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Chromosome phylogeny of *Zamia* and *Ceratozamia* by means of Robertsonian changes detected by fluorescence *in situ* hybridization (FISH) technique of rDNA

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Abstract. Appearance and location of 45S rDNA and 5S rDNA signals were compared in chromosomes of nine species of the aneuploid *Zamia* and their taxonomically and phylogenetically closely related *Ceratozamia mexicana*. The 45S rDNA signal was detected in the proximal region of six chromosomes in *Zamia angustifolia*, *Z. integrifolia*, *Z. pumila* and *Z. pygmaea* (all $2n = 16$); in the proximal region of 6–14 chromosomes in *Z. furfuracea*, *Z. loddigesii*, *Z. skinneri* and *Z. vazquezii* (all $2n = 18$); and on the proximal region of 20 chromosomes in *Z. muricata* ($2n = 23$). The 5S rDNA signals were commonly seen near the terminal region of the short arm of two metacentric chromosomes in the four species with $2n = 16$ and *Z. furfuracea*, *Z. loddigesii* and *Z. vazquezii* with $2n = 18$. Other 5S rDNA signals were seen near the terminal region of two terminal-centromeric chromosomes in *Z. skinneri* and near the terminal region of a metacentric and a telocentric chromosomes in *Z. muricata*. In contrast, those with 45S and 5S rDNA signals were exhibited in chromosomes of *Ceratozamia mexicana* in a different manner from those in the nine species of *Zamia*; the 45S rDNA signal in the terminal region of four metacentric and two submetacentric chromosomes and the 5S rDNA signal near the proximal region of two metacentric chromosomes.

Key words: Cycadales, generic aneuploid, FISH, 45S rDNA, 5S rDNA, Zamiaceae, *Zamia*.

Among the cycads, *Zamia*, taxonomically placed in the Zamiaceae, is the most diversified genus ecologically, and found from humid to dry areas and from seaside to mountain districts; geographically, in Mexico, Central and South America (Norstog and Nicholls 1997); and morphological variation ranges from large arborescent species to small subterranean ones (Jones 1993). The genus consists of more than 50 species (Norstog and Nicholls 1997).

Among Cycadales, *Zamia* is the only genus which shows inter- and intra-specific aneuploidy, ranging from $2n = 16$ to 28 (Marchant 1968; Norstog 1980, 1981; Vovides 1983; Moretti and Sabato 1984; Moretti 1990a, b; Moretti et al. 1991; Caputo et al. 1996; Vovides and Olivares 1996). This aneuploidy might be performed by centric fission (Moretti and Sabato 1984; Moretti 1990a, b; Moretti et al. 1991; Caputo et al. 1996) and fusion (Marchant 1968, Norstog 1980).

Molecular cytogenetics using fluorescence *in situ* hybridization (FISH) to analyze chromosome structures and phylogenetic relation-

ships in cycads have been carried out in *Ceratozamia* (Kokubugata and Kondo 1998), *Cycas* (Hizume 1995; Hizume et al. 1992, 1998), *Microcycas* (Kokubugata and Kondo 1998) and *Zamia* (Fuchs et al. 1995, Kondo and Tagashira 1998). Those results of FISH have demonstrated the positions of the tandem repeat sequence regions of 18S rDNA, 45S rDNA, 5S rDNA and telomere sequences on certain chromosomes. *Microcycas calocoma* and *Ceratozamia mexicana* showed that the CMA-positive bands and the 45S rDNA signals occurred at the same place on the chromosomes within the respective species, although these regions between two species were different regions in chromosome from each other (Kokubugata and Kondo 1998). In contrast, *Zamia furfuracea*, *Z. integrifolia* and *Z. muricata* displayed some large telomere sequence repeat regions near the centromere of certain chromosomes (Kondo and Tagashira 1998).

Materials and methods

Plant materials. The nine species of *Zamia* and *Ceratozamia mexicana* studied in the Zamiaceae are shown in Table 1. Classification follows Stevenson and Sabato (1980) and Stevenson (1987), with the exception of *Z. vazquezii*, which follows Stevenson et al. (1995–1996).

Preparation of somatic chromosomes at metaphase. Meristematic tissues were harvested from young leaflets by the method described in Kokubugata and Kondo (1994): Young leaflets were chopped into small pieces ca 1.5 mm across and treated with 0.002 M 8-hydroxyquinoline solution at 4 °C for 13 h before being fixed in 1:3 acetic ethanol at 4 °C overnight. They were then washed in distilled water and stored in 70% ethanol. Materials were rinsed in distilled water and 45% acetic acid for 10 min each at room temperature and macerated in 45% acetic acid at 60 °C for 4 min. Trichomes on the epidermis were removed and small pieces of leaflets were chopped into smaller pieces about 0.5 mm across. Those pieces were again macerated in the enzyme mixture of 3% cellulase Onozuka R-10 (Yakult) and 0.25% Pectolyase Y-23 (Seishin) in distilled water (w/v) at 37 °C for 20 min. They were then washed in distilled water for 10 min and squashed in 45% acetic acid on glass slides. After the coverslips were removed from the slides by the dry-ice method, the preparations were air-dried.

Preparation of DNA probes. The 18S-5.8S-26S rRNA gene of wheat, pTa71, which contained the spacer region, was used as 45S rDNA probe (Gerlach and Bedbrook 1979).

The method of total DNA extraction followed Kawahara et al. (1995). Young leaflets of the male *Zamia integrifolia* (2.5 g fresh weight) were harvested, ground, and homogenized before being frozen in liquid nitrogen. The homogenized powder was transferred to the centrifugation tube, which contained 10 ml washing buffer [0.1 M Tris-HCl pH 8.0, 2% 2-mercaptoethanol (w/v), 1% polyvinylpyrolidone X-30 (w/v), 0.05 M L-ascorbic acid, dissolved in distilled water] before applying the CTAB method.

The 5S rDNA probe was produced by the polymerase chain reaction (PCR) method modified by Hizume (1993). Two primers of 5S rDNA gene were selected on the basis of sequences of *Pisum sativum* of 5′-CGGTGCATTAATGCTGGTAT-3′ and 5′-CCATCAGAACTCCGCAGTTA-3′. The PCR reaction mixture consisted of 0.2 M dNTP (TAKARA), 0.5 mM each of the primers, 0.05 μg of total DNA extracted from *Zamia integrifolia*, and 1.25 units of *Taq* polymerase (TAKARA Taq; TAKARA) in 50 μl PCR buffer (TAKARA), and cycles of the PCR were described as follows: The first cycle was denaturation at 95 °C for 90 sec, annealing at 55 °C for 30 sec and primer extension at 75 °C for 30 sec; and the last 30 cycles were denaturation at 95 °C for 30 sec, annealing 55 °C for 30 sec and primer extension at 75 °C for 30 sec. The amplified oligonucleotides were precipitated by ethanol, air-dried and resuspended in TE solution.

The 45S rDNA and 5S rDNA were labeled by nick translation (Bionick labeling system: Life Technologies, GIBCO BRL).

FISH. The FISH method followed Kokubugata and Kondo (1998): The preparations were treated by 50 μl RNase solution containing 0.1 mg/ml RNase A (Sigma) in 2 × SSC at 37 °C for 1 h. After washing in 2 × SSC for 10 min, they were refixed in 4% paraformaldehyde dissolved in PBS at room temperature for 10 min. The preparations were then washed in sterilized water and treated with an ethanol series (70%, 100%). After they were air-dried, 10 μl of the predenatured 45S or 5S rDNA probe mixture were applied to the slides and

Table 1. Nine species of *Zamia* and *Ceratozamia mexicana* used

Species	Distribution (after Jones 1993)	Cultivation source	Accession No. (voucher specimen No.): Sex	Present count of chromosome No.
Zamiaceae				
Zamia				
Z. angustifolia Jacq.	Elenthea Island, Eastern Cuba	Satake Corporation	1091800-S1 (NT-068)	2n = 16
Z. integrifolia L. F. in Aiton	America (South-Eastern Georgia and Florida), Western Cuba, South-Central Puerto Rico, Cayman Islands, Bahamas	LPCGS	1091900-3 (NT-003), 4 (NT-004), 6 (NT-006), 7 (NT-007), 11 (NT-011), 26 (NT-026), 31 (NT-031), 32 (NT-032) : ♂ 1091900-45 (NT-045) : ♀	2n = 16
Z. pumila L.	Central Cuba	LPCGS	1092501-1 (NT-050), 2 (NT-051) : ♂	2n = 16
Z. pygmaea Sims	Central Western Cuba	Satake Corporation	1092100-S1 (NT-065), S2 (NT-067) : ♂	2n = 16
Z. furfuracea L. f. in Aiton	Mexico (Veracruz)	LPCGS	1093400-2 (NT-054), 3 (NT-055) : ♂	2n = 18
Z. loddigesii Miq.	Mexico (Oaxaca, Chiapas, Campeche, Quintana Roo), Belize, Guatemala	Satake Corporation	1094201-S1 (NT-082) : ♂	2n = 18
Z. skinneri Wersz. ex A. Dietrich	Southern Nicaragua, Costa Rica, Panama	Satake Corporation	1090700-S1 (NT-070), S2 (NT-071) : ♂	2n = 18
Z. vazquezii D. Stevenson Sabato & Moretti	Mexico (Veracruz)	LPCGS and Satake Corporation	1091500-1 (NT-052), 2 (NT-053) : ♂ 1091500-S3 (NT-072), S6 (NT-075) : ♂	2n = 18
Z. muricata Willd.	Northern Venezuela and adjacent Colombia	Satake Corporation	1094901-S2 (NT-080) : ♂ S3 (NT-081): ?	2n = 23
Ceratozamia				
C. mexicana Brongn.	Mexico (North-central Veracruz)	Satake Corporation	1081400-1 (NT-095) : ♂	2n = 16

LPCGS: Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University

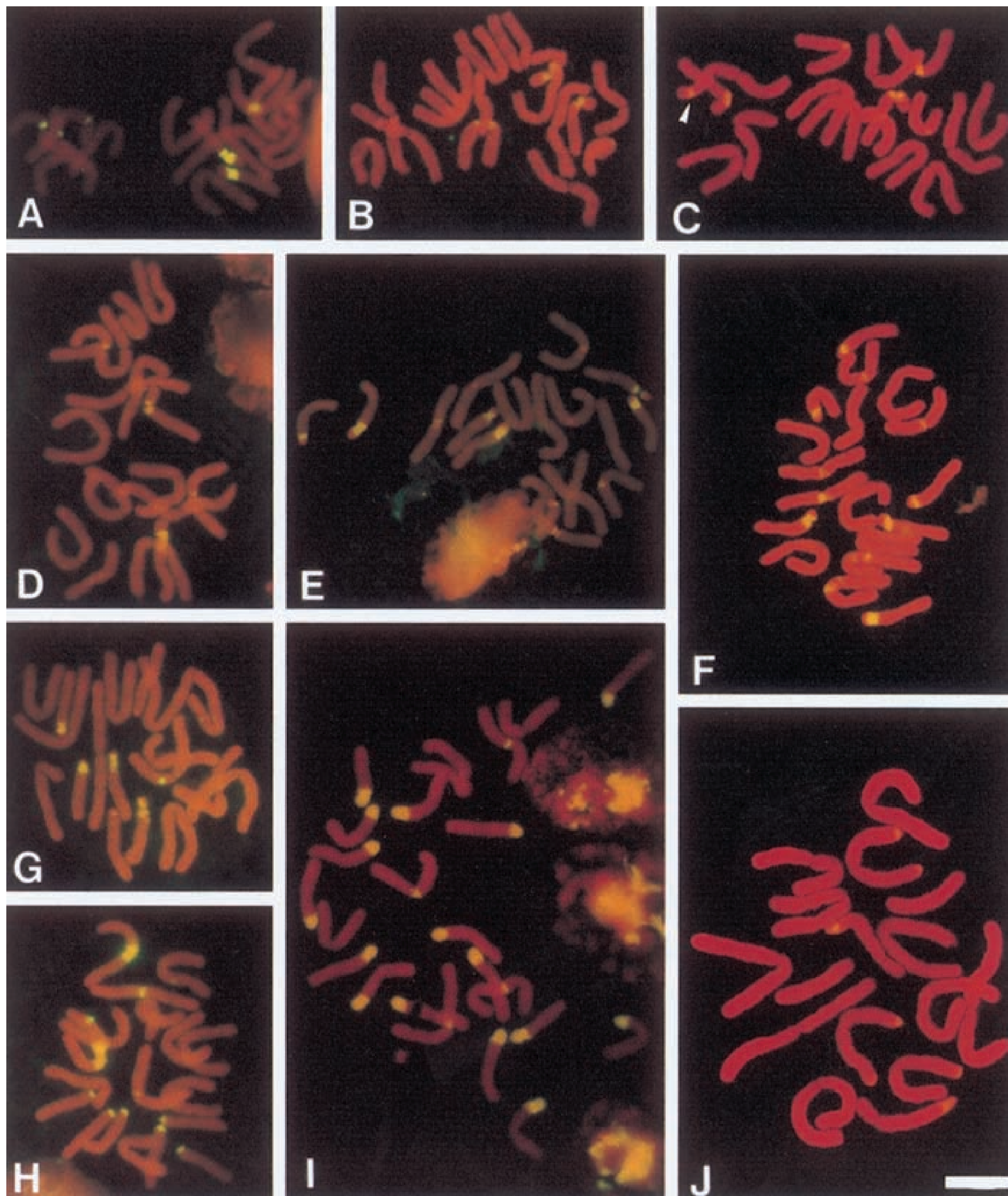

Fig. 1. Fluorescence *in situ* hybridization with biotinilated pTa71 probe to somatic metaphase chromosomes of nine species of *Zamia* and *Ceratozamia mexicana*. **A** *Z. angustifolia*, **B** *Z. integrifolia*, **C** *Z. pumila*, arrowhead points to the 45S rDNA signal on the secondary constriction in the short arm of one chromosome, **D** *Z. pygmaea*, **E** *Z. furfuracea* **F** *Z. loddigesii*, **G** *Z. skinneri* **H** *Z. vazquezii*, **I** *Z. muricata*, **J** *C. mexicana*. **A–D**, the species with the chromosome number of 2n = 16; **E–H**, the species with the chromosome number of 2n = 18; **I**, the species with the chromosome number of 2n = 23. The majority of the 45S rDNA signals appeared around the centromere on several chromosomes in the species of *Zamia* studied, while some appeared on the terminal region of six chromosomes. Bar: 10 μm

covered with a sealing film (Novix; Iwaki). The slides were denatured at 80 °C for 10 min and incubated at 37 °C overnight in Omnislide (HYBAID). They were washed in 2 × SSC and 4 × SSC at 37 °C for 10 min each. The hybridization probes were detected by 10 μg/ml avidin-FITC (Boehringer Mannheim) and counterstained by 1 μg/ml propidium iodide (PI; Sigma). The slides were observed under fluorescence microscope using a blue filter (B-2A; Nikon). Microphotographs were taken on Super G400 (FUJI) film.

Observation of chromosomes. Chromosome classification followed Levan et al. (1964). Among the terminal-centromeric chromosomes, t-chromosome meant acrocentric chromosome characterized by the presence of the heterochromatic short arm, and T-chromosome meant telocentric chromosome characterized by the presence of an extremely small short arm.

Results

FISH using the pTa71 probe on metaphase chromosomes in the species of *Zamia* studied showed interspecific difference in number of chromosomes which contained 45S rDNA signal sites.

Zamia angustifolia, *Z. integrifolia*, *Z. pumila* and *Z. pygmaea* had the chromosome number of 2n = 16 and the karyotype of 12 m-, two sm- and two st-chromosomes. They showed the 45S rDNA signal on the proximal region of both arms of the 11th and the 12th chromosomes (m-chromosomes), the proximal region of the short arms of the 13th and the 14th chromosomes (sm-chromosomes) and the proximal region of both arms of the 15th and 16th chromosomes (st-chromosomes) (Fig. 1A, B, C, D). Additionally *Zamia pumila* showed one extra 45S rDNA signal on the secondary constriction of the 1st chromosome (m-chromosome) (Fig. 1C).

Zamia furfuracea and *Z. loddigesii* had the chromosome number of 2n = 18 and the karyotype of ten m-, two sm- and six t- chromosomes. They showed the 45S rDNA signal on the proximal region of both arms of the 3rd to the 10th chromosomes (m-chromosomes), the whole region of the short arm and the proximal region of the long arm of the 13th to the 18th chromosomes (t-chromosomes) (Fig. 1E, F). However, the two species displayed commonly weaker 45S rDNA signals on the 5th and 6th chromosomes (m-chromosomes) than those on the other six signaled m-chromosomes (Fig. 1E, F). No size variation was observed in the heterochromatic short arms of the 13th to the 18th chromosomes (t-chromosomes), which showed the 45S rDNA signal (Fig. 1E, F). However, this result was different from the previous reports (Moretti and Sabato 1984, Moretti et al. 1991, Caputo et al. 1996): They pointed out that sizes of the heterochromatic short arms of the 13th to the 18th chromosomes (t-chromosomes) displayed intra- and inter-specific variabilities in the four species of *Zamia* with the chromosome number of 2n = 18. *Zamia furfuracea* and *Z. loddigesii* showed the same chromosome which were described above and could be correlated with hybridization between the two species (Jones 1993). An artificial hybrid between *Z. furfuracea* and *Z. spartea*, made and characterized by Norstog (1990), was quite similar morphologically to plants in some populations of *Z. loddigesii* (Schutzman 1987). Furthermore, Schutzman (1987) stated that *Z. furfuracea* and *Z. loddigesii* might have performed introgressive hybridization in those populations not so long ago.

Zamia skinneri had the chromosome number of 2n = 18 and the karyotype of ten m-, two sm-, four t- and two T-chromosomes. The species showed the 45S rDNA signal on the whole region of the short arm and the proximal region of the long arms of the 13th to the 16th chromosomes (t-chromosomes), which would correspond to the 13th to 18th chromosomes (t-chromosomes) in *Z. furfuracea* and *Z. loddigesii* described above. Additionally, a minute, dotted 45S rDNA signal appeared on the (rather) short arms of the 17th and 18th chromosomes (T-chromosomes) (Fig. 1G).

Zamia vazquezii had the chromosome number of 2n = 18 and the karyotype of ten m-, two sm- and six T-chromosomes. The species showed the 45S rDNA signal on the

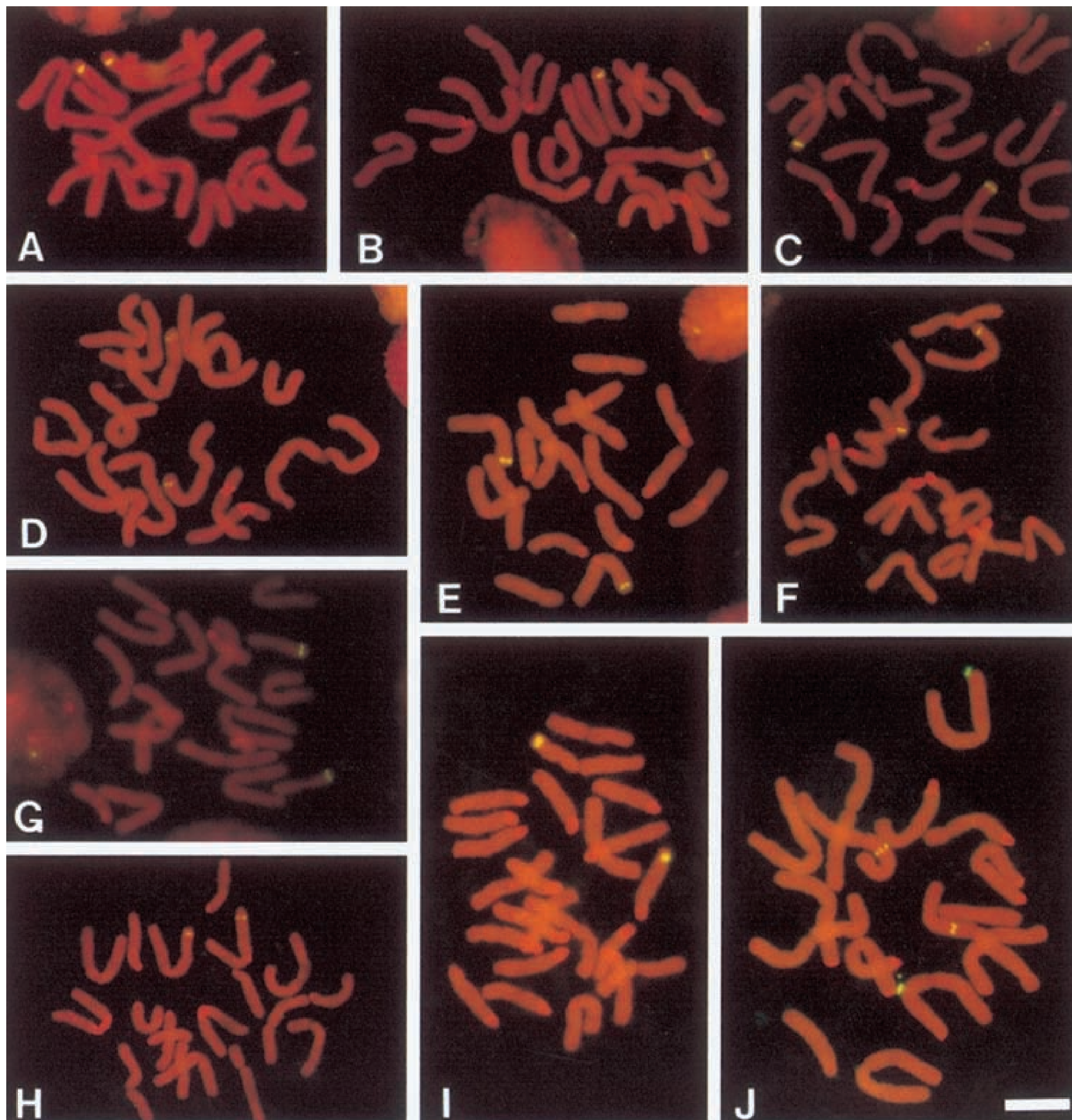

Fig. 2. Fluorescence *in situ* hybridization with biotinilated 5S rDNA probe to somatic metaphase chromosomes of nine species of *Zamia* and *Ceratozamia mexicana*. **A** *Z. angustifolia*, **B** *Z. integrifolia*, **C** *Z. pumila*, **D** *Z. pygmaea*, **E** *Z. furfuracea*, **F** *Z. loddigesii*, **G** *Z. skinneri*, 5S rDNA signals (yellow) appeared on two T-chromosomes, **H** *Z. vazquezii*, **I** *Z. muricata*, the one m-chromosome and one t-chromosome had the 5S rDNA signal, **J** *C. mexicana*. **A–D**, the species with the chromosome number of 2n = 16; **E–H**, the species with the chromosome number of 2n = 18; **I**, the species with the chromosome number of 2n = 23. Seven species of *Zamia* studied excluding *Z. skinneri* and *Z. muricata* had the 5S rDNA signal near the terminal region of the short arm of two m-chromosomes. In contrast, *C. mexicana* had the 5S rDNA signal near the distal region of the short arm of two m-chromosomes. Bar: 10 μm

proximal region of both arms of the 3rd to the 6th chromosomes and additional 45S rDNA dots on the much shorter arms of the 13th to the 18th chromosomes (Fig. 1H). The 45S rDNA signals of the 3rd and the 4th chromosomes occupied relatively larger regions than did the other signalled regions.

According to the above results, the four species of *Zamia* with the chromosome number of $2n = 18$ studied had interspecific variability in the position of the 45S rDNA signal, but the other four species of the genus with the chromosome number of $2n = 16$ did not.

Zamia muricata had the chromosome number of $2n = 23$ and the karyotype of seven m- and 16 t-chromosomes. The species showed the 45S rDNA signal on the proximal region of both arms of the 2nd to 4th chromosomes and the whole region of the short arms and the proximal region of the long arms of the 8th to the 23rd chromosomes (Fig. 1I). The signals found on the 8th to the 23rd chromosomes were similar in shape to each other and were brighter than any signals seen in the other eight species.

Ceratozamia mexicana, another member of the Zamiaceae, had the chromosome number of $2n = 16$ and the karyotype of 12m-, two sm- and two T-chromosomes. The species showed the 45S rDNA signal on the terminal region of the long arm of the 9th and the 10th chromosomes, the terminal region of both the arms and the satellite region of the 11th and the 12th chromosomes and the terminal region of the long arm and the satellite region of the 13th and the 14th chromosomes (Fig. 1J). The 45S rDNA signals in the 9th and the 10th chromosomes were different in size from each other.

During the investigation, FISH using the 5S probe showed three different colors in the chromosomes of the nine species of *Zamia* studied: The yellowish FITC fluorescent signals of the 5S rDNA regions; dark red colored heterochromatic regions counter-stained by propidium iodide (PI), which corresponds with the C-banded regions and the Chromomycin A_3-positive banded region and furthermore 45S rDNA regions; and pale red colored region, other than heterochromatic regions, counter-stained by PI (Fig. 2). Thus, FISH combined with 45S and/or 5S rDNA regions made it possible to identify the chromosomes on each nuclear plate. In the nine species of *Zamia* studied, the 5S rDNA signal appeared commonly on two chromosomes, which varied in size. The four species of *Zamia* which had the chromosome number of $2n = 16$ showed the 5S rDNA signal in the distal region of the short arms of the 7th and the 8th chromosomes (Fig. 2A–D).

In contrast, the other four species of *Zamia* which had the chromosome number of $2n = 18$ showed the 5S rDNA signal on the distal region of two chromosomes, each of which differed morphologically depending on their respective species. *Zamia furfuracea* and *Z. loddigesii* showed the 5S rDNA signal on the distal region of the short arms of the 3rd and the 4th chromosomes, classified as m-chromosomes. *Zamia skinneri* had the 5S rDNA signal on the distal region of the long arms of the 17th and the 18th chromosomes, classified as T-chromosomes. *Zamia vazquezii* exhibited the 5S rDNA signal on the distal region of the short arms of the 5th and the 6th chromosomes, classified as m-chromosomes.

Zamia muricata displayed the 5S rDNA signal on the distal region of the short arm of the 1st chromosome, classified as the m-chromosome, and on the distal region of the long arm of the 14th chromosome, classified as the t-chromosome.

Ceratozamia mexicana showed the 5S rDNA signal on the interstitial region near the proximal region of the short arms of the 1st and the 2nd chromosomes.

Discussion

The four species of *Zamia* with the chromosome number of $2n = 16$ and the same relative location and number of 45S rDNA signals, except for a signal on the secondary constriction in *Z. pumila*, and the other species with $2n = 18$ and 23 and different relative location

and number of 45S rDNA signals can be correlated with their geographical distribution: The four species with 2n = 16 were distributed among Islands of the Caribbean Sea, while the four species with 2n = 18 were distributed in Mexico and Central America (Jones 1993). A similar tendency was found in the position of 5S rDNA signals (Fig. 3). Moretti et al. (1991), Caputo et al. (1996) and Vovides and Olivales (1996) stated that the species of *Zamia* found in uniform and stable environments had the chromosome number of 2n = 16, while the species found in a wide range of environments had the chromosome number of 2n = 18 or more; the species with 2n = 16 might progress and differentiate toward the species with 2n = 18 or more for occupying and adapting in a wide range of environments. Schutzman et al. (1988) indicated that karyotypes with high chromosome numbers in *Zamia* were commonly found in inhospitable environments. Vovides and Olivares (1996) also postulated that the onset of drier ecological conditions could have exerted selective pressures, resulting in chromosomal change in *Zamia*. However, whether chromosome number in *Zamia* has increased or decreased was difficult to determine since progress of centric fission and fusion might occur over a timespan of karyotype evolution (Jones 1977).

The karyotype variation in *Zamia* might have occurred by centric fission (Moretti 1990a, b; Moretti et al. 1991; Caputo et al. 1996) or fusion (Marchant 1968; Norstog 1980), and pericentric inversions and unequal translocation (Vovides and Olivares 1996). An instance of karyomorphological change by either centric fission or fusion was observed in *Z. muricata* by FISH (Fig. 3l): The largest m-chromosome and one t-chromosome in *Z. muricata* showed the 5S rDNA signal. The former, largest m-chromosome (or the first chromosome), could not make a homologous pair with the any other m-chromosome, and thus it might have arisen by centric fusion of two t-chromosomes, of which one chromosome contained the 5S rDNA region. Two T-chromosomes in *Z. skinneri* (2n = 18) had the 5S rDNA region, while two m-chromosomes in the other three species which contain the chromosome number of 2n = 18 had the 5S rDNA. These differences in 5S rDNA region might be caused by the centric fission and/or fusion.

Because the 1st chromosome of *Z. muricata* had the 5S rDNA signal but no 45S rDNA signal, while the 13th chromosome had the 5S rDNA signal and the 45S rDNA signal existed around the centromere, the 45S rDNA region may have been added or deleted by chromosome fission or fusion. This kind of structural change in chromosome may occur in the species of *Zamia* studied, because the large 45S rDNA region was observed on other t-chromosomes of *Z. muricata* and t- or T-chromosomes of the four species with the chromosome number of 2n = 18. Addition and deletion of rDNA regions have been reported on the fused chromosome in humans (Gravholt et al. 1992, Wolff and Schwartz 1992) or the fissioned chromosome in the plant *Hypochoeris radicata* (Hall and Parker 1995). In contrast, the rDNA regions of *Z. muricata* were much larger than those of humans and *Hypochoeris radicata*.

FISH using the *Arabidopsis*-type telomere sequence repeats was applied to metaphase chromosomes of *Z. integrifolia*, *Z. furfuracea and Z. muricata* by Kondo and Tagashira (1998), who demonstrated the telomere sequence region on their several chromosomes. Comparing the 45S rDNA regions and the telomere sequence repeat regions, the telomere sequence regions were found around the 45S rDNA region on certain chromosomes or on the centromere of the chromosomes, which did not contain the 45S rDNA region.

All the species of *Zamia* studied with an exception of *Z. pumila* commonly showed the 45S rDNA signal near the centromeric region of certain chromosomes and the 5S rDNA signal near the terminal region of certain chromosomes. In contrast, *Ceratozamia mexicana* had the 45S rDNA signal on the terminal region of four m-chromosomes and two sm-chromosomes and the 5S rDNA signal near

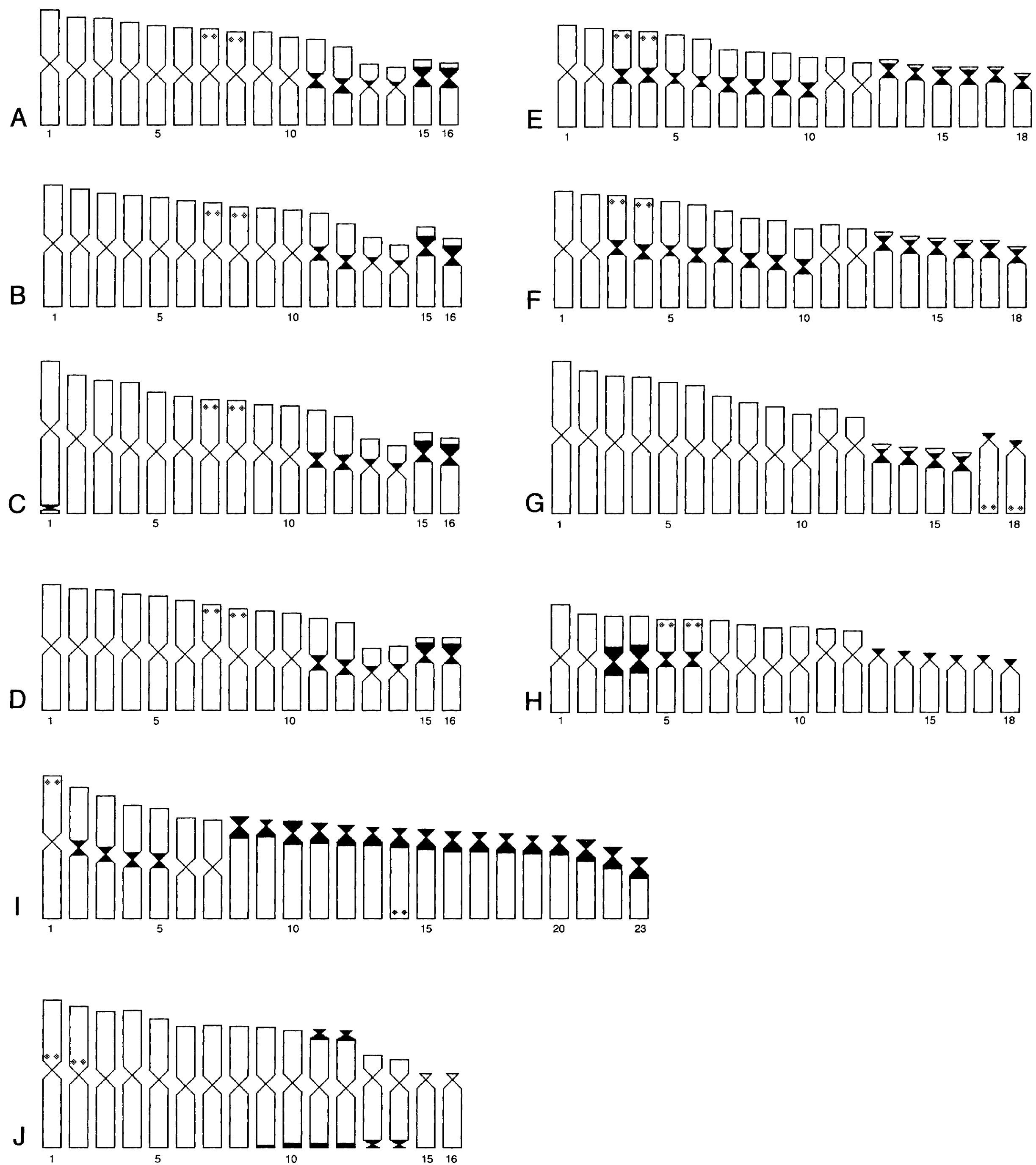

Fig. 3. Idiograms of the metaphase chromosome sets with locations of 45S rDNA and 5S rDNA signals in nine species of *Zamia* and *Ceratozamia mexicana*. **A** *Z. angustifolia*, **B** *Z. integrifolia*, **C** *Z. pumila*, **D** *Z. pygmaea*, **E** *Z. furfuracea*, **F** *Z. loddigesii*, **G** *Z. skinneri*, **H** *Z. vazquezii*, **I** *Z. muricata*, **J** *C. mexicana*. Black solid areas represent the 45S rDNA signals and small dotted areas represent the 5S rDNA signals. **A–D**, high similarity of appearance of 45S rDNA and 5S rDNA regions among the chromosome sets of the four species of *Zamia* with the chromosome number of 2n = 16; **E–H**, differences of appearance of 45S rDNA and 5S rDNA regions among the chromosome sets of the four species of *Zamia* with the chromosome number of 2n = 18

the centromere of two m-chromosomes. These positions in *Ceratozamia* have already been reported by Kokubugata and Kondo (1998). Moretti (1990b) stated that T-chromosomes of *Ceratozamia* might occur due to deletion of the short arm of st-chromosomes on karyotype evolution from *Zamia* species with the chromosome number of 2n = 16 toward *Ceratozamia*. The present demonstration of relationships between the chromosomes of 2n = 16 of four species of *Zamia* and *Ceratozamia mexicana* has led to a speculation that the terminal region and the proximal region of the 45S and 5S rDNA- signalled chromosomes were inverted. Thus, chromosome evolution in *Zamia* and *Ceratozamia mexicana* with the chromosome number of 2n = 16 could be explained not only by Moretti's speculation (1990b) but also by the present results. This inverting mechanism might confirm the pericentric inversion and unequal translocation proposed by Vovides and Olivares (1996). *Microcycas calocoma*, which was considered more closely related to *Zamia* than *Ceratozamia*, displayed the 45S rDNA signal on the proximal region of certain chromosomes, as did *Zamia* (Kokubugata and Kondo 1998). Consequently, although *Ceratozamia* and *Zamia* had various morphological similarities, the progress of karyotypic evolution differed greatly.

Khoshoo (1969) hypothesized that *Zamia* with the chromosome number of 2n = 16 might not develop karyotype evolution, while *Microcycas calocoma* with the chromosome number of 2n = 26 might. In contrast, Moretti (1990a) speculated that the karyotype of *Bowenia* with the chromosome number of 2n = 18 could be the most primitive among the cycad members studied, except for *Cycas*, and might have given rise to the karyotypes of *Stangeria* and *Zamia* with the chromosome number of 2n = 16. Then, the karyotype of *Zamia* might be transformed into that of *Ceratozamia* with the chromosome number of 2n = 16 and that of *Microcycas* with the chromosome number of 2n = 26. The present results support Moretti's hypothesis (1990a) described above.

The present study has shown that the aneuploid *Zamia* resulted from centric fission or fusion of chromosome with structural differentiation of heterochromatic 45S rDNA regions. Further detection and comparison of the 45S rDNA regions and other heterochromatic sequence regions in chromosomes involved with centric fission and fusion among intraspecific aneuploid individuals in *Zamia* should be made by FISH to clarify and justify the progress of speciation and phylogenetic relationships.

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