Molecular Cytogenetic Organization of 5S and 18S rDNA Loci in *Aster ageratoides* var. *ageratoides*, *A. iinumae* (= *Kalimeris pinnatifida*) and *A. microcephalus* var. *ovatus* in Japan

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Summary Detailed karyotypes of diploid *Aster ageratoides* var. *ageratoides* ($2n=18$), diploid *A. iinumae* (= *Kalimeris pinnatifida*) ($2n=18$) and tetraploid *A. microcephalus* var. *ovatus* ($2n=36$) were constructed on the basis of chromosome lengths, arm ratios and multi color fluorescence *in situ* hybridization (McFISH) with 5S and 18S ribosomal RNA gene sequences as probes. The karyotype of *A. iinumae* was morphologically similar to those of *A. ageratoides* var. *ageratoides*, however, the size of the chromosomes of *A. iinumae* was apparently smaller (S-type chromosomes) compared to those of *A. ageratoides* (L-type chromosomes). The chromosomes of *A. microcephalus* var. *ovatus*, a putative amphidiploid between them consisted of the 18 large chromosomes (L-type chromosomes) and the 18 smaller chromosomes (S-type chromosomes). The McFISH with 5S rDNA and 18S rDNA of each *A. ageratoides* and *A. iinumae* chromosomes tagged 4 out of 18 chromosomes. Whereas *A. microcephalus* var. *ovatus* have 5S rDNA and 18S rDNA sites equal to the sum of the numbers in the two diploids (*A. ageratoides* and *A. iinumae*), 4 on the L-type chromosomes and 4 on the S-type chromosomes. The locations of the rDNA sites in *A. microcephalus* var. *ovatus* are corresponding to those of *A. ageratoides* and *A. iinumae*.

Key words Ag-NOR, *Aster s.l.*, Asteraceae, Chromosome evolution, 18S rDNA, 5S rDNA, *Kalimeris*, Karyotype analysis, McFISH.

Aster L. is one of the representative genera of the family Asteraceae, which is well-known as distributed widely in Eurasia (Richard and Rieseberg 1999). But the generic circumscription of Eurasian *Aster* has been still controversial because of the existence of many closely related genera (Bremer 1994). In the Eastern Asia, three small genera, *Kalimeris* Cass., *Heteropappus* Less., and *Miyamayomena* Kitamura (= *Gymnaster* Kitamura) were segregated from *Aster* by Kitamura (1937), primarily based on the length of the pappus. However, molecular works of Ito *et al.* (1995 and 1998) showed that *Kalimeris* and *Miyamayomena* have multiple origins respectively, each derived from different ancestors of *Aster*, and *Heteropappus* from another species of *Aster*. In conclusion, they suggested that these three genera and *Aster sensu strict* should be merged together to form *Aster sensu lato*. Their results indicated that the length of the pappus could have been shortened independently in parallel evolution. But what is more interesting evolutionary trend they showed was the direction of chromosome evolution. Previous studies (Huziwara 1957a,b, 1958, 1967, Shindo 1967) have revealed that some species of *Kalimeris* had karyotype consisted of signif-

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icantly small chromosomes (S-type karyotype) comparing to those of other species of Japanese *Aster s.s* (L-type karyotype). It means that chromosome size reduction occurred within the clade of *Aster s.l.* In addition to the L-type and S-type karyotypes, LS-type karyotype, considered to be amphidiploid between them, is known in *A. microcephalus* (Miq.) Franch. et Sav. and its intraspecific taxa (Huziwaru 1957b, Tara 1977). Ito *et al.* (1995 and 1998) supposed that *A. ageratoides* Turcz. var. *ageratoides* (designated as *A. leiophyllus* Franch. et Sav.) and *A. iinumae* Kitam. ex Hara (designated as *K. pinnatifida* (Matsum.) Makino) could be the parents of *A. microcephalus* var. *ovatus* (Franch. et Sav.) Soejima et Mot. Ito based on chloroplast DNA data.

Fluorescence *in situ* hybridization (FISH) with rDNA is a useful marker for chromosome identification, which provides the phylogenetic information of closely related taxa (*e.g.*, Ansari *et al.* 1999, Singh 2001). In plant, FISH for ribosomal RNA genes have been widely used for karyotyping (Leitch and Heslop-Harrison 1993, Matoba *et al.* 2001, Raina *et al.* 2001, Hajdera *et al.* 2003, Navrátilová *et al.* 2003), and for studying genome organization and chromosome evolution within many genera (Ali *et al.* 2000, Anamthawat-Jónsson and Bödvarsdóttir 2001, Hasterok *et al.* 2001, Raina *et al.* 2001, Ran *et al.* 2001, Singh *et al.* 2001, Li and Zhang 2002). The gene for 18S-5.8S-26S rRNA occur as a large number of tandem repeats that form a single transcription unit. Usually, these sequences are located in the NORs of eukaryotes (Long and Dawid 1980). The 5S rDNA is organized in tandem array with repeat units composed of a coding region, and generally located independently of 18S-5.8S-26S rRNA cluster in eukaryotes (Ansari *et al.* 1999). The physical mapping of these two rDNA greatly contribute to chromosomal identification in plant with small or morphologically similar chromosomes (Murata *et al.* 1997, Hasterok *et al.* 2002, Hajdera *et al.* 2003). Especially, McFISH is a powerful tool for the molecular cytogenetics, which allows simultaneous detection of several DNA probes.

Recently, Kokubugata *et al.* (2003) have determined the number of 18S rDNA loci in 2 species of *Aster s. l.*, but they did not refer on the chromosomal evolution nor karyological homogeneity at all. The aim of this study is to investigate the chromosome homology between *A. ageratoides* var. *ageratoides* (referred as *A. ageratoides* hereafter), *A. iinumae* and *A. microcephalus* var. *ovatus* (referred as *A. microcephalus* hereafter) using karyotyping and McFISH with 5S and 18S rDNA, and consider the mechanism of chromosomal evolution in *Aster s. l.*

Materials and methods

Plant materials and chromosome preparation

Aster ageratoides, *A. iinumae* and *A. microcephalus* were collected in Japan, and were cultivated in the Department of Plant Science, Kyushu Tokai University (Table 1). The seedlings were pre-treated with 2 mM 8-hydroxyquinoline at room temperature for 3 h before they were fixed in ethanol-acetic acid (3 : 1) at 4 °C for 24 h. Chromosomal preparations were prepared by the modified method of Leitch and Heslop-Harrison (1992). Fixed seedlings were washed 3 times in distilled

Table 1. Plant materials

Species	Collection	Chromosome number
<i>Aster ageratoides</i> Turcz. var. <i>ageratoides</i>	Japan: Mt. Kiyosumi, Tenshinkominato T., Chiba Pref.	2n=18
<i>A. iinumae</i> Kitam. ex Hara (= <i>Kalimeris pinnatifida</i> (Matsum.) Kitam.)	Japan: Nishiurabara, Niigata Pref.	2n=18
<i>A. microcephalus</i> var. <i>ovatus</i>	Japan: Kawanisi City, Hyougo Pref.	2n=36

water and their root-tips were cut into 2–3 mm long. Ten to thirty root-tips were placed in a microcentrifuge tube containing 500 μ l of enzyme solution (1% Cellulase Onozuka-RS (Yakult Honsha) and 0.5% Pectolyase Y-23 (Kikkoman), pH 4.2) and incubated at 37 °C for 1 h. They were washed 3 times with distilled water. Ten to 20 μ l of the fixative was dropped onto root-tip and air-dried.

Chromosome classification

Karyotype formulas at mitotic metaphase were based on the data of measurements of the chromosomes taken from the photographs. Chromosomes at mitotic metaphase were classified by arm ratio calculated by long arm/short arm following Levan *et al.* (1964): m=median-centromeric chromosome with arm ratio of 1.0–1.7; sm=submedian-centromeric chromosome with arm ratio of 1.8–3.0; st=subterminal-centromeric chromosome with arm ratio of 3.1–7.0, t=terminal-centromeric chromosome with arm ratio 7.1 or more.

PCR amplification of 5S and 18S ribosomal DNA

Total genomic DNA was extracted according to the method of Shaw (1988) from young growing leaves of *Aster ageratoides*. PCR amplification of 5S and 18S rDNA followed the procedure of Hizume (1993) and Sogin (1990), respectively. The sequences of the primers were as follows: 5'-CGGTGCATTAATGCTGGTAT-3' and 5'-CCATCAGAACTCCGCAGTTA-3' for the repeating units in 5S rRNA gene clusters, and 5'-AACCTGGTTGATCCTGCCAGT-3' and 5'-TGATCCTTCTGCAGGTTACCTAC-3' for the 18S rRNA coding regions. PCR reaction mixture (total 50 μ l) contained 60 ng of template DNA, 200 pmol of each primer, 0.1 mM of each dNTP, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 1 unit of Taq polymerase (TOYOBO). Amplification was performed in a T1 Thermocycler (Biometra) programmed for 1 cycle of 4 min at 94 °C, 30 cycles of 30 sec at 94 °C, 30 sec at 60 °C, 60 sec at 72 °C, and 1 cycle of 5 min at 72 °C.

Fluorescence in situ hybridization (FISH)

The 5S and 18S rDNA probes amplified by PCR were labeled by means of random primer DNA labeling with digoxigenin-dUTP (Takara) and nick translation with biotin-14-dATP (invitrogen), respectively, following the supplier's instructions. Labeled DNA probes were denatured at 95 °C for 10 min. Hybridization mixtures contained 50% formamide, 10% dextran sulfate and each probe at a concentration of 4 ng/ μ l in 2 $\times$ SSC. 25 μ l of hybridization mixture was put on a chromosomal preparation and covered with a cover slip and sealed with rubber gum. The slides were denatured at 80 °C for 3 min on a hot plate and then incubated overnight at 37 °C in a humid chamber. After overnight incubation, coverslips were floated off in 2 $\times$ SSC and slides were rinsed in 2 $\times$ SSC at 42 °C for 10 min, 1 $\times$ SSC at 42 °C for 10 min, and 2 $\times$ SSC/0.2% Tween 20 at room temperature for 10 min twice. Slides were blocked with 5% (w/v) bovine serum albumen in 2 $\times$ SSC/0.2% Tween 20 for 10 min at room temperature. Signal was detected with 100 μ l of 5 μ g/ml anti-digoxigenin-rhodamine (Roche) and 2 μ g/ml avidin-FITC (Roche) in 2 $\times$ SSC in each slide for 1 h at 37 °C in a humid chamber. Slides were washed in 2 $\times$ SSC/0.2% Tween 20 for 10 min twice, 2 $\times$ SSC at room temperature. The slides were then mounted in Vectashield Mounting Medium (Vector Lab.) containing 500 ng/ml of 4',6-diamidino-2-phenylindole (DAPI).

Results

Karyotype

The chromosome number of *Aster ageratoides* was counted to be $2n=18$ (Fig. 1A). The chromosomes of the complement in the species indicated a gradual decrease in length from the largest (6.90 μ m) to the shortest (4.84 μ m) chromosomes and consist of 2 sat-median-, 14 median- and 2 submedian-centromeric chromosomes (Fig. 2A).

Fig. 1. Somatic metaphase chromosomes of 3 species of *Aster*. A: *A. ageratoides* var. *ageratoides* ($2n=18$). B: *A. iinumae* ($2n=18$). C: *A. microcephalus* var. *ovatus* ($2n=36$). Scale bar represents $10\ \mu\text{m}$ for A to C.

The chromosome number of *A. iinumae* counted here was $2n=18$ (Fig. 1B). The chromosomes of the complement indicated a gradual decrease in length from the largest ($4.15\ \mu\text{m}$) to the shortest ($2.45\ \mu\text{m}$) chromosomes and consist of 2 sat-subterminal-, 14 median- and 2 submedian-centromeric chromosomes (Fig. 2B).

The chromosome number of *A. microcephalus* observed was $2n=36$ (Fig. 1C). The chromosomes of the complement in the species showed bimodal distribution in length; larger ones were between $6.64\text{--}4.51\ \mu\text{m}$, the shorter ones between $3.27\text{--}1.88\ \mu\text{m}$, and consist of 2 sat-median-, 2 sat-subterminal-, 28 median- and 4 submedian-centromeric chromosomes (Fig. 2C).

FISH with 18S and 5S ribosomal DNA probes

The chromosomal positions of 5S rRNA and 18S rRNA genes of *A. ageratoides* and *A. iinumae* were detected by McFISH. One pair of 5S rDNA site was at the interstitial region of the short arm of the submedian-centromeric chromosome in *A. ageratoides* (Fig. 3A), and at the interstitial region of the short arm of the median-centromeric chromosome in *A. iinumae* (Fig. 3B). One pair of 18S rDNA cluster was found in the secondary constriction on satellite chromosomes of each of the species (Figs. 3D and 3E). The McFISH of *A. microcephalus* chromosomes tagged 8 out of 36 chromosomes (Figs. 3C and 3F). The 5S rDNA were localized on the interstitial region of the short arm of one pair of the submedian-centromeric L-chromosomes and one pair of the median-centromeric S-chromosomes. A pair of 18S rDNA sites was detected at the secondary constriction on L-satellite chromosomes and another pair was detected at the secondary constriction on S-satellite

Fig. 2. Karyotypes of *Aster* species observed. A: *A. ageratoides* var. *ageratoides* ($2n=18=18L$). B: *A. iinumae* ($2n=18=18S$). C: *A. microcephalus* var. *ovatus* ($2n=36=18L+18S$). Scale bar represents 10 μm for A to C.

chromosomes.

Discussion

The plants of *Aster ageratoides* and *A. iinumae* examined had the same chromosome number, $2n=18$ (Fig. 1A and 1B), which implied diploid based on $x=9$. But there observed a significant difference in the chromosome size between *A. ageratoides* (L-type chromosomes) and *A. iinumae* (S-type chromosomes). The longest chromosome of *A. iinumae* (4.15 μm) is shorter than the shortest one of *A. ageratoides* (4.84 μm), and the total length of all the chromosomes of *A. iinumae* (60.49 μm) is 55.4% of the latter (109.1 μm). In spite of the critical difference in chromosome size, their karyotypes are relatively similar. One pair of satellite chromosomes could be easily recognized in each species, but the definitive identification of 14 median chromosomes of each species was difficult due to their similar chromosome lengths and arm ratios (Figs. 1C & 1D). These results agreed with the previous reports (Huziwaru 1957b and 1958, Shindo 1967, Tara 1977).

According to the molecular tree of Ito *et al.* (1995 and 1998), the large reduction of genome size has occurred on the evolutionary way to the several species called as *Kalimeris* which have short chromosomes. It is known that the genome size of plant species is greatly affected by the amounts of repetitive sequences and ploidy levels. The difference in the number of repetitive sequences contributes significantly to genome size, organization of chromosomes, and evolution of plant species (Bennetzen 2000). And it is also known that the difference of the amount of repetitive sequences could be caused by retrotransposons (Kamm *et al.* 1996, Galasso *et al.* 1997, Heslop-Harrison *et al.* 1997, Vicient *et al.* 1999, Hanson *et al.* 2003). For example, retrotransposons showed more than 40% of the *Vicia* genome (Pearce *et al.* 1996) and more than 90% of the wheat genome (Flavell 1986). In these cases, retrotransposons caused increase in genome size. On the

Fig. 3. Somatic metaphase chromosomes of *Aster ageratoides* var. *ageratoides* (A, D), *A. iinumae* (B, E) and *A. microcephalus* var. *ovatus* (C, F). A-C, Localization of rRNA gene by McFISH of 5S rDNA (red) and 18S rDNA (green) probes; D-F, Partial karyotype showing 18S rDNA and 5S rDNA carrying chromosomes. Chromosomes were counter stained with DAPI. Scale bar represents 10 μ m for A to F.

other hand, the case in *Aster s. l.* mentioned above shows the genome size reduction, which is a relatively rare example in chromosome evolution. To resolve the mechanism of genome reduction in *Aster s. l.*, to compare the chromosome structure between the both karyotypes is needed. In this study, the McFISH with 5S rDNA and 18S rDNA of *A. ageratoides* and *A. iinumae* chromosomes tagged 4 out of 18 chromosomes (Fig. 3). Chromosomal sites of 5S rDNA were on the short arms of the submedian-centromeric chromosomes in *A. ageratoides*, and on the short arms of the median centromeric chromosomes in *A. iinumae*. And the sites of 18S rDNA were on the satellite chromosomes, which could be considered as homologous position in both species. The similarity of the karyotypes and the position of 18S rDNA sites may support the possibility that the reduction of the genome size was caused through a parallel miniaturization at least for the satellite chromosomes.

To identify the chromosome homology between them, additional markers are required such as McFISH with bacterial artificial chromosomes and transformation-competent artificial chromosome as probes (Jiang *et al.* 1995, Hanson *et al.* 1995, Lapitan *et al.* 1997, Ohmido *et al.* 1998, Shen *et al.* 1998, Suzuki *et al.* 2001). And to elucidate the qualitative difference between them, measurement of DNA content is also necessary.

Tara (1977) suggested that *A. microcephalus* var. *ovatus* ($2n=36$) (referred as *A. ageratoides* subsp. *ovatus*) was a tetraploid amphidiploid produced by the combination of the L-type chromosomes of *Aster* and S-type chromosomes of *Kalimeris*. The chromosome number of *A. microcephalus* observed in this study was $2n=36$, composed of LS-type chromosome set which is considered to be a combined set of *A. ageratoides* and *A. iinumae* (Figs. 1C and 2C). The McFISH with 5S rDNA and 18S rDNA of *A. microcephalus* showed 2 pairs of 5S rDNA and 18S rDNA sites (Fig. 3C and 3F), respectively. *Astermicrocephalus* (LS-type karyotype) has 5S rDNA and 18S rDNA sites equal to the sum of the numbers of the 2 diploid species (*A. ageratoides* and *A. iinumae*) (Fig. 3). One pairs of 18S rDNA detected at the secondary constrictions of the each satellite chromosomes pair in L-type and S-type chromosome set (Fig. 3F). The 5S rDNA probes were hybridized 2 pairs of 2 different non-satellite chromosomes, one on the short arms of the submedian-centromeric chromosomes in L-type chromosome set, and the other on the short arms of the median centromeric chromosomes in S-type chromosome set (Fig. 3F). Variability in number and distribution of 5S and 18S rRNA genes on chromosomes had been observed in several species and its relatives (Vaughan *et al.* 1993, Leggett and Markand 1995, Snowdon *et al.* 1997, Raina *et al.* 2001, Li and Zhang 2002). In this study, however, we demonstrated that the number and loci of 5S rDNA and 18S rDNA had remained stable during the evolutionary process in chromosome reduction of *Aster*. Our results provide additional evidence for *A. microcephalus* being a tetraploid amphidiploid produced by the combination of *Aster* and *Kalimeris*.

References

- Ali, H. B. M., Meister, A. and Schubert, I. 2000. DNA content, rDNA loci and DAPI bands reflect the phylogenetic distance between *Lathyrus* species. *Genome* **43**: 1027–1032.
- Anamthawat-Jónsson, K. and Bödvarsdóttir, S. K. 2001. Genomic and genetic relationships among species of *Leymus* (Poaceae: Triticeae) inferred from 18S-26S ribosomal genes. *Amer. J. Bot.* **88**: 553–559.
- Ansari, H. A., Ellison, N. W., Reader, M. S., Badaeva, E. D., Friebe, B., Miller, T. E. and Williams, W. M. 1999. Molecular Cytogenetic Organizations of 5S and 18S-26S rDNA Loci in White Clover (*Trifolium repens* L.) and Related Species. *Amer. J. Bot.* **83**: 199–206.
- Bennetzen, J. L. 2000. Transposable element contributions to plant gene and genome evolution. *Plant. Mol. Biol.* **42**: 251–269.
- Bremer, K. 1994. *Asteraceae. Cladistics & Classification*. Timber Press, Portland.
- Flavell, R. B. 1986. Repetitive DNA and chromosome evolution in plants. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **312**: 227–242.
- Galasso, I., Harrison, G. E., Pignone, D., Brandes, A. and Heslop-Harrison, J. S. 1997. The distribution and organization of Ty1-copia-like retrotransposable elements in the genome of *Vigna unguiculata* (L.) Walp. (Cowpea) and its relatives. *Ann. Bot.* **80**: 327–333.
- Hajdera, I., Siwinska, D. and Hasterok, R. 2003. Molecular cytogenetic analysis of genome structure in *Lupinus angustifolius* and *Lupinus cosentinii*. *Theor. Appl. Genet.* **107**: 988–996.
- Hanson, L., Brown, R. L., Boyd, A., Johnson, M. A. T. and Bennett, M. D. 2003. First nuclear DNA C-values for 28 angiosperm genera. *Ann. Bot.* **91**: 31–38.
- Hanson, R. E., Zwick, M. S., Choi, S., Islam-Faridi, M. N., McKnight, T. D., Wing, R. A., Price, H. J. and Stelly, D. M. 1995. Fluorescent *in situ* hybridization of a bacterial artificial chromosome. *Genome* **38**: 646–651.
- Hasterok, R., Jenkins, G., Langdon, T., Jones, R. N. and Maluszynska, J. 2001. Ribosomal DNA is an effective marker of Brassica chromosomes. *Theor. Appl. Genet.* **103**: 486–490.
- , Langdon, T., Taylor, S. and Jenkins, G. 2002. Combinatorial labeling of DNA probes enables multicolour fluorescence *in situ* hybridization in plants. *Folia Histochem. Cytobiol.* **40**: 319–323.
- Heslop-Harrison, J. S., Brandes, A., Taketa, S., Schmidt, T., Vershinin, A. V., Alkhimova, E. G., Kamm, A., Doudrick, R. L., Schwarzacher, T., Katsiotis, A., Kubis, S., Kumar, A., Pearce, S. R., Flavell, A. J. and Harrison, G. E. 1997. The chromosomal distribution of Ty1-copia group retrotransposable elements in higher plants and their implications for genome evolution. *Genetica* **100**: 197–204.
- Hizume, M. 1993. Chromosomal localization of 5S rRNA genes in *Vicia fava* and *Crepis capillaris*. *Cytologia* **58**: 417–421.
- Huziwar, Y. 1957a. Karyotype analysis in some genera of Compositae. II. The karyotype of Japanese *Aster* species. *Cytologia* **22**: 96–112.
- , 1957b. Karyotype analysis in some genera of Compositae. III. The karyotype of the *Aster ageratoides* group. *Amer. J. Bot.* **44**: 783–790.
- , 1958. Karyotype analysis in some genera of Compositae. IV. The karyotypes within the genera Compositae. IV. The karyotypes within the genera *Gymnaster*, *Kalimeris* and *Heteropappus*. *Cytologia* **23**: 33–45.

- . 1967. Chromosomal evolution in *Aster* and related genera. *Taxon* **16**: 303–304.
- Ito, M., Soejima, A., Hasebe, M. and Watanabe, K. (1995). A Chloroplast-DNA Phylogeny of *Kalimeris* and *Aster*, with Reference to Generic Circumscription. *J. Plant Res.* **108**: 93–96.
- , —, and Watanabe, K. 1998. Phylogenetic relationships of Japanese *Aster* (Asteraceae, Astereae) *sensu lato* based on chloroplast-DNA restriction site mutations. *J. Plant Res.* **111**: 217–223.
- Jiang, J., Gill, B. S., Wang, G. L., Ronald, P. C. and Ward, D. C. 1995. Metaphase and interphase fluorescence *in situ* hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proc. Natl. Acad. Sci. USA* **92**: 4487–4491.
- Kamm, A., Doudrick, R. L., Heslop-Harrison, J. S. and Schmidt, T. 1996. The genomic and physical organization of *Ty1-copia*-like sequences as a component of large genomes in *Pinus elliotii* var. *elliottii* and other gymnosperms. *Proc. Natl. Acad. Sci. USA* **93**: 2708–2713.
- Kitamura, S. 1937. Compositae Japonicae. I. Men. Coll. Sci. Kyoto Imp. Univ. Ser. B. **13**: 337–357.
- Kokubugata, G., Kondo, K., Tatarenko, I. V., Kulikov, P. V., Verkholat, V. P., Gontcharov, A., Ogura, H., Funamoto, T., Hoshi, Y. and Suzuki, R. 2003. Diploid cytotypes in two species of *Aster sensu lato* (Asteraceae) from Primorye Territory, Russia. *Chrom. Sci.* **7**: 23–28.
- Lapitan, N. L. V., Brown, S. E., Kennard, W., Stephens, J. L. and Knudson, D. L. 1997. FISH physical mapping with barley BAC clones. *Plant J.* **11**: 149–156.
- Leggett, J. M. and Markand, G. S. 1995. The genomic identification of some monosomics of *Avena sativa* L. cv. Sun using genomic *in situ* hybridization. *Genome* **38**: 747–751.
- Leitch, I. J. and Heslop-Harrison, J. S. 1992. Physical mapping of the 18S-5.8S-26S rRNA genes in barley by *in situ* hybridization. *Genome* **35**: 1013–1018.
- , and —. 1993. Physical mapping for four sites of 5S rDNA sequences and one side of the alpha-amylase gene in barley (*Hordeum vulgare*). *Genome* **36**: 517–523.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosome. *Hereditas* **52**: 201–220.
- Li, D. and Zhang, X. 2002. Physical Localization of the 18S-5.8S-26S rDNA and Sequence Analysis of ITS Regions in *Thinopyrum ponticum* (Poaceae: Triticeae): Implications for Concerted Evolution. *Amer. J. Bot.* **90**: 445–452.
- Long, E. O. and Dawid, B. 1980. Repeated genes in eukaryotes. *Annu. Rev. Biochem.* **49**: 727–764.
- Matoba, H., Uchiyama, H. and Koyama, T. 2001. Physical mapping of 5S and 18S rDNA in lettuce, *Lactuca sativa* L. (Asteraceae). *Chrom. Sci.* **5**: 73–77.
- Murata, M., Heslop-Harrison, J. S. and Motoyoshi, F. 1997. Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence *in situ* hybridization with cosmid clone. *Plant J.* **12**: 31–37.
- Navrátilová, A., Neumann, P. and Macas, J. 2003. Karyotype analysis of four *Vicia* species using *in situ* hybridization with repetitive sequences. *Ann. Bot.* **91**: 921–926.
- Ohmido, N., Akiyama, Y. and Fukui, K. 1998. Physical mapping of unique nucleotide sequences on identified rice chromosomes. *Plant Mol. Biol.* **38**: 1043–1052.
- Pearce, S. R., Harrison, G., Li, D., Heslop-Harrison, J. S., Kumar, A. and Flavell, A. J. 1996. The *Ty1-copia* group retrotransposons in *Vicia* species: copy number, sequence heterogeneity and chromosomal location. *Mol. Gen. Genet.* **250**: 305–315.
- Raina, S. N., Mukai, Y., Kawaguchi, K., Goel, S. and Jain, A. 2001. Physical mapping of 18-5.8-26S and 5S ribosomal RNA gene families in three important vetches (*Vicia species*) and their allied taxa constituting three species complexes. *Theor. Appl. Genet.* **103**: 839–845.
- Ran, Y., Hammett, R. W. and Murray B. G. 2001. Phylogenetic Analysis and Karyotype Evolution in the Genus *Clivia* (Amaryllidaceae). *Ann. Bot.* **87**: 823–830.
- Richard, D. and Rieseberg, L.H. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster s. l.* *Amer. J. Bot.* **83**: 398–412.
- Shaw, C. H. (ed.) 1988. *Plant Molecular Biology. A Practical Approach*. Oxford Univ. Press, Oxford.
- Shen, K. A., Meyers, B. C., Islam-Faridi, M. N., Chin, D. B., Stelly, D. M. and Micheltore, R. W. 1998. Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. *Mol. Plant-Microbe Interact.* **11**: 815–823.
- Shindo, K. 1967. Cytological, morphological and geographical studies on the differentiation of species in section *Asteromoea* of *Kalimeris* in Japan. *J. Sci. Hiroshima Univ., Ser. B. Div. 2*, **11**: 127–199.
- Singh, R. J., Kim, H. H. and Hymowitz, T. 2001. Distribution of rDNA loci in the genus *Glycine* Willd. *Theor. Appl. Genet.* **103**: 212–218.
- Snowdon, R. J., Köhler, W. and Köhler, A. 1997. Chromosomal localization and characterization of rDNA loci in the *Brassica* A and C genomes. *Genome* **40**: 582–587.
- Sogin, M. L. 1990. Amplification of ribosomal RNA genes for molecular evolution studies. In *RCR Protocols. A Guide to Methods and Applications* (eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J.), pp. 307–322, Academic Press, Inc.
- Suzuki, G., Ura, A., Saito, N., Do, G.S., Seo, B.B., Yamamoto, M. and Mukai, Y. 2001. BAC FISH analysis in *Allium cepa*. *Genes Genet. Syst.* **76**: 251–255.
- Tara, M. 1977. Cytogenetic studies on natural intergeneric hybridization on *Aster* alliances. IV. Experimental confirmation of the hybrid origin of *Aster ageratoides* subsp. *ovatus*. *Bot. Mag. Tokyo* **90**: 253–258.
- Vicient, C. M., Suoniemi, A., Ananthawat-Jönsson, K., Tanskanen, J., Beharav, A., Nevo, E. and Schulman, A. H. 1999. Retrotransposon *BARE-1* and Its Role in Genome Evolution in the Genus *Hordeum*. **11**: 1769–1784.
- Vaughan, H. E., Jamilena, M., Ruiz Rejon, C., Parker, J. S. and Garrido-Ramos, M. A. 1993. Loss of nucleolus-organizer regions during polyploid evolution in *Schilla autumnalis*. *Heredity* **71**: 574–580.