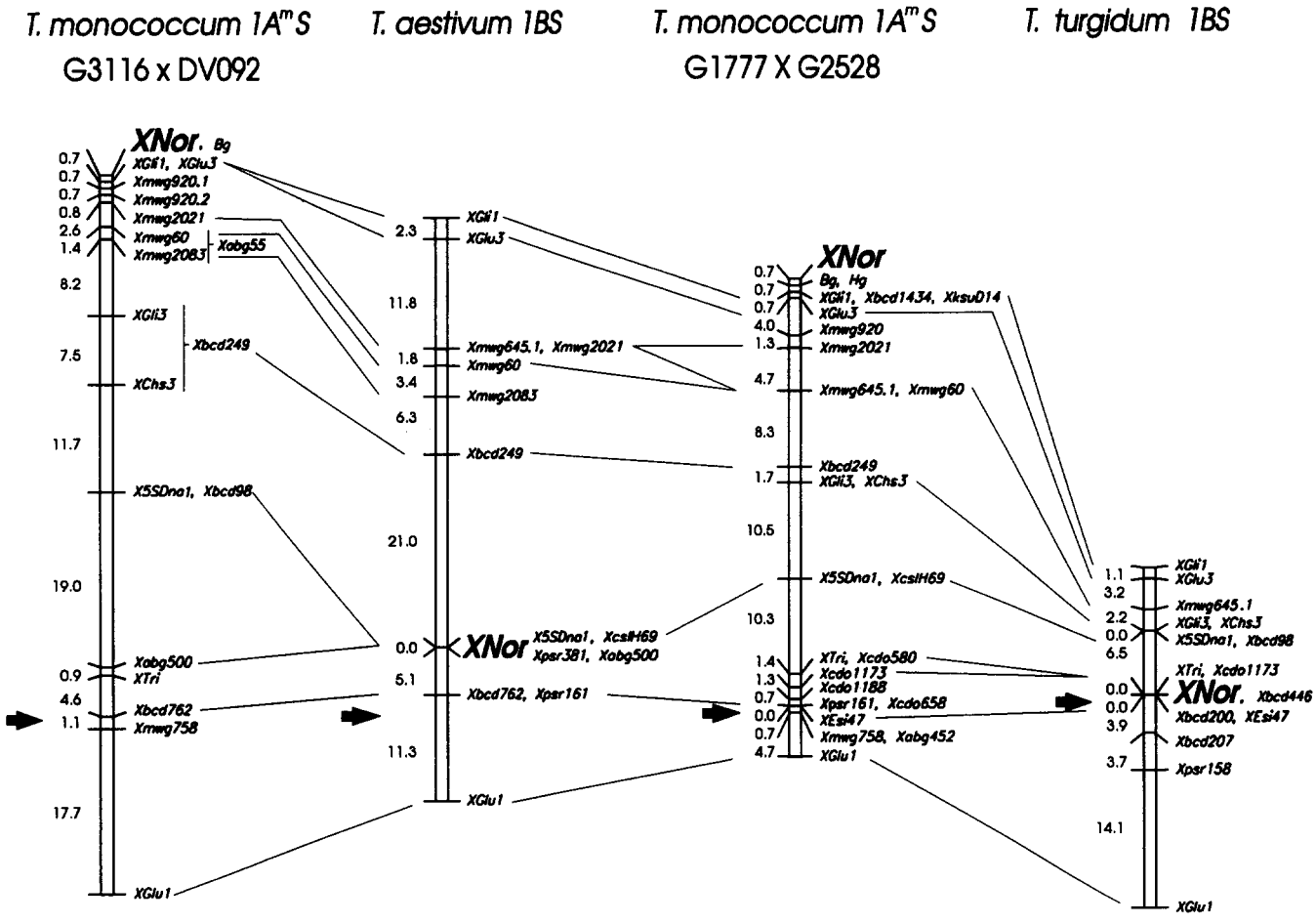

FIGURE 2.—Comparative linkage maps of chromosome arm *1A^mS* in two *T. monococcum* mapping populations and of chromosome arm *1BS* in a *T. aestivum* cv. Chinese Spring × cv. Cheyenne mapping population (*T. aestivum 1BS*) and *T. turgidum* ssp. *durum* cv. Langdon × disomic substitution of *T. turgidum* ssp. *dicoccoides 1B* in cv. Langdon mapping population (*T. turgidum 1BS*). Morphological loci were *Bg* (black glume) and *Hg* (hairy glume). The remaining loci were mapped by hybridization of DNA probes. Loci of unknown function were mapped with the following clones. Those designated *Xabc* were mapped with cDNA clones from barley (KLEINHOFS *et al.* 1993), *Xbcd* and *Xcdo* with cDNA clones from barley and oat, respectively (ANDERSON *et al.* 1992); *XcsIH* with a cDNA probe from wheat (LAGUDAH *et al.* 1991), *Xmwg* with cDNA or genomic clones from barley (GRANER *et al.* 1991); *Xksu* with genomic clones from *T. tauschii* (GILL *et al.* 1991); *Xwg* with genomic clones from wheat (ANDERSON *et al.* 1992); and *Xpsr* with cDNA or genomic clones from wheat (WANG et al. 1991). The following loci of known function were mapped (probe and reference): *X5SDna* (pTa794) (GERLACH and DYER 1980), *XChs* (pcChS11) (ROHDE *et al.* 1991), *XEsi47* (pESI47) (GULICK and DVOŘÁK 1990), *XGli1* and *XGli3* (pcP387) (FORDE *et al.* 1985), *XGlu1* (pDY10A/KS-) (ANDERSON *et al.* 1989), *XGlu3* (pTdUCD1) (CASSIDY and DVOŘÁK 1991), and *XTri* (Tri25–11) (SINGH *et al.* 1993). Distances are in centimorgans. The positions of the centromeres, inferred by mapping of wheat ditelosomic stocks and *T. monococcum*/wheat ditelosomic substitution lines, are indicated by arrows.

served 0.75 FL (fraction of the total arm length from the centromere) in a medium-sized metacentric chromosome. Comparison of the morphology of this chromosome with the consensus karyotype of the Triticum-Aegilops group (DVOŘÁK *et al.* 1986) suggested that this might be chromosome *6A^m*. The same site was probably also observed by JIANG and GILL (1994), who concluded that it might be on chromosome *7A^m* and tentatively designated it *Norx* (Table 1). A minor site, observed less consistently, was in the telomeric region of the long arm of one of the chromosomes bearing a major rDNA site and probably is the same site as that observed by JIANG and GILL (1994) and named *Nor-A7*. Additional potential minor sites are indicated in Figure 4. In spite of observing these minor rDNA sites, no minor sites were proximal to the major sites on the short arms of chromosomes *1A^m* and *5A^m*.

DISCUSSION

The rDNA loci on chromosome arms *1BS* of wheat and *1RS* of rye are located interstitially, and comparative linkage mapping suggested that the loci are orthologous (VAN DEYNZE *et al.* 1995). Because the divergence of diploid wheats, *T. monococcum* and *T. urartu*, from the *S*-genome Aegilops species must be more re-

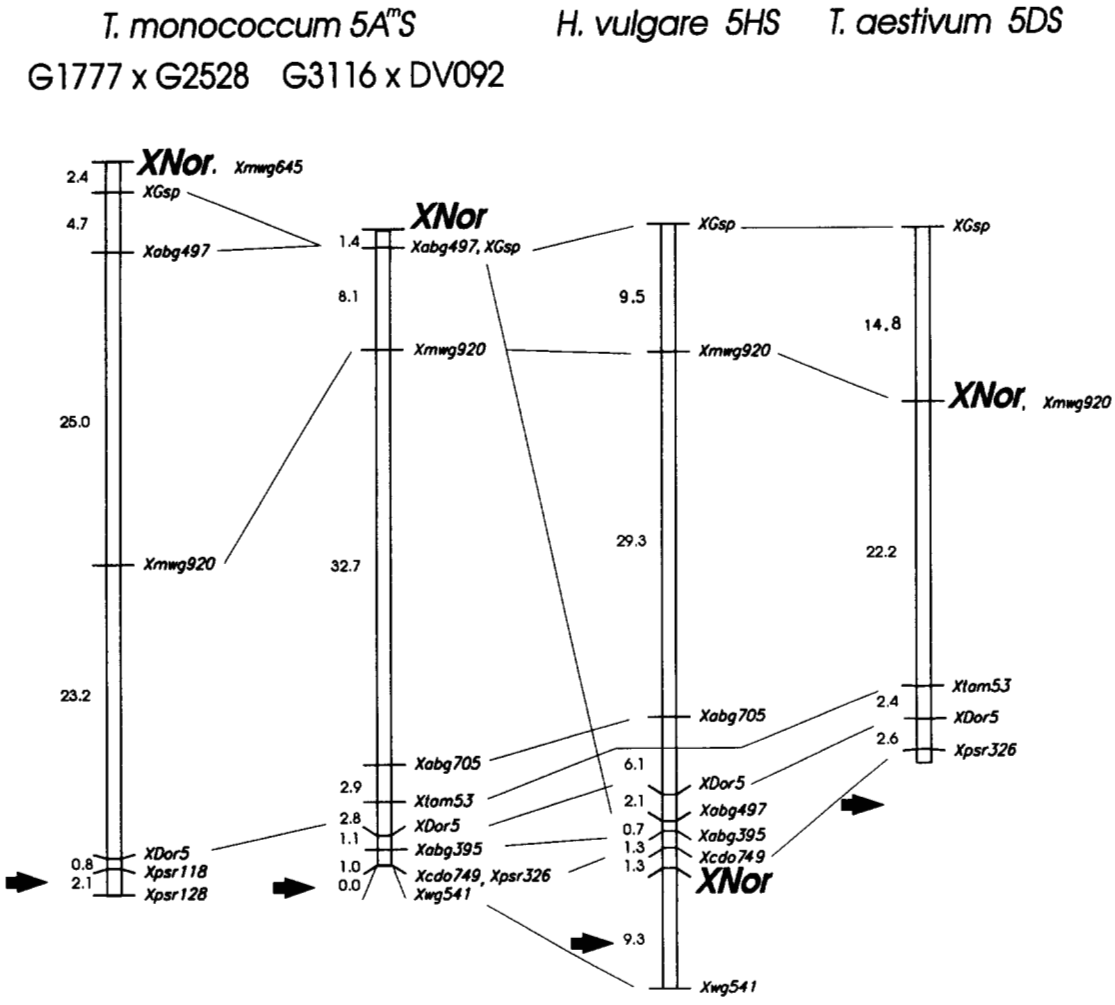

FIGURE 3.—Comparative linkage maps of chromosome arm *5A^mS* in two mapping populations of *T. monococcum*, *5HS* in the Steptoe × Morex barley mapping population (*H. vulgare 5HS*) (KLEINHOFS *et al.* 1993 in MATTHEWS and ANDERSON 1994) and *5DS* in the *T. aestivum* cv. Chinese Spring × RL5406 mapping population (*T. aestivum 5DS*). The designation of loci, probes and their sources are described in Figure 2 except for loci designated *Xtam*, which were mapped with probes produced by DEVEY and HART (1993). The following loci of known function were mapped (probe and reference): *XDor5* (pMA1951) (MORRIS *et al.* 1991), and *XGsp* (pGsp) (RAHMAN *et al.* 1994). The distances are given in centimorgans. The positions of the centromeres, inferred by telocentric mapping, are indicated by arrows.

cent than their divergence from rye, the interstitial position of the homoeologous group *1 Nor* locus is likely ancestral and the terminal position, as in the *A^m* genome of *T. monococcum* (GERLACH *et al.* 1980) and the *A* genome of *T. urartu* (MILLER *et al.* 1983) and wheat (MUKAI *et al.* 1991), is likely derived. Except for the change in the position of the *Nor* locus, chromosome arms *1A^mS* and *1BS* are colinear. Both appear to be also colinear with the short arm of the consensus chromosome *1* in the tribe Triticeae, including wheat, rye and barley (VAN DEYNZE *et al.* 1995).

These findings make it very unlikely that the terminal position of the *Nor* locus in *T. monococcum* chromosome *1A^m* and wheat chromosome *1A* is a result of structural changes in the short arm. To place the *Nor* locus terminally by a paracentric inversion and not to perturb the colinearity of the arm would require reinversion by breaks in a close vicinity of the original breaks that resulted in the inversion of the *Nor* locus terminally. While this is not impossible, it is unlikely. What makes this possibility even more unlikely is that the same paracentric inversion and reinversion would have to be postulated for the *5A^mS* chromosome arm. In chromosome arm *5A^mS*, the order of markers is colinear with those in the *D* genomes of wheat and *Ae. tauschii* and the barley genome. Yet, the *Nor* locus on the *5A^m* chromosome is terminal whereas it is interstitial in the *D* genome and the barley genome. The *Nor* locus on *T. monococcum* chromosome *5A^m*, like that on chromosome *1A^m*, has moved distally without a concomitant inversion in the arm.

GILL and APPELS (1988) showed that the nucleotide sequences of nontranscribed spacers of rDNA on chromosomes *1A^m* and *5A^m* could not be distinguished by thermal

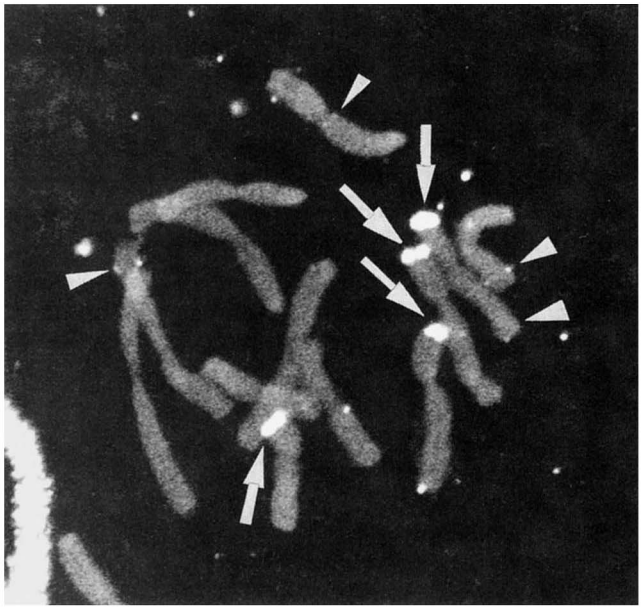

FIGURE 4.—Fluorescent *in situ* hybridization of rDNA clone pTa71 with *T. monococcum* metaphase chromosomes. The major rDNA hybridization sites are indicated by arrows and the minor sites by arrowheads. Note the absence of any minor rDNA site proximal to the major ones.

stability studies of reassociated duplexes in Southern blots and concluded that this is due to recent duplication of the *Nor* loci via duplication of chromosome arm or its portion. If their hypothesis were true, the terminal position of the *Nor* locus on $1A^m$ could be the result of duplication of the terminal position of the *Nor* locus on chromosome $5A^m$, or vice versa. An analogous mechanism was suggested to be responsible for homogenization among the five *Nor* loci in the human genome (ARNHEIM 1983). While the duplication hypothesis might explain the origin of the terminal position of the *Nor* locus on one of the two *T. monococcum* NOR chromosomes, it would not explain the terminal position of both loci. Markers in the short arms of chromosomes $1A^m$ and $5A^m$, except for the *XNor*, *Xmwg920* and *X5SDna* loci, were not duplicated (Figures 2 and 3).

The changes in the positions of the *Nor* loci without concomitant structural changes in the chromosome arms have not been limited to the *T. monococcum* genome. Although the *Nor* loci on the *5DS* and *5HS* arms are both interstitial, they are not in identical positions; the *5HS* locus is more proximal than the *5DS* locus. The low level of polymorphism between Chinese Spring and RL5406 limited the number of loci that could be mapped on the *5DS* arm. Nevertheless, the position of the *Nor-D3* locus on the *5DS* arm inferred here agrees with the inferred position of the *Nor-D3* locus on *Ae. tauschii* chromosome *5D* reported by LAGUDAH *et al.* (1991). The more distal location of the *Nor* locus on *5DS* than on *5HS* is consistent with the smaller size of the *5DS* satellite than that of the *5HS* satellite.

It is possible that rDNA loci change position by the same, albeit unknown, mechanism that results in the dispersion of heterochromatic sequences through a genome. *In situ* rDNA hybridization with the wheat, barley and *T. monococcum* chromosomes revealed existence of minor rDNA sites in these genomes (MUKAI *et al.* 1991; LEITCH and HESLOP-HARRISON 1992; JIANG and GILL 1994; present data). One *T. monococcum* minor locus, probably the same as that previously reported by JIANG and GILL (1994) to be on $7A^m$, may alternatively be on chromosome $6A^m$. In that case, it could be a reduced *Nor2* locus that was mapped on wheat chromosome *6B* (DVOŘÁK and CHEN 1984; DVOŘÁK and APPELS 1986). In addition to this locus, other minor rDNA loci were found in the *T. monococcum* genome in the centromeric or telomeric regions. If minor rDNA loci contain functional rDNA units, their copy numbers can potentially magnify by unequal crossing over to become major rDNA loci. The competition among the loci for regulatory proteins can result in suppression of the original *Nor* locus. Because hypostatic *Nor* loci are prone to deletion (DVOŘÁK 1989), the copy number of the rDNA units in the hypostatic locus is likely to become reduced or the locus entirely deleted. A result of this process would be an apparent movement of a major *Nor* locus without any structural changes.

Movement of a major *Nor* locus may be, as hypothesized above, associated with temporal existence of two *Nor* loci in a chromosome arm. Comparative mapping of the tomato (*Lycopersicon esculentum* Mill.) genome with that of a hybrid between *Capsicum annuum* L. × *C. chinense* Jacq. showed that the three genomes share a common rDNA site that is on chromosome *2* in tomato and is on a translocated segment of chromosome *1/2* in Capsicum (TANKSLEY *et al.* 1988). While there is a single rDNA site on the tomato chromosome *2*, there is a duplicated rDNA site located distally on the Capsicum chromosome *1/2* (TANKSLEY *et al.* 1988). In *C. chinense,* but not in *C. annuum,* there is another site terminally located in the linkage group V (TANKSLEY *et al.* 1988). The two rDNA loci in Capsicum chromosome *1/2* may represent an intermediate state of the distal movement of the *Nor* locus in the Capsicum chromosome.

If major *Nor* loci in the *T. monococcum* genome have changed position by the above process, there should be minor rDNA loci at the sites of the original *Nor*-A1 and *Nor*-A3 loci on chromosome arms $1A^mS$ and $5A^mS$, respectively. However, such loci were not observed by *in situ* hybridization, even though other minor sites were observed. Nor was a minor site reported near the major locus on chromosome arm *5DS* or chromosome arm *5HS* (MUKAI *et al.* 1991; LEITCH and HESLOP-HARRISON 1992). Although minor rDNA loci that could possibly be orthologous to the major loci, and might have originated by the reduction of major loci, were observed on

barley chromosome *1H* (LEITCH and HESLOP-HARRISON 1992; however, see below), *Ae. longissima* Sweinf. et Muschl. chromosome *1S*^{l} (FRIEBE *et al.* 1993) and possibly *T. monococcum* chromosome *6A*^{m} (present data), in other cases no such reduced loci were detected, such as on *Ae. tauschii* chromosomes *1D* and *6D* (MUKAI *et al.* 1991). In the case of *1D* and *6D*, major *Nor1* and *Nor2* loci must have existed in the phylogenetic lineage leading to *Ae. tauschii* because the species of the genus Aegilops are monophyletic (DVOŘÁK and ZHANG 1992) and because major loci do exist on short arms of chromosomes *1* and *6* in positions similar to those of *Nor1* and *Nor2* in other Aegilops species, such as *Ae. speltoides* Tausch (DVOŘÁK *et al.* 1984). A possible explanation for the absence of minor loci corresponding to the major *Nor1, Nor2*, or *Nor3* loci is that they have been reduced below the detection level of the *in situ* DNA hybridization techniques or that they have been entirely eliminated during evolution.

A puzzling observation is that the movement of NORs appears to be nonrandom. The new NORs tend to be in the same chromosome arms as the lost NORs; in the present cases the short arms of chromosomes *1* and *5*. The transposition of the mobile genetic elements also shows a tendency to transpose preferentially to nearby loci on the same chromosome (VAN SCHAIK and BRINK 1959; GREENBLATT and BRINK 1962). Whether this reflects a commonalty between the mechanism of the movement of *Nor* loci and transposition or is caused by natural selection for new positions of *Nor* loci in chromosome regions that permit full expression and function of the NORs is not clear. The demonstration that NORs tend to occur preferentially in the short arms and in preferred positions relative to the centromere and the telomere (LIMA-DE-FARIA 1976) suggests that the positions of NORs within chromosome arms is constrained during evolution. It must be pointed out that it is not known whether the *Nor* loci in the new positions on chromosomes *1A*^{m} and *5A*^{m} originated from the *Nor* loci on the same chromosomes or from *Nor* loci on nonhomologous chromosomes. That dilemma and, hence, the question of orthology and paralogy of nucleotide sequences in the loci, can be resolved only by isolation and sequencing of rDNA units from each rDNA site in the *T. monococcum* genome and from rDNA sites in genomes of relevant *T. monococcum* relatives.

It has been tacitly assumed that rDNA loci present at the same approximate locations within metaphase chromosome arms of homoeologous chromosomes are the same and they have been named as such (Table 1), without a direct evidence. The present findings illustrate fallacy of that assumption. The *Nor* loci on *T. monococcum* chromosome arm *1A*^{m}*S* and wheat chromosome arm *1AS* are not the same as the *Nor1* locus on chromosome arm *1BS* and are not *Nor1*, as concluded earlier (MUKAI *et al.* 1991; JIANG and GILL 1994), but a new locus *Nor9* (eight rDNA loci have thus far been designated as *Nors*; Table 1). Likewise, the locus on chromosome *5A* is not the same as locus *Nor3* on wheat chromosome *5D* and should be designated *Nor10*, not *Nor-A3* as it was designated by JIANG and GILL (1994). We prefer not to designate the minor rDNA loci as *Nor* loci without evidence that they contain functional rDNA units and can indeed function as NORs. Because the *Nor* locus on barley chromosome *5H* is not the same as the *Nor3* locus, the traditional designation *Rrn2* (SAGHAI-MAROOF *et al.* 1984; KLEINHOFS *et al.* 1993) or *Nor11* are preferable to a designation implying that it is the same locus as *Nor3*, as done by LEITCH and HESLOP-HARRISON (1992). The assumption of orthology between the reduced rDNA site on barley chromosome *1H* and *Nor1* (LEITCH and HESLOP-HARRISON 1992) should be treated with great caution, particularly because *in situ* DNA hybridization showed that the barley locus is more proximal in the metaphase chromosome than *Nor-B1* (LEITCH and HESLOP-HARRISON 1992).

A relevant question is whether the mobility of *Nor* loci is unique to the tribe Triticeae or whether it is their general attribute. Indirect evidence exists that in *Allium cepa* L. and *A. fistulosum* L. NORs "jump" around the genome (SCHUBERT 1984; SCHUBERT and WOBUS 1985). The inference was based on the variability in the size, number, and chromosomal position of NORs in cultivars of *A. cepa* and hybrids between *A. cepa* and *A. fistulosum*. Unfortunately, the possibility of translocations involving the terminal regions of the chromosomes, where all the Allium *Nor* loci were found, cannot be ruled out in the absence of comparative gene mapping. Moreover, magnification of minor rDNA sites, which have been shown to be numerous in Triticeae and which would not had been detected by the *in situ* DNA hybridization technique employed by SCHUBERT and WOBUS (1985), and reductions of major rDNA sites could result in the observed intraspecific and interspecific polymorphism in the positions of NORs without any actual "jumping" of NORs. Although more work is needed to clarify the nature of the polymorphism for the NOR locations in the Allium genomes, data presented here for Triticeae showed that NORs do change position without structural rearrangements of chromosomes on the evolutionary time scale and it is likely that a similar situation will be found for the Allium NORs when rigorously scrutinized.

A number of mechanisms have been implicated in the homogenization of repeated nucleotide sequences, including rDNA. Saltatory replication and deletions of sequences (BRITTEN and KOHNE 1968), unequal exchanges (TARTOF 1975; SMITH 1976; PETES 1980), replication slippage (FARABAUGH *et al.* 1978), nucleotide sequence conversions (BIRKY and SKAVARIL 1976; KLEIN and PETES 1981; DVOŘÁK *et al.* 1987), intrachromoso-

mal recombination and insertion of circular intermediates into another locus (DVOŘÁK 1989), and translocations of distal chromosome regions (ARNHEIM 1983) are few of the suggested mechanisms. The latter mechanism was suggested to be responsible for the homogenization among and the origin of new rDNA loci in the primate genomes (ARNHEIM 1983). It is possible, however, that primate rDNA loci may move among chromosomes in a similar fashion as those in Triticeae. That would account for the observation that the distribution of NORs is not entirely consistent with the chromosome homoeologies in primate genomes (TANTRAVAHI *et al.* 1976; HENDERSON *et al.* 1977; SEUANEZ 1979). This cold be one more mechanism for rDNA homogenization.

There is also disagreement in the location of rDNA loci in *Drosophila melanogaster* and its closest relative, *D. simulans* (LOHE and ROBERTS 1990). Although it is possible that the absence of rDNA on the *D. simulans Y* chromosome was caused by a deletion of the locus, it is also possible that the *Y*-chromosome rDNA in *D. melanogaster* originated by movement of rDNA from the *X* chromosome. A circular intermediate has been speculated to be potentially responsible for the insertion of large number of copies of the 240-bp repeats from the rDNA nontranscibed spacer to the terminus of the long arm of the *D. simulans Y* chromosome (LOHE and ROBERTS 1990). The same mechanism may also result in movements of the entire rDNA units.

The finding that major *Nor* loci may move within and among chromosomes and that their movements may potentially occur via magnification of minor loci consisting of a few rDNA copies—the numbers of repeated rDNA units in the barley minor sites were estimated to vary between 5 and 100 copies, depending on the site (LEITCH and HESLOP-HARRISON 1992)—is of a serious concern for the use of rDNA sequences in phylogenetic reconstructions at the generic level. The sequences of the rDNA internal transcribed spacer (ITS) have been used for such purpose (BALDWIN 1992). Because this phylogenetic strategy employs a single molecule for phylogenetic inferences and, hence, is a single-trait method, violation of the assumed orthology would have serious consequences for the analyses. The deletions of major rDNA sites and their replacements by magnified minor, potentially paralogous, rDNA sites can lead to sudden, stochastic fluctuations in the rDNA consensus sequence in an evolutionary lineage. This could result in discontinuities in the rDNA lineages at the evolutionary time scale. This strategy of phylogenetic reconstructions should, therefore, be treated with a great deal of caution.

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