Differential amplification of rDNA repeats in barley translocation and duplication lines: role of a specific segment

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Variation in restriction pattern, relative amounts of the two ribosomal DNA (rDNA) repeats, and the overall content of rDNA were compared among twelve segmental duplications and eleven parental translocations involving NOR6 and NOR7 of cultivated barley. Southern blot hybridization revealed two rDNA repeats of 9.9 kb and 9.0 kb. While all duplications showed dimers for these rDNA repeats, the duplication lines D29 and D47 displayed trimers in addition to a higher proportion of rDNA repeats as dimers. The rDNA of D1, D29 and D47 showed resistance to Bam HI and Taq I digestion, indicating possible methylation of cytosine and adenine. Densitometric scans of autoradiographs revealed variations in the relative amounts of the 9.0 kb and 9.9 kb rDNA repeats among different karyotypes. Dot blot hybridizations indicated variation in the overall rDNA content. Comparison of the 9.0/9.9 kb ratios and the percentage of genomic DNA hybridizing to an rDNA clone of barley illustrates differential amplification for the two rDNA repeats. When the segmental composition of these deviating lines were compared, it was evident that the relative position of the segment 12–16 of chromosome 6 determines differential amplification while duplication of the same segment controls the overall rDNA content.

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Genes coding for the 18S, 5.8S, and 25S rRNAs are organized in tandem arrays of repeating units in enormous amounts in higher plants. The number of units per haploid genome varies from 570 in *Arabidopsis thaliana* to over 32,000 copies in *Hyacinthus orientalis* (INGLE et al. 1975; PRUITT and MEYEROWITZ 1986). There is also a distinct variation within the same species (FLAVELL and SMITH 1974; CULLIS 1976; PHILLIPS 1978; SUBRAHMANYAM and AZAD 1978).

The number of arrays of rDNA repeats per genome is usually small, e.g., in barley where chromosomes 6 and 7 carry these two loci (SUBRAHMANYAM and AZAD 1978; APPELS et al. 1980). The length of the rDNA repeats varies between species from 7 kb in soybean (VARSANYI-BREINER et al. 1979) to over 12 kb in wheat (APPELS et al. 1980). This length variation is due to different amounts of intergenic subrepeats as shown in wheat (GERLACH and BEDBROOK 1979; APPELS et al. 1980), barley (GERLACH and BEDBROOK 1979; SAGHAI-MAROOF et al. 1984), and *Vicia faba* (YAKURA and TANIFUJI 1983; ROGERS et al. 1986;

ROGERS and BENDICH 1987). In spite of the variation for rDNA repeat lengths within species, and even within a plant, at individual loci there is considerable homogeneity for the number of subrepeats of intergenic DNA (APPELS et al. 1980; APPELS and DVORAK 1982).

Duplication of the Nucleolar Organizer Region (NOR) in maize results in a larger pachytene nucleolus and a double copy number of rRNA genes (PHILLIPS et al. 1971), while the monosomic for the nucleolar organizer (chromosome 6) contains half the number of rRNA genes. In barley, NOR6 contains 1,600 copies and NOR7 2,600 copies (SUBRAHMANYAM and AZAD 1978), yet chromosome 6 organizes a larger nucleolus than chromosome 7. This fact suggests that the size of the nucleolus is related to the proportion of total active rRNA genes rather than the absolute number of rRNA genes (SUBRAHMANYAM and AZAD 1978). This conclusion is supported by the findings in wheat (FLAVELL and O'DELL 1979). Thus, most of the rRNA genes are inactive and they are condensed in heterochromatin (RAMIREZ and

SINCLAIR 1975; GIVENS and PHILLIPS 1976; DOERSCHUG 1976; PHILLIPS 1978).

The active NOR loci in wheat have a higher proportion of rRNA genes with unmethylated cytosine residues in comparison with less active or inactive loci. The proportion of genes with methylated cytosine residues at CCGG sites also increases as the total rDNA increases (FLAVELL et al. 1988).

The total number of rRNA genes in barley is known to be influenced by the increased dosage of chromosomes, not only NOR6 and NOR7 but also other chromosomes not known to carry any rRNA genes (SUBRAHMANYAM and AZAD 1978). In wheat, deleting or duplicating 14 of the 17 chromosomes with no rDNA loci brings about alteration in the number of nucleoli formed (FLAVELL and O'DELL 1979). In barley, when both NOR6 and NOR7 are brought on to the same chromosome through translocation, the activity of NOR7 is reduced (ANASTASSOVA-KRISTEVA et al. 1980). However, in maize the translocation of NOR to other chromosomes does not prevent its activity (RAMIREZ and SINCLAIR 1975; GIVENS and PHILLIPS 1976; DOERSCHUG 1976; PHILLIPS 1978). Investigations on the location of different restriction sites in cereal rDNA repeats (GERLACH and BEDBROOK 1979; APPELS et al. 1980) and the rDNA spacer length polymorphisms (SAGHAI-MA-ROOF et al. 1984) revealed two rDNA repeat lengths at the two rDNA loci Rrn1 and Rrn2.

Information on the specific role of structural alterations such as translocations or duplications on rDNA variation is lacking. In this paper we present evidence for: (i) differential amplification at the two rDNA loci in barley, (ii) a chromosomal segment other than NOR modulating the amplification of rDNA, and (iii) alterations in the methylation pattern of rDNA in duplication lines.

Materials and methods

Plant material

The barley material included 11 homozygous translocation lines (Table 1), involving breakpoints in chromosomes 6 and 7 (HAGBERG et al. 1978), and 12 homozygous duplication lines (Table 2) derived from intercrosses of the translocation lines in the genetic background of cv. 'Betzes' and/or 'Bonus' (HAGBERG and HAGBERG 1978, 1991,

1992). Since 'Bonus' and 'Betzes' do not differ in their rDNA content and repeat length pattern, the F_1 hybrid between 'Bonus' and 'Betzes' was used as the standard karyotype (Fig. 1). It has to be stressed that the translocation breakpoint positions indicated in the chromosomes in Fig. 1 are based on cytogenetic observations. The chromosome segments between breakpoint positions have been designated numbers for the simplicity of discussion. Breakpoints and numbers might be subject to future adjustments.

## DNA purification and hybridization techniques

Shoots from three-week old greenhouse-grown plants were freeze dried and powdered using a mechanical mill. DNA was extracted essentially as described by MURRAY and THOMPSON (1980) and further purified on ethidium bromide-CsCl gradients.

Total genomic DNA ($3 \mu g$) was digested with restriction enzyme (15–25 units), separated on 0.8 % agarose gels (2 V/cm), and transferred to GeneScreen membranes (DuPont) by electroblotting. The 9.9 kb barley rDNA repeat in pHv294 (GERLACH and BEDBROOK 1979) was labelled by random priming using α^{32}P-dCTP. The membranes were prehybridized at 65°C for 5 h in 2 × SSC, 1 % SDS, 10 × Denhardt's solution, 50 mM sodium phosphate pH 6.8, and 0.1 mg/ml of salmon sperm DNA. Hybridization was carried out by the addition of ^{32}P-labelled probe and incubation for 12 h at 65°C. The filters were first washed with two changes of 1 × SSC, 1 % SDS, and then twice with 0.1 × SSC, 1 % SDS at 65°C. After drying, the filters were exposed to X-ray film. The autoradiograms were scanned using an LKB 2202 ultrascan laser densitometer. The peak heights were used to determine the relative level of hybridization in each band.

The rDNA copy number was determined using ribonuclease A treated DNA. Samples of 0, 40, 100, 200, and 400 ng of DNA from all barley lines and 0, 0.4, 1, 2, and 4 ng of Eco RI digest of the rDNA clone pHv294 were immobilized on to GeneScreen membranes in a dot blot apparatus and hybridized as above. Each dot was cut out and assayed by liquid scintillation counting. Taking the extent of hybridization of rDNA probe to itself as 100 %, the relative proportion of genomic DNA hybridized to rDNA was determined.
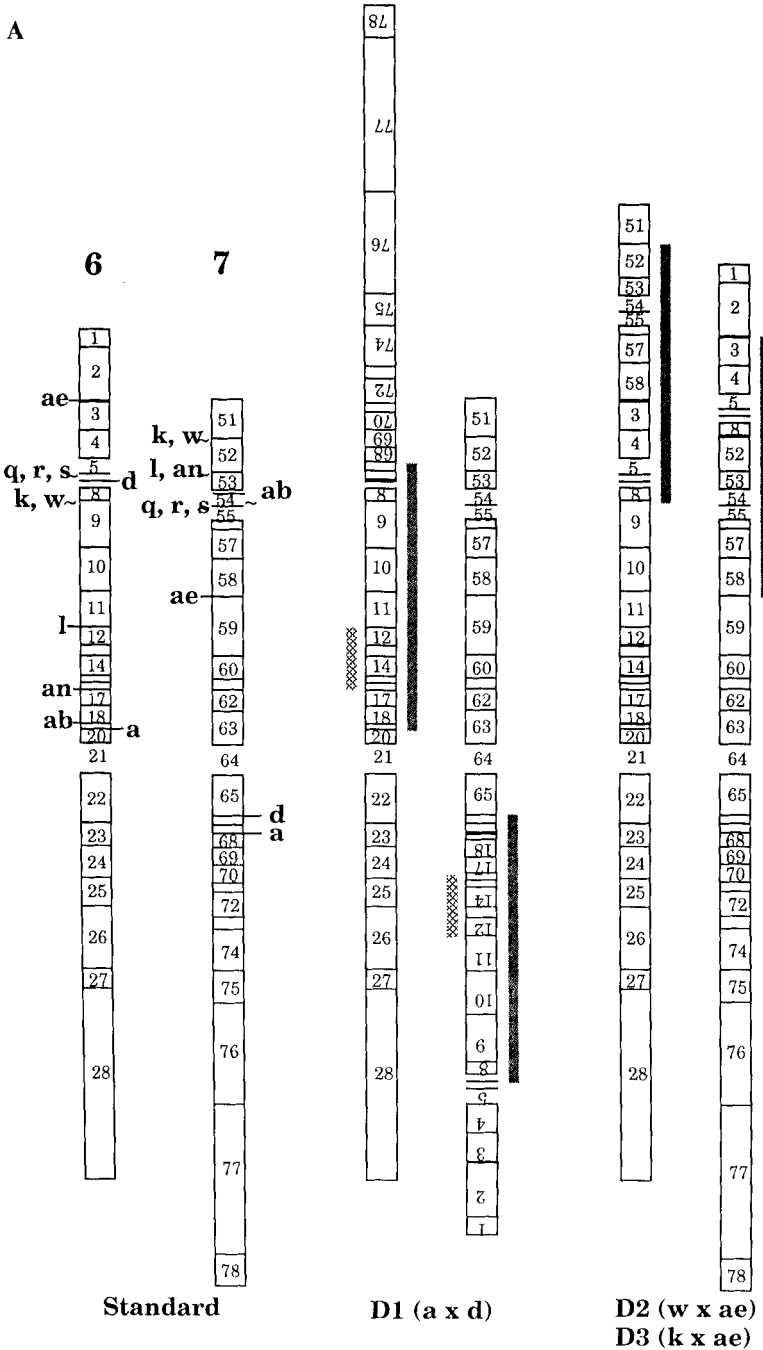
Fig. 1A–C. Idiograms of chromosomes 6 and 7 in the standard karyotype and in 12 duplication lines. Segments 1–28 and 51–78 in chromosome 6 and 7, respectively, are between translocation break points located in the chromosomes, with all available information in consideration. By crosses between translocation lines with suitably located breaks, duplications were obtained in the F_2-generation and identified cytologically. The break points of translocations are indicated by letters. T6–7a, d, k, l, q, r, s, w, ab, ae, and an are indicated in the standard karyotype (**A**).

B

D5 (an x ae)   **D7 (l x ae)**   **D8 (l x an)**
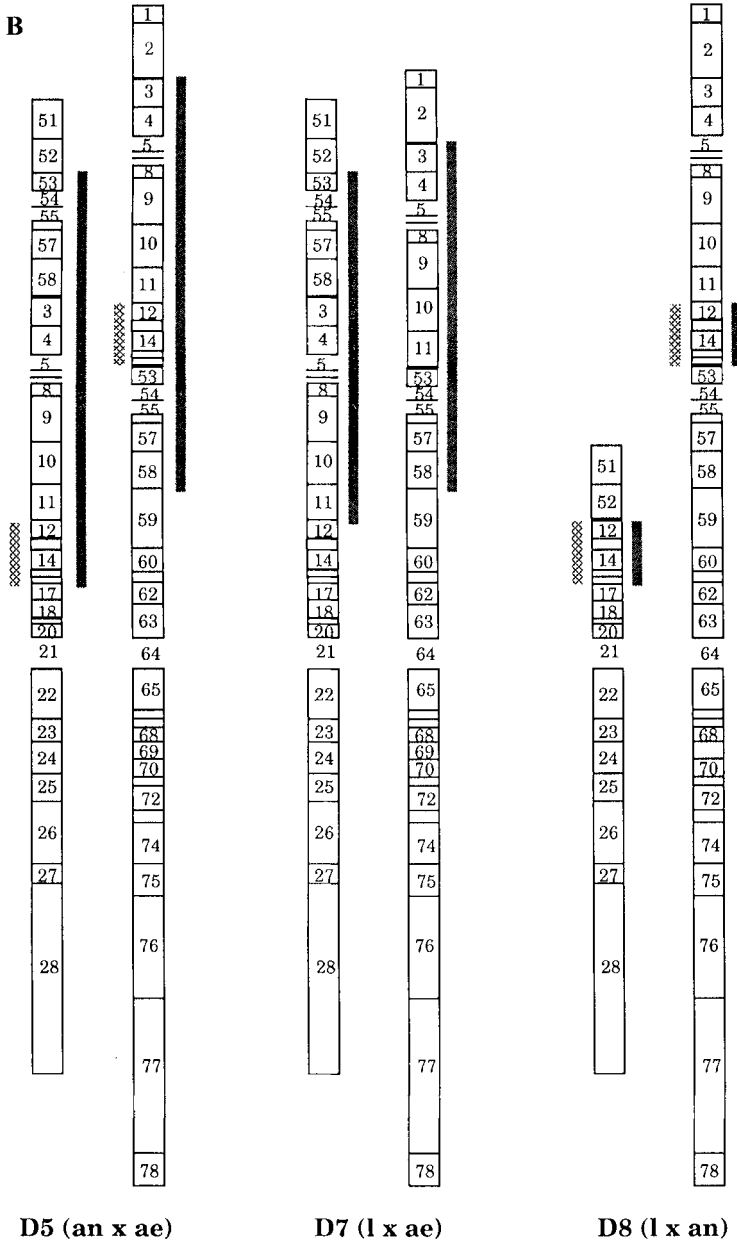
Fig. 1. cont. Included are also the duplications D1 (from T6–7a × d), D2 (T6–7w × ae), and D3 (T6 7k × ae). In (**B**) D5, D7, and D8 are presented, and in (**C**) D24, D25, D29, D26, D46, and D47. The duplicated segments in each duplication line are marked by bars. The position of segment 12–16 is marked by a hatched bar when duplicated (D1, D5, D8, and D26).

C

D24 (q x ae)   D26 (r x an)   D46 (q x w)
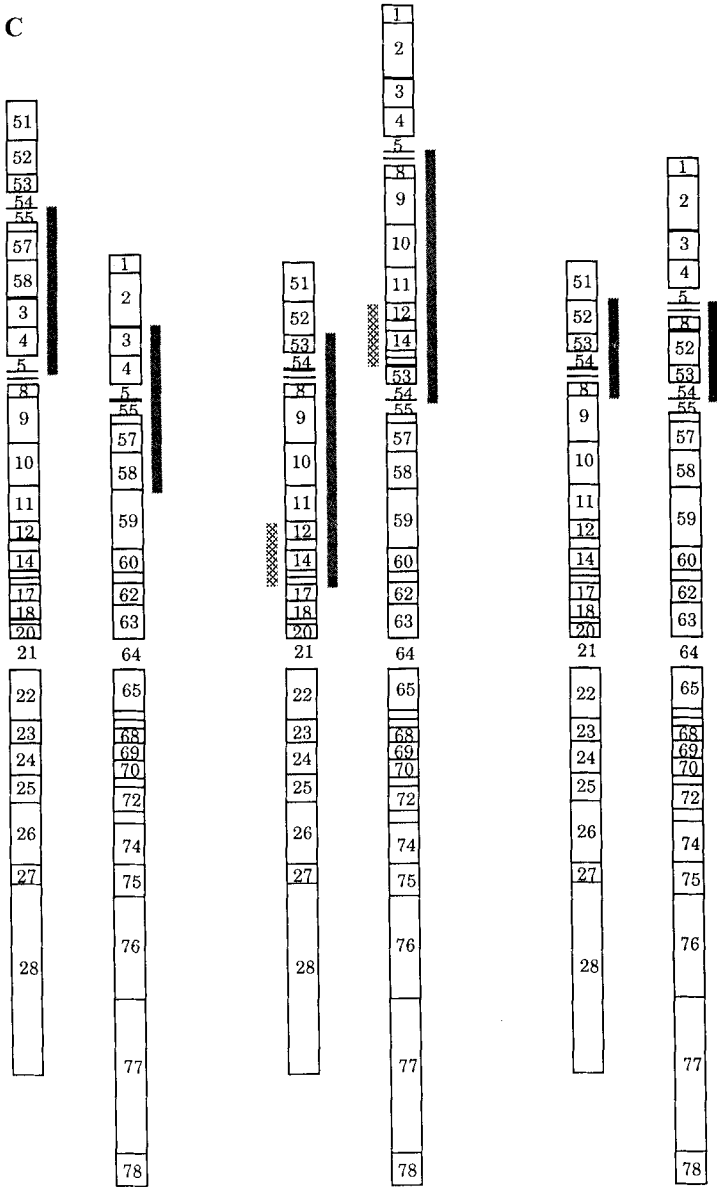D25 (r x ae)   D47 (s x w)
D29 (s x ae)

Fig. 1 cont.

Results

Twelve different homozygous duplication lines (Fig. 1), covering the short arm of chromosomes 6 and 7 of barley, were chosen to assess the effect of differing segments on rDNA. To differentiate dosage effect from transpositional effect, eleven parental translocation lines from which these duplications were derived, and a standard karyotype, as a control, were included (Table 1). Density scans of the autoradiographs, following Southern blot

Table 1 Origin of used translocation lines (see also HAGBERG et al. 1978)

Karyotype	Source variety	Mode of induction	Stage of development	Year of induction
T6–7a	Bonus	X-rays, 7580r + 0.1% colch.	Seeds	1946
T6–7d	Bonus	Acute γ-rays, 8070r	Seeds	1954
T6–7k	Bonus	X-rays, 4000r	Seeds	1957
T6–7l	Bonus	Chronic γ-rays, 3925r	Plants	1957
T6–7q	Betzes	Spontaneous		
T6–7r	Betzes	Spontaneous		
T6–7s	Betzes	Spontaneous		
T6–7w	Bonus	Acute γ-rays, 600r	Meiosis	1958
T6–7ab	Bonus	Acute γ-rays, 800r	Meiosis	1960
T6–7ae	Bonus	Chronic γ-rays, 2675r	Plants	1958
T6–7an	Bonus	Acute γ-rays, 800r	Meiosis	1963

Table 2. Origin and characteristics of used duplication lines. For numbering of segments, see Fig. 1. NOR6 consists of the segments 5–7 and NOR7 of 54–55

| Dupli-cation	Parental combination	Duplicated segments	Duplication of NOR	Duplication of segment 12–16
D1	T6–7a × T6–7d	7–19 and 66–67	7	+
D2	T6–7w × T6–7ae	3–8 and 52–58	5–7 + 54–55	−
D3	T6–7k × T6–7ae	3–8 and 52–58	5–7 + 54–55	−
D5	T6–7an × T6–7ae	3–16 and 53–58	5–7 + 54–55	+
D7	T6–7l × T6–7ae	3–11 and 53–58	5–7 + 54–55	+
D8	T6–7l × T6–7an	12–16	...	+
D24	T6–7q × T6–7ae	3–5 and 55–58	5 + 55	−
D25	T6–7r × T6–7ae	3–5 and 55–58	5 + 55	−
D26	T6–7r × T6–7an	6–16 and 53–54	6–7 + 54	+
D29	T6–7s × T6–7ae	3–5 and 55–58	5 + 55	−
D46	T6–7q × T6–7w	6–8 and 52–54	6–7 + 54	−
D47	T6–7s × T6–7w	6–8 and 52–54	6–7 + 54	−

hybridization to genomic DNA from each line, were used to determine the ratio between the amount of rDNA in the 9.0 kb and 9.9 kb repeats (Fig. 2 and 3). The relative levels of these two repeats in the standard karyotype (Fig. 4, lane c) are in agreement with trisomic analysis data, which shows that chromosome 6 has a lower copy number of rDNA than chromosome 7 (SUBRAHMANYAM and AZAD 1978). The less intense band (9.9 kb repeat) is on chromosome 6 and the 9.0 kb repeat is on chromosome 7 since the same background genotypes were used in both studies.

A visual comparison of the hybridization intensities of the bands corresponding to the 9.0 and 9.9 kb repeat units, revealed clear differences among the translocation lines. Density scans of each lane revealed a shift in the band ratio from 1.5 in the control to 0.5 in T6–7an and 1.06 in T6–7d (Fig. 2), indicating a deamplification of the 9 kb repeats and/or amplification of the 9.9 kb repeats. On the other hand, the translocation lines T6–7q and T6–7r, with breakpoints in NOR6 and NOR7, showed an increase in the ratio to 2.3 and 2.2,

respectively. However, only marginal or no shifts are evident in the other lines.

Comparison of the 9.0/9.9 kb ratio among the duplication lines demonstrated striking shifts in the hybridization signals as compared to the control (Fig. 3). The ratios were lower in ten of the twelve duplication lines. Prominent among these shifts are 0.5 for D26, 0.7 for D1 and 0.9 for D8. There was no indication of any increase in the 9.0/9.9 kb repeat ratio above the control in any of the duplications. A characteristic feature of the duplication lines is the appearance of dimers and trimers of the two rDNA repeat units. The duplications D1, D29, and D47 had 16, 30, and 32 % of their rDNA repeats as dimers. D29 and D47 displayed an additional 4 % and 7 % of the two rDNA repeats as trimers following Eco RI digestion (Fig. 4). Interestingly, rDNA from the duplications D1, D29, and D47 were resistant to digestion with Bam HI and Taq I (Fig. 5 and 6), indicating possible methylation in the recognition sequences.

The proportion of genomic DNA hybridizing to the rDNA clone was determined by dot blot analy-
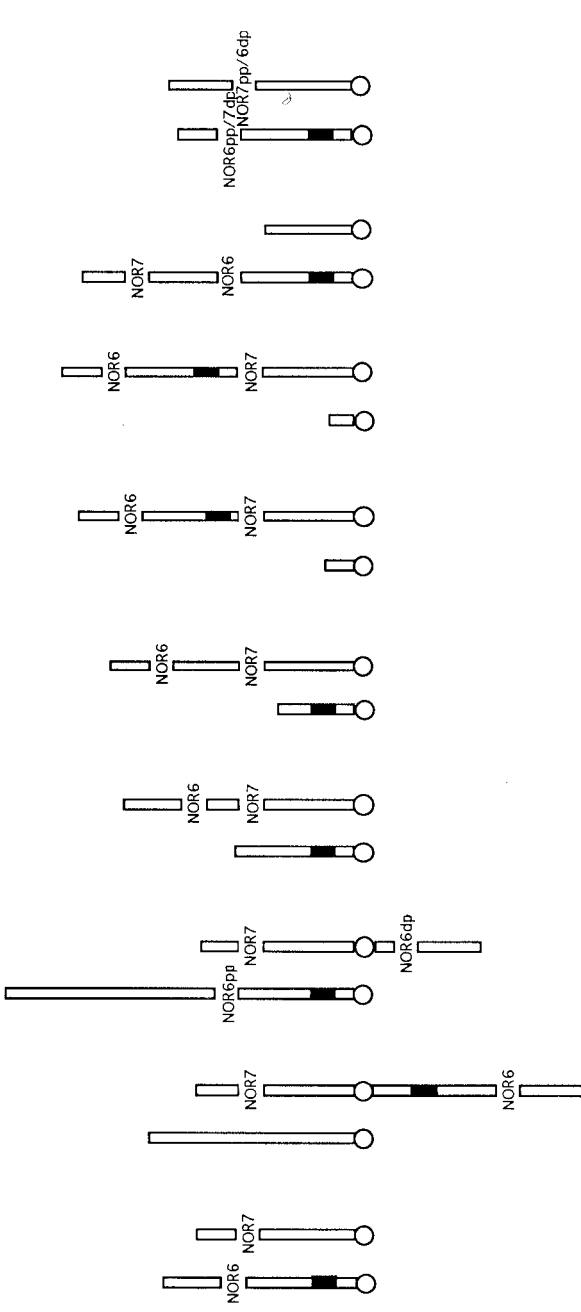
| Karyotype:	Control	T6-7a	T6-7d	T6-7k; T6-7w	T6-7l	T6-7an	T6-7ab	T6-7ae	T6-7q, T6-7r, T6-7s
NOR 6 (9.9 kb):		⇒	⇑	⇑		⇑			
NOR 7 (9.0 kb):						⇓			
9.0/9.9 kb ratio:	1.5	–	1.1	1.5; 1.3	1.7	0.5	1.6	1.7	2.3; 2.2; 1.6
rDNA (%):	1.2 ± 0.4	0.9 ± 0.2	1.4 ± 0.3	1.9 ± 0.4*↑ 1.8 ± 0.4	1.7 ± 0.3	1.0 ± 0.2	1.4 ± 0.3	1.7 ± 0.4	2.6 ± 0.5*↑ 2.0 ± 0.4*↑ 1.9 ± 0.4*↑

Fig. 2. Idiograms of chromosomes 6 and 7 of the standard karyotype and eight translocation lines, limited to the chromosome arms involved in the translocations, i.e., the short arms including NOR 6 and NOR7. The segment 12–16 is shaded. Changes in hybridization intensity of the 9.9 and 9.0 kb rDNA repeats and the proportion of genomic DNA from translocation lines hybridizing to the rDNA clone pHv294 of barley are indicated. An increased hybridization signal compared to the control (standard karyotype) is indicated by ⇑ and a decreased signal by ⇓. Significant changes are indicated by * (P < 0.05). In T6–7d the break has occurred in NOR6; the two new NORs are marked NOR6pp for proximal part and NOR6dp for distal part. In T6–7q, r, and s both NORs are broken. This means that the translocation lines have NOR6pp/7dp and NOR7pp/6dp.
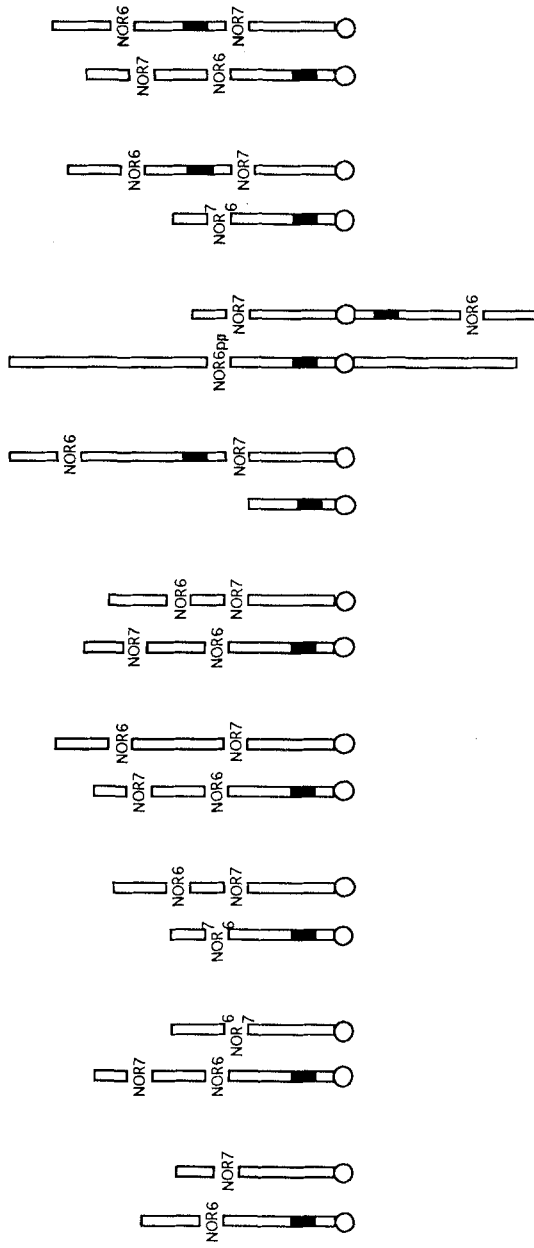
Karyotype:	Control	D24, D25, D29	D46, D47	D7	D2, D3	D8	D1	D26	D5
NOR duplication:	– –	NOR 6 dp NOR 7 pp	NOR 6 pp NOR 7 dp	NOR 6 NOR 7	NOR 6 NOR 7	– –	NOR 6 pp –	NOR 6 pp NOR 7 dp	NOR 6 NOR 7
"12-16" duplication:	–	–	–	–	–	"12-16"	"12-16"	"12-16"	"12-16"
NOR 6 (9.9 kb):		⇑⇑⇑	⇑⇑	⇑	⇑⇑	⇑	⇑	⇑	⇑
NOR 7 (9.0 kb):		⇑⇑⇑	⇑⇑	⇑	⇑⇑	⇓	⇓	⇓	
9.0/9.9 kb ratio:	1.5	1.2; 1.1; 1.6	1.0; 0.9	1.1	1.4; 1.1	0.9	0.7	0.5	1.0
rDNA (%):	1.2 ± 0.4	2.9 ± 0.4 2.7 ± 0.3 2.7 ± 0.3	2.7 ± 0.3 2.1 ± 0.1	2.5 ± 0.4	3.0 ± 0.6 3.0 ± 0.6	1.2 ± 0.2	1.4 ± 0.3	1.5 ± 0.2**↓	1.8 ± 0.4*↓

Fig. 3. Idiograms of chromosomes 6 and 7 of the standard karyotype and twelve duplication lines, limited to the chromosome arms involved in the duplications, i.e., the short arms including NOR6 and NOR7. The segment 12–16 is shaded. D24, D25, and D29 are similar in karyotype and grouped together as are D46 and D47 and also D2 and D3. Significant changes are indicated by * (P < 0.05) and ** (P < 0.01). See also Fig. 2 for explanation of symbols.
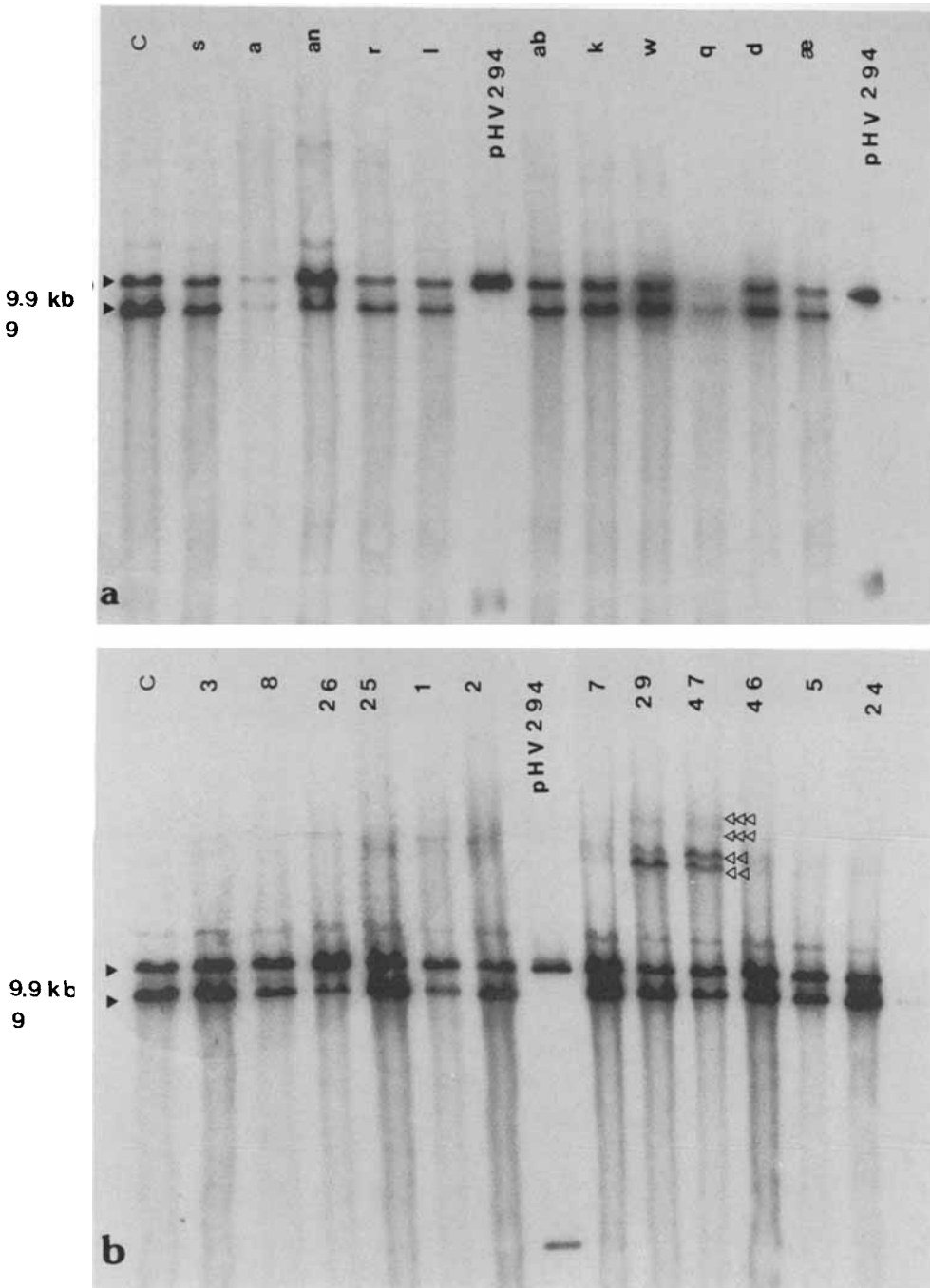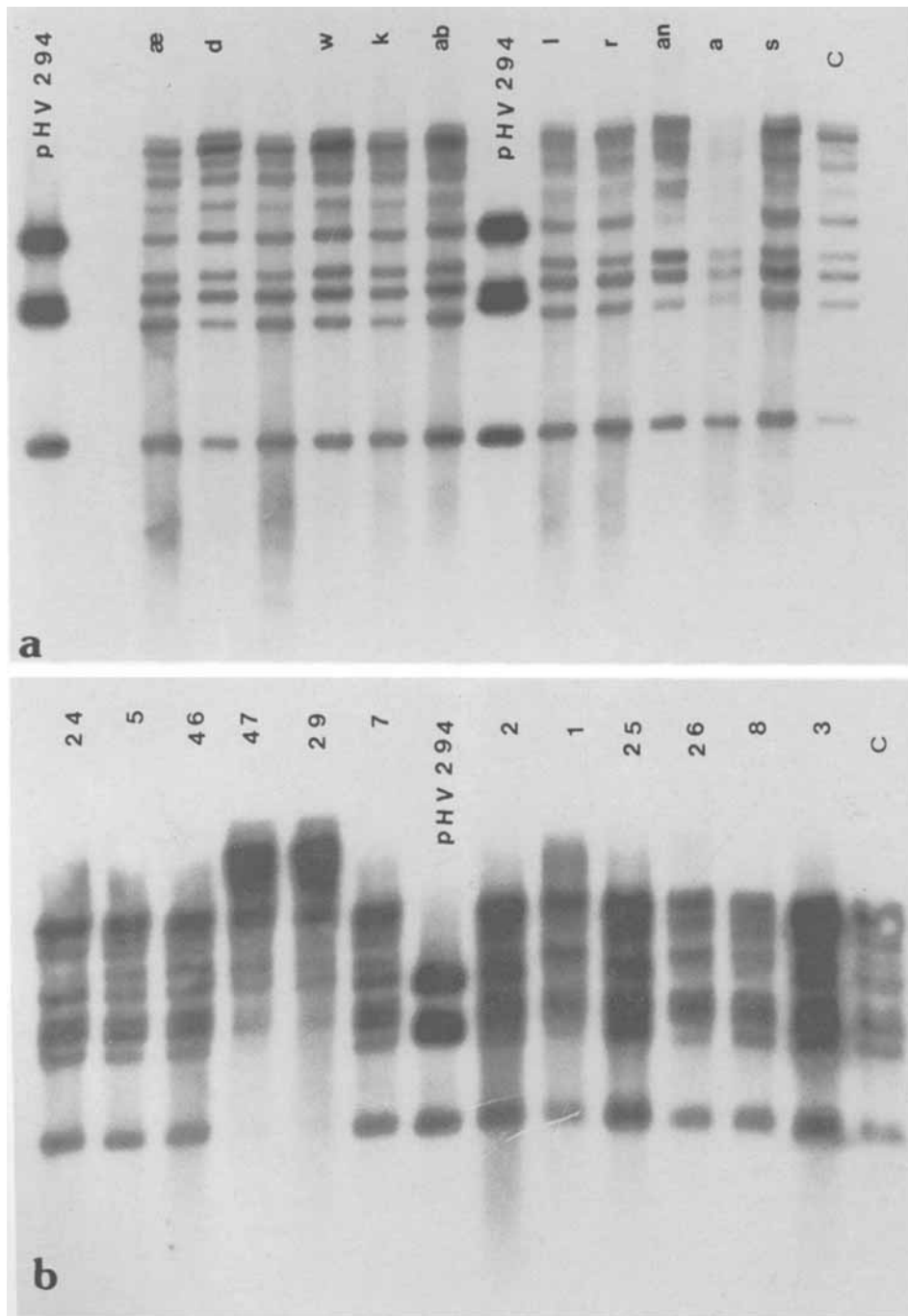
Fig. 4a and b. Southern blot hybridization with the rDNA from **(a)** translocation lines and **(b)** duplication lines following Eco RI digestion. Digestion of pHV294 with Eco RI results in the 9.9 kb barley rDNA repeat and 4 kb vector DNA. C: standard karyotype. Note intensity changes and shifts in the two rDNA repeats. Additional bands in duplications 47 and 29 are indicated by white arrows.
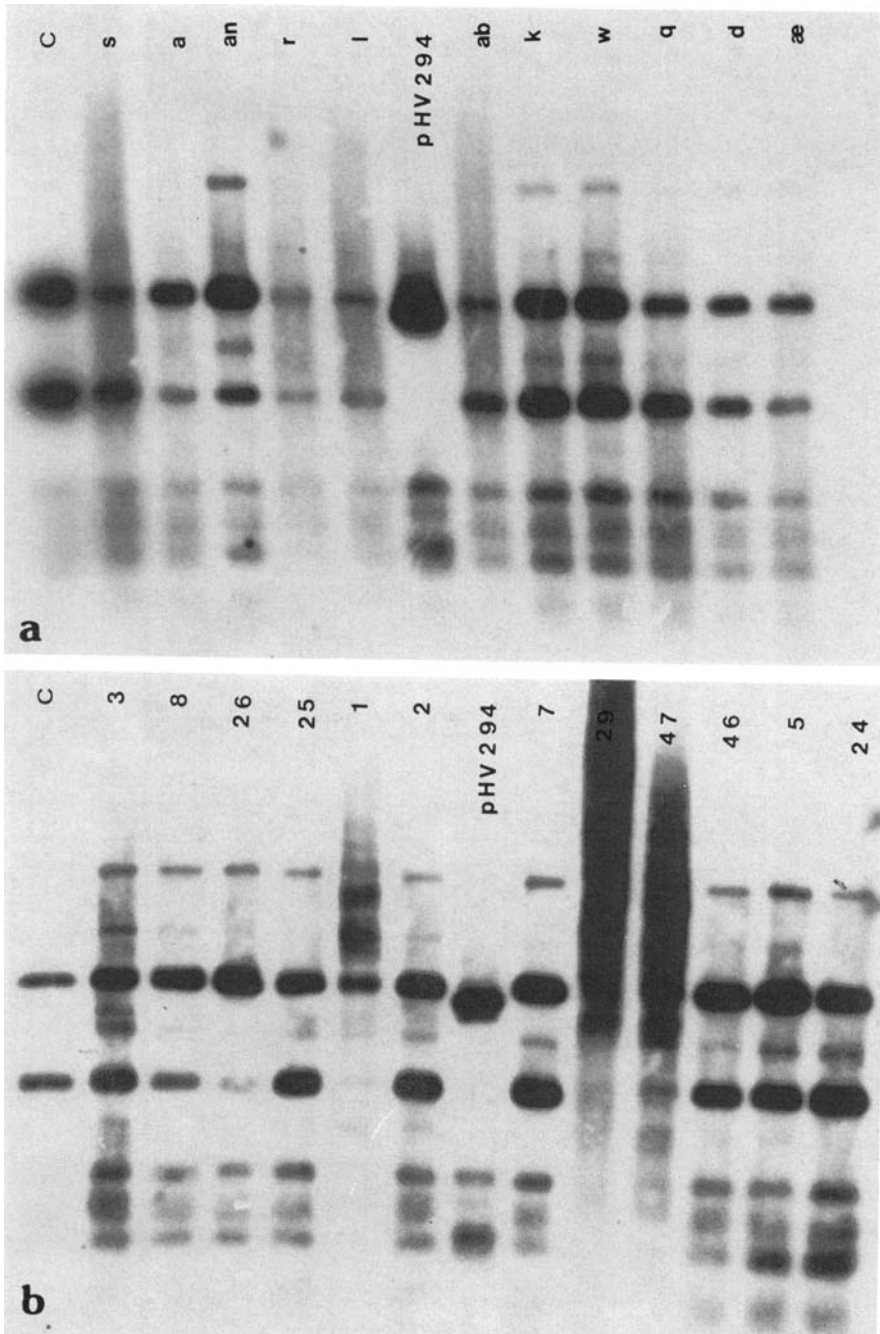
Fig. 5a and b. Southern blot hybridization of genomic DNA from (a) translocation lines and (b) duplication lines following Taq I digestion and probing with the rDNA clone pHV294. Lanes marked pHV294 include a double digest with Eco RI and Taq I. C: standard karyotype. Note intensity changes among different karyotypes and resistance to digestion.

Fig. 6a and b. Southern blot hybridization of genomic DNA from **(a)** translocation lines and **(b)** duplication lines following Bam HI digestion and probing with the rDNA clone pHV294. Lanes marked pHV294 include a double digest with Eco RI and Bam HI. C: standard karyotype. Note resistance to digestion of DNA from duplications 29, 47, and 1.

sis. Two batches of DNA extractions and four replicates were used for each sample. The level of rDNA in seven of the eleven translocation lines was not significantly different from the control (1.2 %) (Fig. 2). The translocation lines T6–7q, r, and s showed a significantly higher level (2.6 %, 2.0 %, and 1.9 %, respectively) of rDNA and these three lines apparently have break points within the NOR in both chromosomes. The translocation T6–7k also had a significantly increased level of rDNA, 1.9 %.

Among the twelve duplications, nine showed substantial increases in the proportion of DNA hybridizing to rDNA (Fig. 3). Looking at the nature of the segments duplicated, D2, D3, D5, and D7 were expected to show twice the value of the control. However, D5 showed a significant decrease from this expectation. D24, D25, and D29, with an extra dose of a mixed NOR7–NOR6 (proximal NOR7, distal NOR6) segment, were expected to have comparable levels among themselves. These three duplications showed a nearly doubled value compared to the control. Similarly, another set of three duplications, D26, D46, and D47, which are complementary in their NOR composition to D24, D25, and D29, with an extra dose of a mixed NOR6–NOR7 (proximal NOR6, distal NOR7) segment, are expected to have comparable levels, but displayed differences. While D46 and D47 have 2.7 % and 2.1 % rDNA (not significantly different from double the standard value), D26 showed a significantly lower level (1.5 %) of rDNA, not different from that of the control.

Discussion

The results of Southern blot analysis and dot blot analysis provided information on the relative amounts of the two rDNA repeats and the overall rDNA levels in each of the karyotypes. A comparison of the two parameters in each of the deviating karyotypes, for instance the translocation lines T6–7an and T6–7d, with low ratios (0.5 and 1.1, respectively) of the 9.0/9.9 kb repeats without any significant change in the overall rDNA level, is indicative of differential amplification of the two repeats, whereas the translocation lines T6–7q and T6–7r, with high ratios (2.3 and 2.2) of 9.0/9.9 kb repeats and an overall increase in total rDNA, may imply differential amplification of the two repeats or only amplification for the 9.0 kb repeats.

A look at the karyotypes (Fig. 1a) reveals that T6–7an results from an exchange of a major por-

tion (segment 1 to 16) of the short arm of chromosome 6 and a small portion of the satellite of chromosome 7 whereby both NORs are brought on to chromosome 7. A comparison of this translocation with T6–7l, which represents an exchange of a relatively short segment (1–11) of chromosome 6, reveals that the breakpoint (an) itself or the position of the segment 12–16 from chromosome 6 is crucial in bringing about differential amplification for the two rDNA repeats. T6–7d, with a shift of part of NOR6 on to chromosome 7, also shows a differential amplification. In T6–7an perhaps the 9.0 kb repeat is brought under the influence of the crucial segment 12–16, while in T6–7d, part of the locus for the 9.9 kb repeat is shifted away from the influence of that segment. In the translocation lines T6–7q and T6–7r, part of the 9.9 kb repeat locus is shifted away from this segment in exchange of part of the 9.0 kb repeat locus. The overall increase in the rDNA suggests that a break in the locus itself triggers the amplification. It is likely that amplification of the rDNA repeats on chromosome 7 takes place, while limited or no amplification at the rDNA locus on chromosome 6 occurs. Although T6–7q and T6–7r are cytologically indistinguishable from T6–7s, the latter has not deviated from the standard karyotype. This may indicate that T6–7s carries an exchange with a break slightly proximal of NOR6, the other break also slightly proximal of, but close to NOR7.

Similar comparative analyses of the results among the duplications and their karyotypic differences (Fig. 1) revealed that all deviating duplications (with low ratios of two rDNA repeats and reductions in the overall rDNA from expected values) share a common feature, i.e., two doses of the segment 12–16. For example, D1 is expected to have an increased rDNA level in view of the extra dose of the proximal part of NOR6 which remains on chromosome 6 along with the crucial segment 12–16 in its original position, whereas a full NOR6 is shifted to chromosome 7 along with the segment 8–19 of chromosome 6. Thus, two doses of the 12–16 segment deamplifies the 9.0 kb rDNA repeat, resulting in no alteration in the overall rDNA level. The duplication D5 showed a significantly lower level of the total rDNA in spite of possessing a duplication for both NOR6 and NOR7 as in the duplications D2, D3, and D7. The low ratio of 9.0 to 9.9 kb repeats (1.0) and a lower overall rDNA level implies deamplification. A distinguishing feature of D5 from D2, D3, and D7 is the double dose of segment 12–16, one in its original position

and the second dose on chromosome 7, each of which possesses NOR6 and NOR7. D8 contains a duplication for the 12–16 segment exclusively, one in its original position and the second transpositioned on to chromosome 7. This brings about the alteration in the ratio of 9.0/9.9 kb repeat units (0.9) through differential amplification since there was no overall increase in the rDNA. Among the other duplications, D26, which is expected to show similarities with D46 and D47 based on the duplications of the NOR segments, showed a very low ratio of 9 to 9.9 kb repeats (0.5) in addition to the very low level of total rDNA. This also implies differential amplification for the two rDNA repeats. Again, in D26 the segment 9–16 is present in two doses while it is present in the normal dose in D46 and D47. Thus duplication of the segment 12–16, common to all the deviating duplications in their rDNA levels and/or the relative levels of the two rDNA repeats, plays a vital role in amplification of the rDNA repeats in barley.

A comparison of the salient features of all the karyotypes deviating in their rDNA (Fig. 2 and 3) revealed that the segment 12–16 of chromosome 6 plays a vital role in bringing about amplification or deamplication at the two rDNA loci. As evident from T6–7an, transposition of this segment to chromosome 7 alone brings about differential amplification. The results from duplications are consistent with the conclusion that a double dose of the same segment, the second dose being positioned on NOR7, leads to an overall reduction (deamplification) in the rDNA level. Thus the transposition of a specific segment from NOR6 to NOR 7 brings about deamplification of rDNA at the Rrn2 locus while the presence of this segment on both the NOR chromosomes results in the overall deamplification as in D1, D5, and D26.

Considering the nature of the material and the time lag after their initial establishment and their use in the present study, we have not investigated the possible mechanism(s) of differential amplification. Several hypotheses have been invoked to explain the variations in the amount of specific repeated sequences (BRETTELL et al. 1986). Direct evidence comes from the nearest neighbour analysis of R-loop experiments in *Vicia faba* (ROGERS and BENDICH 1987) which indicate sister chromatid exchange as the primary mechanism for altering the rDNA copy number as well as causing variation in the intergenic spacer.

A genetic locus (cr^{+} — compensatory response) having *trans* and contiguous *cis* functions that

control the compensation of rRNA genes in Drosophila has been proposed (PROCUNIER and TARTOF 1978). The compensatory increase in rRNA genes occurs when an X-chromosome carrying wild or partially bobbed (deleted NOR) locus is placed in X/O males or in females in which the other X chromosome carries a complete deletion for the NOR. The intriguing feature of our findings in barley is that the segment 12–16 has contiguous *cis* function and its relative position with respect to the two NOR loci determines differential amplification. We are not aware of any other reports of this kind.

Differential amplification of genes at different NOR loci and the altered methylation pattern in rDNA of structurally altered karyotypes of barley have an important bearing on the role of karyotypic changes in speciation in general since differential amplification of different genes leads to increases and/or decreases in specific sequences and altered methylation patterns may lead to different expression of specific genes, as evident in wheat (FLAVELL et al. 1988).

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