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Genome plasticity in *Festuca arundinacea:* direct response to temperature changes by redundancy modulation of interspersed DNA repeats

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Abstract The response of the genome of *Festuca arundinacea* seedlings to changes in the temperature at which they were grown was investigated. Fifteen repeated sequences in the nuclear DNA were isolated and hybridized to the genomic DNA of seedlings grown at 10 °C or 30 °C. The redundancies of sequences recognized by four probes (*Fa*A5, *Fa*H8, *Fa*H13 and *Fa*H14), were found to differ significantly in the two DNAs. DNA sequences recognized by *Fa*H8, *Fa*H13 and *Fa*H14 were more represented in the genome of the 30 °C-raised seedlings than in the genome of the 10 °C-raised seedlings (76.5×10^3, 1.9×10^3, and 111.8×10^3 copies per haploid, 1C genome vs 62.7×10^3, 1.3×10^3, and 80.8×10^3 copies, respectively). In contrast, *Fa*A5-related sequences were more represented in the genome of seedlings grown at the lower temperature (15.5×10^3 vs 10.2×10^3 copies, respectively). Southern-blot hybridization of these repeats to digested genomic DNA produced patterns which indicated that the probe sequences were part of longer repeated sequences having a limited degree of structural heterogeneity. These patterns were partly different when the probes were hybridized to the DNA from seedlings grown at 10 °C or 30 °C. In situ hybridization showed that the DNA sequences recognized by each probe were scattered along the length of all the chromosomes, with preferential location of *Fa*A5- and *Fa*H13-related sequences at given, mainly centromeric, regions of certain chromosomes. These findings suggest that redundancy modulations of interspersed repeated sequences allow direct responses of the genome of *F. arundinacea* to changes in environmental temperature.

Keywords *Festuca arundinacea* · Genome plasticity · Intraspecific genomic changes · Repeated DNA sequences · Environmental adaptation

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Introduction

Based on reports which have been accumulating in the literature since the 1980s, there has been a growing consensus of opinion that, in addition to more stable portions, fluid domains may also exist in eukaryotic genomes. Rapid changes in the redundancy of repeated sequences in these domains may alter the basic amount and organization of nuclear DNA, which is intrinsically plastic due to its content of independent replicative units. Genomic changes of this kind in response to environmental and/or developmental stimuli may not be exceptional, particularly in plants (Walbot and Cullis 1985; Cionini 1989; Bassi 1990, 1991; Nagl 1990).

Genome size and organization differ between natural populations of hexaploid *Festuca arundinacea* in correlation with both the mean temperature during the year at the sampling stations and changes in plant development (Ceccarelli et al. 1992, 1993). A more recent study of genome plasticity in this species has shown that seed germination is a specific, limited time in development within which genomic alterations can be produced in response to changing temperature. The DNA content of seedlings increases significantly with the increase in the temperature at which seeds are germinated. Preferential synthesis of guanine+cytosine- and adenine+thymine-enriched DNA sequences occurs when seeds are germi-

nated at 30 °C, while only guanine+cytosine-enriched sequences are amplified when seeds are germinated at 10 °C (Ceccarelli et al. 1997). These findings agree with many reports of intraspecific changes in the organization of the nuclear DNA of plants exposed to environmental stimuli (reviewed by Bassi 1999; Cullis 1999), which indicate that these changes may be direct responses of the genome rather than the result of a selection process. Thus, the idea that the effect of the environment on an organism is, as a rule, physiological, with the genome remaining unchanged, is now being discarded after having been considered a tenet of biology.

On account of the preceding, the changes in the amount and organization of nuclear DNA which express genome plasticity in *F. arundinacea* seemed to be worth studying in more detail. We here report results which suggest that different repeats interspersed in the nuclear DNA are specifically involved in the response of the genome to changes in environmental temperature.

Materials and methods

Plant material and DNA extraction

Seeds (caryopses) of *F. arundinacea* cv 'Sibilla' were germinated on wet paper in Petri dishes under sterile conditions at 10 °C, 20 °C, or 30 °C, and the seedlings were grown at these temperatures for 20 days. Genomic DNA was extracted from these seedlings, which were pulverized with a pestle and mortar in the presence of liquid nitrogen, and homogenized in a pH 8.0 buffer containing 0.1 M Tris-HCl, 0.005 M EDTA, 0.5 M NaCl, and 0.01 M C_2H_6OS. DNA extraction and purification were performed as described by Maggini et al. (1978). Roots (5–6 mm) to be squashed for in situ hybridization were treated with a saturated aqueous solution of alpha-bromonaphtalene for 4 h at room temperature and fixed in ethanol-acetic acid 3:1 (v/v).

DNA fractionation and cloning

Genomic DNA was fractionated in two ways. It was solubilized in 0.12 M Na phosphate buffer at pH 7.0 and then sheared by sonication in an MSE sonicator at a medium energy output for 5 × 5 s with 10-s intervals at 4 °C. The DNA was then denatured for 10 min at 103 °C, allowed to reassociate up to a C_0t 1/2 value of 1.73×10^{-3}, and fractionated at room temperature by elution through a hydroxylapatite (DNA grade; Biorad) column equilibrated in the same buffer as above (Britten et al. 1974). Single-strand DNA was eluted with the same buffer, and reassociated sequences were recovered by elution with 0.5 M Na phosphate buffer. The latter sequences were cloned in pUC18/*Sma*I BAP vectors using a Sureclone ligation kit (Pharmacia Biotech).

Alternatively, genomic DNA was digested with *Hae*III (Gibco-BRL) and fractionated by electrophoresis in a 1% agarose gel. The DNA in a satellite band of about 25 kb, visualized under UV light after ethidium bromide staining, was recovered by a Prep-a-gene kit (Bio-Rad) and digested with *Alu*I (Roche). The digested DNA was ligated in the presence of pUC18/*Sma*I BAP and the ligation mixture was used to transform *Escherichia coli* DH5 alpha.

Probe DNA preparation and sequencing

Recombinant clones containing repeated DNA sequences were identified by colony hybridization with genomic DNA which had been labelled with digoxigenin-11-dUTP using a random primed DNA labelling kit (Roche). The inserts of positive clones were amplified and labelled with digoxigenin-11-dUTP by polymerase chain reaction.

Sequencing was carried out using the dideoxy method of Sanger et al. (1977) as modified by Chen and Seburg (1985) for double-stranded plasmid DNA. Computer analysis of the sequence data and sequence comparisons were carried out using the PC/Gene program (IntelliGenetics). The search for homology to sequences found in the EMBL database was carried out using the Fasta 3 program.

Southern-blot hybridization

Genomic DNA was digested with *Alu*I, *Rsa*I or *Sau*3AI (Roche), electrophoresized in 1% agarose gels, and blotted on nitrocellulose filters (BA85; Schleicher and Schuell) according to Southern (1975). The blots were hybridized with digoxigenin-labelled DNA probes as described by Ceccarelli et al. (1995). High-stringency conditions were obtained by washing the filters after hybridization twice for 5 min at room temperature with 2 × SSC containing 0.1% SDS and twice for 15 min at 65 °C with 0.1 × SSC containing 0.1% SDS.

Digoxigenin haptens in DNA-DNA hybrids were detected using a Dig-DNA detection kit (Roche) by enzyme-linked immunoassay using an antidigoxigenin-alkaline phosphate conjugate.

DNA methylation analysis

Samples of 200 ng of genomic DNA and solutions of cytosine and 5-methylcytosine as standards were prepared as described by Singer et al. (1979) with minor modifications and analyzed using a HP6890/5973 gas chromatograph-mass spectrometer. The gas cromatograph was outfitted with a 25 m capillary column HP5-MS (250 μm internal diameter and 0.25 μm film thickness). The carrier gas (high-purity helium) was run at a constant flow of 1 ml/min. An electron impact ionization source chamber was utilized for ion formation at a filament potential of 70 eV. Programmed separations were performed (120 °C for 3 min, 20 °C per min up to 320 °C). The injector temperature was 260 °C.

In situ hybridization

Root apices were treated with a solution of pectinase (1%; Fluka) and cellulase (3%; Calbiochem) in citrate buffer pH 4.6 for 90 min at 37 °C, and squashed under a coverslip in a drop of 45% acetic acid. After removing the coverslips by the solid CO_2 method, the preparations were processed and hybridized according to Schwarzacher et al. (1989). The DNA of nuclei and chromosomes was denatured in 70% (v/v) deionized formamide in 2 × SSC for 2 min at 70 °C, and the preparations were incubated overnight at 37 °C with 2 ng/μl of heat-denatured DNA probes which had been labelled with digoxigenin-11-dUTP by polymerase chain reaction. The digoxigenin at the hybridization sites was detected by using sheep antidigoxigenin-fluorescein (Roche). The preparations were then counterstained with DAPI (2 μg/ml), and mounted in antifade solution (AF1; Citifluor).

Dot-blot hybridization and calculation of sequence copy numbers

Replicate samples of 30, 60 or 120 ng of genomic DNA and serial dilutions (from 10^6 to 10^{10} copies) of the probe DNA sequences (dilution spots) were suspended in 10 μl of TE buffer (0.01 M Tris-HCl, pH 8.0, 0.001 M EDTA, pH 8.0) and loaded on Zeta-Probe (Bio-Rad) filters using a Minifold-I apparatus (Schleicher and Schuell). Loading was controlled by adding lambda DNA to plant DNA (1×10^4 copies of lambda per *F. arundinacea* genome

Table 1 Length and G+C content of the DNA probes in Fig. 3. The copy numbers (calculated per 1C DNA) of related sequences in the genomes of seedlings grown at 10 °C or 30 °C are given

DNA probe	Length (bp)	G+C content (%)	Copy number ($\times 10^3$; ± SE)	
			10 °C	30 °C
*Fa*A5	863	41.95	15.52±0.63	10.20±0.57
*Fa*H8	140	41.43	62.70±1.95	76.46±2.30
*Fa*H13	651	36.25	1.33±0.05	1.92±0.09
*Fa*H14	548	55.29	80.82±3.38	111.82±5.11

equivalent). Each filter was probed in series with 50 ng of *F. arundinacea* probes and lambda DNA which had been digoxigenin-labelled by random priming using a Dig-DNA labelling kit (Roche). Digoxigenin haptens in DNA–DNA hybrids were detected as described above, and the filters were scanned densitometrically (Ceccarelli et al. 1995).

The linear regression equation relating the natural logarithm of the copy number of probe sequences in the dilution spots and the natural logarithm of the corresponding densitometric readings was used to estimate the copy number of the sequence probed in the samples of genomic DNA.

Results

Fifteen repeated sequences in the nuclear DNA of *F. arundinacea* were isolated from either a satellite band of genomic DNA or the repetitive DNA fraction eluted from hydroxylapatite. These sequences were dot-blot hybridized to genomic DNAs from seedlings grown at 10 °C or 30 °C. Densitometric absorptions were linear in the range from 30 to 120 ng of loaded DNA, and sequence frequencies were calculated from these results. Sequences recognized by four probes, one isolated from the satellite band (*Fa*A5) and three from the repetitive DNA fraction (*Fa*H8, *Fa*H13, *Fa*H14), showed significant differences in their frequency in the two DNAs (Fig. 1). The frequency of DNA sequences recognized by *Fa*H8, *Fa*H13 and *Fa*H14 was higher in the genome of seedlings grown at 30 °C than in the genome of seedlings grown at 10 °C. In contrast, the frequency of *Fa*A5-related sequences was higher in the genome of the 10 °C-raised seedlings than in that of the 30 °C-raised seedlings (Fig. 2). Since the genome size of plants of *F. arundinacea* cv 'Sibilla' grown at 10 °C or 30 °C is known (1C = 8.67 pg and 9.14 pg, respectively; Ceccarelli et al. 1997), the redundancies of probe-related DNA sequences can be calculated. The copy number ($\times 10^3$) per haploid, 1C genome of sequences related to *Fa*H8, *Fa*H13 and *Fa*H14 turned out to be 62.70 ± 1.95, 1.33 ± 0.05, and 80.82 ± 3.38, respectively, in plants grown at 10 °C vs 76.46 ± 2.30, 1.92 ± 0.09 and 111.82 ± 5.11 in plants grown at 30 °C. The copy number of *Fa*A5-related sequences was 15.52 ± 0.63 in the genome of the plants grown at 10 °C and 10.20 ± 0.57 in those grown at 30 °C (Table 1).

The nucleotide sequences of *Fa*A5, *Fa*H8, *Fa*H13 and *Fa*H14 are given in Fig. 3. No obvious sequence

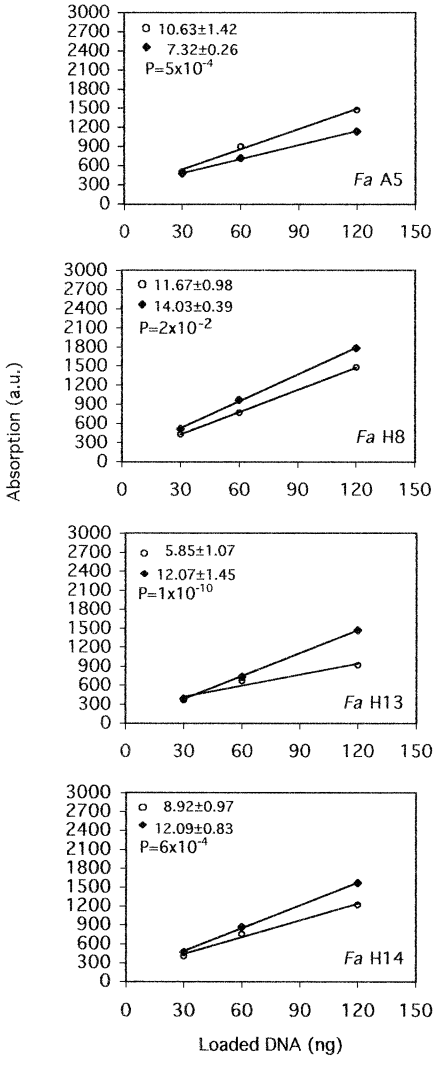

Fig. 1 Densitometric absorptions of dot-blot filters loaded with different amounts of genomic DNA from seedlings grown at 10 °C (○) or 30 °C (◆). The filters were hybridized with different, digoxigenin-labelled DNA probes. Each value is the mean of those obtained in six repetitions, three for each of two DNA extractions. Linear regression lines are plotted, and their angular coefficients and the results of statistical analysis (*t*-test) are given

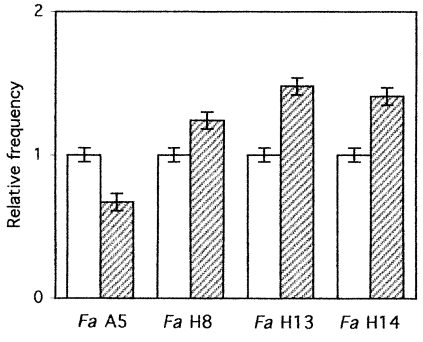

Fig. 2 Frequencies of sequences related to different DNA probes in the genomic DNA from seedlings grown at 10 °C (□) or 30 °C (■) as calculated from the results of dot-blot hybridizations. The values were normalized (□=1) to facilitate comparisons. Confidence limits at $P \leq 0.01$

Fig. 3 Nucleotide sequences of the DNA probes used. The sequences have been registered with EMBL Nucleotide Sequence Database under the accession numbers AJ310136 (*Fa*A5), AJ310137 (*Fa*H8), AJ310138 (*Fa*H13) and AJ310139 (*Fa*H14)

FaA5

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          10         20         30         40         50         60         70         80         90
          '          '          '          '          '          '          '          '          '
CCTTTGCTAG TAACGATAAA ATAGTTCTTT TATAAATAAA GTACCCATCT TGCCGTAGGT TCTATTTGTT TGAATTGTTC AGTGTCTCTA
ATTGAGTGGT TTGCTACTAG TCTTGTGTGT GTGGTTGATG CTATGGCTAT GTGTTGATTG TTCGCCTTTT ATTTTGTGAC GTTAGAATGC
GAACACTATC GGAGAAGAAG GAGAGGATCT TGGTGAGGAT TTCGAAGAGG AAGGAAGAGC AGAGGTTCGT GAAGAAGAAG TCCAGGAACA
AATAGCCAGC CAAGGCAAGC CACCTCTTGA TGCATTCCTA TTCCTATTTG TCTTACTCTT GCATTATTAG GAGTTTTATA AATTGCATGT
CTTTTATTTA TTTATGTGAG TGGTTCCGCC ACTCGGAGTT TTATCGTCCC ACCCTCACGA GTGCTGCCCC TCATTGATAC CTATCAGACC
ACCCCATTAG TGATATGGTG ATTAATACCT TATCTTCTCT TTGTCACCTC TTTTTAAATA GGGTTGGACT ATAGGTTGGG AGCCCTTGGA
AAAATTGAGT TTTGGAAATG GTTTTTAAAG AAAATGAATT CTTTGAAAAC CTTGGAATGG GACAACCTTG CCCAAGTGAG AATTTTATTA
AAAAGGTTTT GAGAAAGATA AAAACTTTGG TGACTGAGTA CCTAACCGGA CCTAGTTAGT GCAACCACAT TACCCCTAAT GGGAAGGGGC
GTAACGTAGT AGTTTGTCTC GCCTTAGTTT GGGTCCTGCA AGTGTAGTGG TAGTCCGACC ATGGACGCTT GCACCGGGGT TCTTCCCGTT
TAAAGGGACG CAGCCCATTT GCATGTTTAG GTACGCAGGG TACAACCTAT GAG
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FaH8

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          10         20         30         40         50         60         70         80         90
          '          '          '          '          '          '          '          '          '
CATTTTCATG CTAACATGTT CTTCGGTTGT TTCGTTGCTA TCTTGCCTTT CTTTGTTCCG GTGCATACAT CAACCTTCTT TCTCCGGAGT
TGTTTCGCCA AGCTCATACC TTGTTGTTTC CTTCCAATAT CGATAACTAG
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FaH13

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          10         20         30         40         50         60         70         80         90
          '          '          '          '          '          '          '          '          '
CTACTACAGG TAATGACATT GTTATTTCTA TCATACTTTG TCGTAAGCTA ACAAAGTAAC TGACTAATAT TAGTTCCTGC ATTCTATCGC
TCTGCTATCC TGCAGTACAA TTTTTTTTAA ACTCGTCACA CTAGCTACTT CGATAATAAA TTTGTGTGCC ATCGGGTTCT CCAAAAGCTG
CAACATTAAC TCGGTTCTCT TACTTTGCAT GGCACTCATA CATGGAATGC TGAACATATT GGTTTGCATT CCCTCTTCAG CTCTTCTACA
AGTTCACAGG TGATCCCACT ATATTATGTA TCTGGTCTAT CCTTAGCTCC AGCATTAACT ATCCTCTGCG TGTTGCTCAT TACAAATTTA
TGTCCCGAAA CATATTGATT TTCATTCCCT GCACAACAAA GTTTAGATTA TAATTCCTAT ATGGTTCATT ACTAGCTATG ATAGTAAAAT
GCTACTATTA TTTAGTTCAT GGCCAATTTA AGAAATTGAG CATGTTGGTT TGCATTCCCT GCTCTATAGT TGTTGCAATT ATAACTTCTA
TCTGGTTCAT TCTTTGTTAC AAAAGTAAGT GCCTAGTATT ATGGAGTAGT TCATGTCAAT CTGGGAAGTA ACTGAACATG CTGGTTTACT
TTTGCTGCAC TCGAAATGAT A
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FaH14

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          10         20         30         40         50         60         70         80         90
          '          '          '          '          '          '          '          '          '
GATCACCGAC GCCGCCACCC GCAGATGCCC TTCTCCGGCG AGCTCCGGTG ACCTCCTGCT AGCGGCGCCA CCTTCTTTTG GTCTGCGTCA
ACGAGTCGAC CACCGCGCGC CCACGTGATC TTCCTGGAAC GCGCTGAGAC CGCCGCCACG GCTCGCGCCC AGCCGCGGTG ACCGCCATCT
CCTGCGCGGC GCACGGCATC GAGCACCTCG TGTGCGTGCG CACTTGGTTG CCACGCGGCG TCCACGTCGG CCCCGCGGAC CCCACCCGTC
AGCGACCACG GGTCAACGGT TTCGGTGAGA AACGTTCACA GATCTAGATC TGGCAGCCTT TTTCAGTTTA GCCCCTGGAA GTTTTAAACC
ATTAACCCGG TGCCCCTGGC TAAACGTTTT TCGGCATAAC CCCCTGGAAC TAAAAGAAAC CAACCCGGGG TCCGTTTTTT TTCAGTAGAT
TTTCGTTCGG GTCCTTTTTA GTTTAAATTC AAATTTCAAA TCTGTTTAAT GCTTTATTAA ATAAAACAAC TTTCTGAACT CCAAATTTAA
CAAATTTT
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similarity exists between these DNA stretches or between any of them and DNA sequences found in the EMBL nucleotide sequence database. It can be seen from Table 1 that *Fa*A5, *Fa*H8 and *Fa*H13 are A+T-enriched (G+C 36.25–41.95%), whereas *Fa*H14 is G+C-enriched (55.29%).

These DNA probes were hybridized to Southern blots of genomic DNA from seedlings grown at 10 °C or 30 °C digested with *Alu*I, *Rsa*I or *Sau*3AI. Each blot showed a few strong bands and several minor bands. It can be seen from Fig. 4 that the strong bands were the same in the blots of both the DNA from 10 °C-raised seedlings and that from the 30 °C-raised ones. In contrast, the patterns of minor bands differed in the blots of the two DNAs. Minor bands were more numerous in the blots of DNA from seedlings grown at 30 °C than in those of DNA from seedlings grown at 10 °C when *Fa*H8, *Fa*H13, and *Fa*H14 were hybridized. Conversely, hybridization with *Fa*A5 produced more minor bands in the blots of DNA from 10 °C-raised seedlings than in those of DNA from the 30 °C-raised ones. The 5-methylcytosine/cytosine ratio, as calculated from the results of gas cromatograph-mass spectrometer analysis in six repetitions, was 0.424 ± 0.11 in the DNA from seedlings grown at 10 °C and 0.431 ± 0.06 in that of seedlings grown at 30 °C.

Fluorescent in situ hybridization of the *Fa*A5, *Fa*H8, *Fa*H13 and *Fa*H14 repeats to metaphase chromosomes of seedlings obtained at 20 °C showed the DNA sequences recognized by each probe to be scattered along

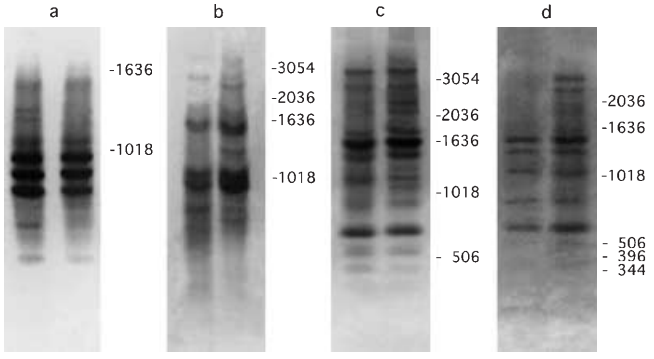

Fig. 4 Southern-blot hybridization of different, digoxigenin-labelled DNA probes to genomic DNA from seedlings grown at 10 °C (left) or 30 °C (right). The DNAs were digested with different restriction endonucleases. *Numerals* indicate molecular sizes in base pairs as determined using a 1-kb DNA ladder as a marker. The figure was assembled using Adobe Photoshop. *a Fa*A5; *Alu*I. *b Fa*H8; *Sau*3AI. *c Fa*H13; *Sau*3AI. *d Fa*H14; *Sau*3AI

the length of all the chromosomes, with preferential hybridization of *Fa*A5- and *Fa*H13-related sequences at given, mainly centromeric, regions of certain chromosomes. Figure 5 shows the results obtained after hybridization with *Fa*A5 (*a* and *b*) and *Fa*H14 (*c* and *d*). Quite similar patterns were obtained by hybridizing *Fa*H13 and *Fa*H8, respectively, apart from the levels of hybridization, which differed in relation to the differences in sequence copy number.

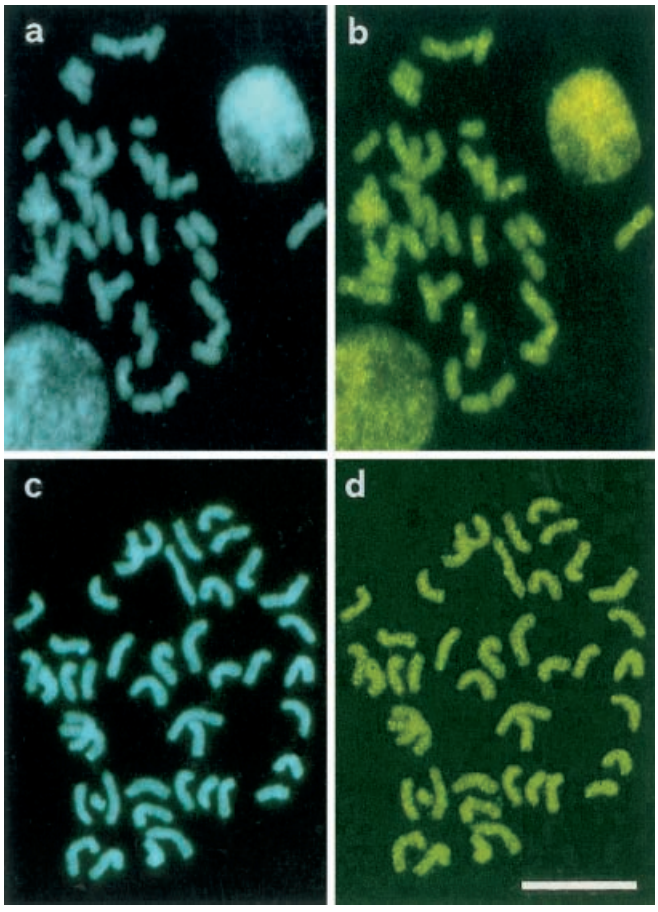

Fig. 5 Metaphase plates after DAPI staining (*a* and *c*) and in situ hybridization with *Fa*A5 (*b*; fluorescein) and *Fa*H14 (*d*; fluorescein). DNA sequences recognized by *Fa*H14 are scattered along the length of all the chromosomes. Preferential location of *Fa*A5-related sequences at given mainly centromeric chromosome regions can be seen. The bar represents 10 μm

Discussion

Four different repeated DNA sequences were recognized by *Fa*A5, *Fa*H8, *Fa*H13 and *Fa*H14, respectively. This is indicated by the differences in the redundancy of repeats related to each probe (Table 1) and the different patterns obtained after Southern-blot hybridization (Fig. 4). The following considerations might suggest that these sequences are mobile DNA elements, even if the nucleotide sequences of the probes did not show any significant similarity with other known sequences of this kind:

1. The major bands in the Southern blots (Fig. 4) may be considered as produced by DNA stretches obtained by cutting repeated sequences at two internal sites. Most of them appeared at molecular sizes higher than those of the probes, and this indicates that all the probe sequences are part of larger repeats having a limited degree of structural heterogeneity.
2. The redundancies of these sequencies (Table 1) are in the ranges of those already documented in plants for mobile DNA elements such as retrotransposons (Frediani et al. 1999).
3. The chromosomal distribution (Fig. 5) clearly resembles that observed when hybridizing in situ DNA sequences belonging to retroelements (Kumar and Bennetzen 1999; Belyayev et al. 2001).
4. Most probes are A+T-enriched (Table 1) and this characteristic has been found in given transposable elements, e.g. the *Ds* elements in maize (Sutton et al. 1984).

That we have found portions of mobile DNA elements by sampling repeated sequences at random would not be surprising, when considering that transposable sequences comprise a large part of the genome of both animals and plants. But to quote two examples, some 50 or 40% of the genomes of *Zea mays* and *Vicia faba*, respectively, are represented by the *copia* group of retrotransposons alone (Pearce 1995; SanMiguel et al. 1996). The fact that we did not isolate highly repeated DNA sequences cannot be surprising either. It is known that the genomes of graminaceous species are poor in highly repeated, tandemly arranged sequences and rich in less-redundant interspersed repeats (Flavell et al. 1974). Therefore, though the presence of tandem repeats in the *F. arundinacea* genome has been reported (Perez-Vicente et al. 1992), our results were not unexpected, and this also in view of the structure of the interphase nuclei, which are typically eureticulate and devoid of heterochromatin.

The results of dot-blot hybridizations show that the redundancies of DNA sequences recognized by *Fa*A5, *Fa*H8, *Fa*H13 and *Fa*H14 undergo quantitative changes in response to variations in the temperature at which the seedlings are grown (Figs. 1 and 2). The differences in hybridization patterns observed in Southern blots of DNA from seedlings obtained at 10 °C or 30 °C (Fig. 4) confirm this finding. These differences cannot be produced by alterations of the endonuclease activity due to changes in the degree of DNA methylation, since the DNAs from seedlings grown at different temperature do not differ in this respect (see Results). Our results find confirmation in previous data, which suggest that the observed changes in sequence copy number are due to amplification processes instead of deletions. The increased redundancy of most sequences (those recognized by *Fa*H8, *Fa*H13 and *Fa*H14) with increasing temperature (Fig. 2) is in line with data already obtained and quoted in the Introduction, which show that: (1) the genome size of *F. arundinacea* populations is positively correlated with the mean temperature during the year at their stations; (2) the genome size of seedlings grown at 30 °C is higher than that of 10 °C-raised seedlings; and (3) extra synthesis of A+T-enriched DNA sequences occurs in seedlings growing at 30 °C (Ceccarelli et al. 1992, 1997). On the basis of these data, the finding that sequences recognized by *Fa*A5 have higher redundancy in the genome of seedlings grown at 10 °C than in that of

seedlings grown at 30 °C (Fig. 2) was somewhat unexpected. However, Ceccarelli et al. (1997) showed that DNA extra synthesis also occurs when *F. arundinacea* seedlings are grown at 10 °C. The finding obtained with *Fa*A5, together with the observation that sequences recognized by 11 repeats do not undergo any quantitative variation with changing temperature, adds reliability to our results. Indeed, these findings exclude the possibility that the variations observed are simply due to the differential loss of repeated sequences in the extracted DNAs.

The number of certain repeated DNA sequences increases at 30 °C, while that of a different repeat increases at 10 °C. This specificity of response seems to exclude a 'selfish' nature of these sequences and does not support the correlated view that possible advantages upon the host genome due to their self-replication must be indirect (Doolittle and Sapienza 1980; Orgel and Crick 1980). Rather, our results suggest that the genomic changes observed occur under plant-level controls and are not independent of the genome as a whole. Findings to this effect have been found in other species. In *Helianthus annuus*, different families of repeated sequences undergo amplification in the genome of seedlings, belonging to single progenies of homozygous plants, obtained from seeds which either develop in the middle of the head or at its periphery (Cavallini et al. 1996). Examples of regulation of the copy number of repeated sequences such as transposable elements by the host genome may be found in the literature dealing with both animals and plants (Kidwell and Lisch 1997). One of these examples is the dynamics of *BARE-1* retrotransposons in the genome of *Hordeum spontaneum*, which belongs to the same family of *F. arundinacea*, the Poaceae. These retroelements have been shown to vary in redundancy in the genome of plants from stations having differing microclimates, thus allowing the genomes adaptive selection in response to climatic divergence (Kalendar et al. 2000). In line with other findings quoted above, our data suggest that these genomic changes may not only be the result of a selection process. These changes may be specific controlled phenomena which carry out direct responses of the plant genome to environmental variation. On one hand, these genomic changes may represent an aspect of the particular necessity of plants to respond to environmental stimuli, due to immobility and their way of reproduction. On the other hand, genomic changes of this kind help explain the extraordinary plasticity in development and physiology which is a common feature of plants and which allows such species as *F. arundinacea* to grow in a wide range of eco-geographical conditions and climates.

References

Bassi P (1990) Quantitative variations of nuclear DNA during plant development: a critical analysis. Biol Rev 65:185–225

Bassi P (1991) Repetitive, non-coding DNA: a possible link between environment and gene expression in plants? Biol Zentralbl 110:1–13

Bassi P (1999) The effect of environmental stress on repetitive DNA behaviour in plants. In: Lerner HR (ed) Plant responses to environmental stresses. M Dekker, New York, Basel, pp 161–170

Belyayev A, Raskina O, Nevo E (2001) Chromosomal distribution of reverse transcriptase-containing retroelements in two *Triticeae* species. Chrom Res 9:129–136

Britten RJ, Graham DE, Neufeld BR (1974) Analysis of repeating DNA sequences by reassociation. Methods Enzymol 29:363–405

Cavallini A, Natali L, Giordani T, Durante M, Cionini PG (1996) Nuclear DNA changes within *Helianthus annuus* L.: variations in the amount and methylation of repetitive DNA within homozygous progenies. Theor Appl Genet 92:285–291

Ceccarelli M, Falistocco E, Cionini PG (1992) Variation of genome size and organization within hexaploid *Festuca arundinacea*. Theor Appl Genet 83:273–278

Ceccarelli M, Minelli S, Falcinelli M, Cionini PG (1993) Genome size and plant development in hexaploid *Festuca arundinacea*. Heredity 71:555–560

Ceccarelli M, Minelli S, Maggini F, Cionini PG (1995) Genome size variation in *Vicia faba*. Heredity 74:180–187

Ceccarelli M, Giordani T, Natali L, Cavallini A, Cionini PG (1997) Genome plasticity during seed germination in *Festuca arundinacea*. Theor Appl Genet 94:309–315

Chen EY, Seburg PH (1985) Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA 4:165–170

Cionini PG (1989) Nuclear DNA changes during plant development. Giorn Bot Ital 123:111–121

Cullis CA (1999) The environment as an active generator of adaptive genomic variation. In: Lerner HR (ed) Plant responses to environmental stressess. Marcel Dekker, New York Basel, pp 149–160

Doolittle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature 284:601–603

Flavell RB, Bennett MD, Smith JB, Smith DB (1974) Genome size and proportion of repeated nucleotide sequences DNA in plants. Biochem Genet 12:257–269

Frediani M, Gelati MT, Maggini F, Galasso I, Minelli S, Ceccarelli M, Cionini PG (1999) A family of dispersed repeats in the genome of *Vicia faba*: structure, chromosomal organization, redundancy modulation, and evolution. Chromosoma 108:317–324

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc Natl Acad Sci USA 97:6603–6607

Kumar A, Bennetzen JL (1999) Plant retrotransposons. Annu Rev Genet 33:479–532

Kidwell MG, Lisch D (1997) Transposable elements as sources of variation in animals and plants. Proc Natl Acad Sci USA 94:7704–7711

Maggini F, Barsanti P, Marazia T (1978) Individual variations of the nucleolus organizer regions in *Allium cepa* and *A. sativum*. Chromosoma 66:173–183

Nagl W (1990) Gene amplification and related events. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 11. Springer, Berlin Heidelberg New York, pp 153–201

Orgel CE, Crick FHC (1980) Selfish DNA: the ultimate parasite. Nature 284:604–607

Pearce SR, Harrison G, Wilkinson M, Li D, Heslop-Harrison JS, Flavell AJ, Kuwar A (1995) The *Ty1-copia* group retrotransposons in *Vicia* species: sequence heterogeneity and chromosomal localization. Mol Gen Genet 250:305–315

Perez-Vicente R, Petris L, Osusky M, Potrykus I, Spangenberg G (1992) Molecular and cytogenetic characterization of repetitive DNA sequences from *Lolium* and *Festuca*: applications in the analysis of *Festulolium* hybrids. Theor Appl Genet 84:145–154

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

SanMiguel P, Tikhonov A, Jin Y-K, Motchoulskaia N, Zacharov D, Melake-Berkan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the maize genome. Science 274:765–768

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ localization of parental genomes in a wide hybrid. Ann Bot 64:315–324

Singer J, Schnute WC Jr, Shively JE, Todd CW, Riggs AD (1979) Sensitive detection of 5-methylcytosine and quantitation of the 5-methylcytosine/cytosine ratio in DNA by gas chromatography mass spectrometry using multiple specific ion monitoring. Anal Biochem 94:297–301

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:1073–1082

Sutton WD, Gerlach WL, Schwarts D, Peacock WJ (1984) Molecular analysis of *Ds* controlling element mutation at the *Adh*1 locus of maize. Science 223:1265–1268

Walbot V, Cullis CA (1985) Rapid genomic changes in higher plants. Annu Rev Plant Physiol 36:367–396