Analysis of Meiotic Chromosome Behaviour in Diploid Individuals of *Chrysanthemum zawadskii* and Related Species (Asteraceae): Evidence for Chromosome Rearrangements

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Summary Diploid individuals ($2n=18$) of *Chrysanthemum zawadskii*, *C. indicum* and *C. boreale*, two or all three of which often occur together in the wild in East Asia, have diverse metaphase karyotypes. We hypothesised that the diverse karyotypes result from chromosome rearrangements. To test this, we investigated chromosomal behaviour during pollen mother cell (PMC) meiosis and pollen fertility, using 33 individuals collected from seven populations in Korea. All individuals contained one or two multivalents (mostly quadrivalents) at late prophase I and metaphase I in some PMCs (approximately 17–37%), and at least one or two bivalents composed of homologous chromosomes of different length and centromere position in all PMCs. This meiotic chromosomal behaviour indicates that chromosome rearrangements, *i.e.*, reciprocal translocation and unequal crossing-over, are prevalent in *Chrysanthemum* species. Nevertheless, because of successful chromosome segregation in anaphase I and II, they produce ample stainable pollen with different chromosome complements. Fertilization by such gametes should result in diverse somatic karyotypes. We also discuss the possibility that older or relatively recent hybridization events have led to a high rate of irregular meiotic chromosome behaviours and pollen sterility, particularly in *C. indicum*.

Key words Chromosome, *Chrysanthemum*, Hybridization, Karyotype, Meiosis, Rearrangement.

The genus *Chrysanthemum* (tribe Anthemideae, Asteraceae), which comprises about 37 perennial species that had previously been treated as *Dendranthema* (Bremer and Humphries 1993, Bremer 1994), is distributed mainly in China, Korea and Japan in East Asia. Some species exhibit notable morphological variation and polyploidy. For instance, *C. zawadskii* Herbach (= *D. zawadskii*), which is widely distributed from East Asia through Russia to Europe (Bremer and Humphries 1993), is highly diverse morphologically, and several infraspecies taxa have been proposed (Lee 1996). The species also has a series of polyploids from $2x$ to $10x$, with the base number $x=9$ (Kim *et al.* 2004). In addition, as previously reported (Kim *et al.* 2003), diploid individuals ($2n=18$) of *C. zawadskii* (= *Dendranthema zawadskii* var. *latilobum*), *C. indicum* L. (= *D. indicum*) and *C. boreale* Makino (= *D. boreale*) have diverse metaphase karyotypes, not only within species but also within populations. These three species are clearly distinguished by inflorescence and flower characters (Kim *et al.* 2003). At least two of the three species often occur together in the wild; especially *C. indicum* always grows with *C. zawadskii* and *C. boreale* and sometimes includes atypical individuals with white ray-flowers (*cf.* Fig. 2d). Thus, hybridization may have occurred among the species.

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However, unlike other diploid species ($2n=18$) such as *Aster iinumae* and *A. ageratoides* which have so distinct karyotypes that can be confirmed in a presumed tetraploid hybrid (Matoba *et al.* 2007), the three *Chrysanthemum* species are characterized by the indistinguishable and diverse karyotypes (Kim *et al.* 2003).

With regard to karyotype changes, chromosomal rearrangements can alter the size and symmetry of chromosomes, resulting in different karyotypes. These rearrangements include chromosomal fusions and fissions, duplications and deletions of chromosome segments due to unequal crossing-over between homologous chromosomes, segment inversions, and segment translocations between non-homologous chromosomes (Stebbins 1971, Raven and Johnson 1999, Rieseberg 2001, Levin 2002). Thus, we hypothesised that the diverse karyotypes in *Chrysanthemum* species result from chromosome rearrangements. To test this, we investigated chromosome behaviour during PMC meiosis in diploid individuals of *C. zawadskii*, *C. indicum* and *C. boreale*. The diploid species *C. boreale* and *C. makinoi* Matsum & Nakai always form nine bivalents at the metaphase I of PMCs (Tanaka 1959a, 1959b, Tanaka and Shimotomai 1961). Any chromosomal rearrangements that did occur should be revealed in unusual chromosome pairings in late prophase I and metaphase I (Stebbins 1971, Levin 2002). For example, chromosomes involved in reciprocal translocation between two non-homologous chromosomes typically form quadrivalents (rings or chains of four chromosomes), while unequal crossing-over should result in bivalents composed of two chromosomes of different length and/or centromere position (Stebbins 1971, Raven and Johnson 1999, Levin 2002). Chromosome rearrangements often generate unbalanced gametes with reduced fertility (Rieseberg 2001, Levin 2002). Thus, we also estimated pollen fertility in these species. Our results will further our understanding of how these species produce fertile gametes to survive and have been sustained in wild populations.

Materials and methods

Plant materials

In total, 33 diploid *Chrysanthemum* individuals ($2n=18$) were collected from seven populations (or localities; Fig. 1), including 14 *C. zawadskii* Herbich, 13 *C. indicum* K. (including eight atypical individuals with white ray-flowers), and six *C. boreale* Makino (Table 1). These individuals are the same as those we used for karyotype analyses in the previous study (Kim *et al.* 2003), in which it was shown that almost all of the individuals examined had different karyotypes of somatic chromosomes. As already stated in the introduction, two or all three of the species often occur together as in the Kwangha population (Figs. 2a–f). The plants were planted individually in pots at the Botanical Garden of the Graduate School of Science, Kyoto University (vouchers at KYO).

Meiosis observations

To obtain PMCs to observe at meiosis, we collected heads with numerous developing flowers from pot-cultivated plants in August–September 2004 and 2005. They were fixed in a ethanol : chloroform : acetic acid (2 : 1 : 1) solution. PMCs undergoing meiosis were separated with forceps on a glass-slide, stained and macerated in a solution of 10 parts 2% acetic orcein and one part 1 N HCl, then smeared and observed with a microscope (Olympus BX51). Chromosome behaviour was analysed at late prophase I, metaphase I and anaphase I, as well as anaphase II and late telophase II.

Pollen fertility

We estimated pollen fertility by the presence or absence of cytoplasm. Some 1,500 pollen grains from a single head per individual were stained with 1% aniline blue in lactophenol solution (Shivanna and Rangaswamy 1992). We also measured the sizes (diameter) of stainable and unstainable pollen grains in typical and atypical *C. indicum* individuals, using 300 grains.

Fig. 1. Map showing the seven sampling sites. Details are: Pochon (Pochon-up, Pochon-kun, Kyunggi-do); Baekun (Baekun-ri, Mitán-myeon, Pyungchang-kun, Kangweon-do); Kwangha (Kwangha-ri, Jungsun-up, Jungsun-kun, Kangweon-do); Youngweol (Munkok-myeon, Youngweol-kun, Kangweon-do); Okgye (Dalsan-myeon, Youngduk-kun, Kyungsangbuk-do); Dalsan (Dalsan-myeon, Youngduk-kun, Kyungsangbuk-do); Mt. Jiri (Byuksoryoung, Mt. Jiri-san, Cheollabuk-do).

Fig. 2. Diploid *Chrysanthemum* individuals collected from the Kwangha population. a–c. *C. zawadskii*. d. Atypical *C. indicum*, with white ray-flowers. e. Typical *C. indicum*. f. *C. boreale*. Scale bar=5 cm.

Fig. 3. Micrographs of chromosome configurations at early metaphase I meiotic divisions of pollen mother cells (PMCs) in diploid *Chrysanthemum* individuals. a–c. PMCs with nine bivalents in *C. zawadskii* (Kim 312), *C. indicum* (Kim 318) and *C. boreale* (Kim 328), respectively. d–f. PMCs containing one or two quadrivalents (white arrows) in *C. zawadskii* (Kim 307, 313, 311). Note that a bivalent in the rectangle (enlarged at the upper right) is composed of two chromosomes differing in length and centromere position (white arrowheads) in Fig. 3d, and a trivalent (arrow) and univalent (arrowhead) appear in Fig. 3f. Asterisks in Figs. 3b, c, e and f indicate a pair composed of two chromosomes of different length and centromere position. Scale bar=10 μ m.

Fig. 4. Micrographs of chromosome segregation in PMCs of *Chrysanthemum zawadskii*. a–c. At meiotic anaphase I. d–f. At meiotic anaphase II. g and h. Tetrads. Note regular segregation in a, d and g, lag-guards (arrows) in b and e, chromosome bridges (arrows) in c and f and a micronucleus (arrow) in h. Scale bars=10 μ m (scale bar in f applies to a–e, and that in h applies to g).

Statistical testing

We performed analysis of variance (ANOVA) and *post hoc* multiple comparisons using Scheffe's test ($p < 0.05$) by SPSS 12.0 program to evaluate the significance of differences in the following frequencies among the species: (1) cells with multivalents at late prophase I and metaphase I, (2) cells exhibiting irregular segregation of chromosomes at anaphase I, anaphase II and late telophase II, and (3) stainable pollen grains. The significance of differences in size between stainable and unstainable pollen grains in *C. indicum* was also evaluated using the *t*-test ($p < 0.01$).

Results

Chromosome pairing at late prophase I and metaphase I

In all plants, PMCs with nine bivalents were most often observed at late prophase I and metaphase I (Fig. 3a–c). Every individual had PMCs with creature quadrivalents, appearing as a chain of four chromosomes, in addition to the bivalents. Fig. 3d shows a PMC with two quadrivalents, and Figs. 3e and 3f show a PMC with a single quadrivalent. A trivalent was occasionally observed with a univalent (Fig. 3f). In addition, all examined PMCs appeared to contain at least creature bivalents composed of two chromosomes of different lengths and/or centromere position (Fig. 3d, upper right).

The frequency of PMCs with multivalents (quadrivalents and trivalents) varied from 16.90 to

Table 1. Data for the occurrence of multivalents at prophase I and metaphase I as well as of irregular chromosome segregation at anaphase I and II, and for pollen stainability in *Chrysanthemum*

Taxon (locality)	Collection	Chromosome pairing at late prophase I and metaphase I		Chromosome segregation				Pollen stainability (%)		
		Number of cells examined	Number of cells with a multi- valent(s)* (%)	Anaphase I		Anaphase II				
				Number of cells examined	Cells showing irregular segregation (%)	Number of cells examined	Cells showing irregular segregation (%)			
<i>C. zawadskii</i> (Okgye)	<i>Kim 258</i>	103	25 (24.27)	157	14 (8.92)	107	9 (8.41)	208	5 (2.40)	82.70
	<i>Kim 307</i>	100	34 (34.00)	159	9 (5.66)	123	12 (9.76)	209	4 (1.91)	82.20
	<i>Kim 308</i>	75	16 (21.33)	168	3 (1.79)	68	1 (1.47)	197	1 (0.51)	91.20
	<i>Kim 260</i>	76	20 (26.32)	147	14 (9.52)	64	1 (1.56)	159	4 (2.52)	81.60
	<i>Kim 309</i>	71	12 (16.90)	105	7 (6.67)	76	5 (6.58)	148	3 (2.03)	93.60
(Dalsan)	<i>Kim 276</i>	63	18 (28.57)	98	2 (2.04)	64	2 (3.13)	125	2 (1.60)	88.20
	<i>Kim 310</i>	47	12 (25.53)	116	5 (4.31)	92	2 (2.17)	280	5 (1.79)	89.50
	<i>Kim 311</i>	113	24 (21.24)	131	10 (7.63)	83	3 (3.61)	153	3 (1.96)	91.50
(Kwangha)	<i>Kim 312</i>	61	19 (31.15)	77	6 (7.79)	110	9 (8.18)	197	9 (4.55)	78.70
	<i>Kim 313</i>	70	26 (37.14)	102	9 (8.82)	74	4 (5.41)	218	2 (0.92)	74.20
	<i>Kim 314</i>	84	25 (29.76)	172	10 (5.81)	195	7 (3.59)	127	2 (1.57)	85.40
	<i>Kim 281</i>	69	26 (37.68)	240	9 (3.75)	135	2 (1.48)	89	1 (1.12)	74.50
	<i>Kim 315</i>	97	20 (20.62)	186	9 (4.84)	328	13 (3.96)	287	9 (3.14)	90.40
	<i>Kim 316</i>	75	23 (30.67)	113	8 (7.08)	92	2 (2.17)	195	2 (1.03)	78.70
	Total (mean)		1104	300 (27.17)	1971	115 (5.83)	1611	72 (4.47)	2592	52 (2.01)
<i>C. indicum</i> (atypical) (Kwangha)	<i>Kim 279</i>	67	16 (23.88)	94	5 (5.32)	137	11 (8.03)	90	1 (1.11)	48.10
	<i>Kim 321</i>	57	26 (45.61)	118	13 (11.02)	349	30 (8.60)	126	5 (3.97)	25.90
	<i>Kim 322</i>	59	27 (45.76)	124	34 (27.42)	135	20 (14.81)	164	17 (10.37)	25.10
	<i>Kim 323</i>	38	10 (26.32)	186	14 (7.53)	223	17 (7.62)	244	16 (6.56)	43.70
	<i>Kim 324</i>	67	26 (38.81)	138	59 (42.75)	112	23 (20.54)	103	4 (3.88)	37.90
	<i>Kim 325</i>	61	18 (29.51)	123	21 (17.07)	191	14 (7.33)	116	7 (6.03)	41.60
	<i>Kim 326</i>	62	28 (45.16)	120	16 (13.33)	111	21 (18.92)	95	9 (9.47)	33.20
	<i>Kim 327</i>	55	24 (43.64)	266	71 (26.69)	104	5 (4.81)	208	11 (5.29)	37.00
	Total (mean)		466	175 (37.55)	1169	233 (19.93)	1362	141 (10.35)	1146	70 (6.11)

Table 1. (Continued)

Taxon (locality)	Collection	Chromosome pairing at late prophase I and metaphase I		Chromosome segregation						Pollen stainability (%)
		Number of cells examined	Number of cells with a multi- valent(s)* (%)	Anaphase I		Anaphase II		Late telophase II		
				Number of cells examined	Cells showing irregular segregation (%)	Number of cells examined	Cells showing irregular segregation (%)	Number of cells examined	Cells with a micronucleus (%)	
<i>C. indicum</i> (Kwangha)	<i>Kim 278</i>	66	20 (30.30)	109	7 (6.42)	159	27 (16.98)	146	8 (5.48)	57.20
	<i>Kim 317</i>	56	21 (37.50)	127	26 (20.47)	208	38 (18.27)	191	26 (13.61)	47.00
	<i>Kim 318</i>	69	25 (36.23)	214	40 (18.69)	159	10 (6.29)	87	2 (2.30)	49.70
	<i>Kim 319</i>	80	32 (40.00)	131	15 (11.45)	201	43 (21.30)	139	22 (15.83)	43.70
	<i>Kim 320</i>	41	6 (14.63)	123	6 (4.88)	237	22 (9.28)	107	9 (8.41)	76.10
Total (mean)		312	104 (33.33)	704	94 (13.35)	964	140 (14.52)	670	67 (10.00)	(54.74)
<i>C. boreale</i> (Kwangha) (Dalsan) (Pochon) (Mt. Jiri) (Youngweol) Total (mesan)	<i>Kim 280</i>	66	13 (19.70)	113	6 (5.31)	77	5 (6.49)	108	1 (0.93)	86.20
	<i>Kim 300</i>	63	11 (17.46)	77	4 (5.19)	72	2 (2.78)	114	1 (0.88)	92.50
	<i>Kim 328</i>	61	9 (14.75)	77	9 (11.69)	75	5 (6.67)	160	2 (1.25)	95.10
	<i>Kim 329</i>	68	12 (17.65)	152	4 (2.63)	79	5 (6.33)	159	5 (3.14)	93.40
	<i>Kim 196</i>	76	13 (17.11)	109	2 (1.83)	112	4 (3.57)	128	4 (3.13)	95.20
	<i>Kim 330</i>	91	16 (17.58)	116	10 (8.62)	165	4 (2.42)	223	1 (0.45)	87.10
		425	74 (17.41)	644	35 (5.43)	580	25 (4.31)	892	14 (1.57)	(91.58)

* Multivalents include quadrivalents and trivalents.

37.68% (mean=27.17%) in *C. zawadskii*, 23.88 to 45.76% (mean=37.55%) in atypical *C. indicum*, 14.63 to 40.00% (mean=33.33%) in typical *C. indicum*, and 14.75 to 19.70% (mean=17.41%) in *C. boreale* (Table 1). Thus, PMC frequencies with multivalent were highest in atypical *C. indicum*, lowest in *C. boreale*, and medium in typical *C. indicum* and *C. zawadskii*. ANOVA showed that a significant difference exists in the mean frequencies of PMCs with multivalents among the species ($p<0.05$). Scheffe's test further gave a significant difference between *C. zawadskii* and atypical *C. indicum* as well as between *C. boreale* and typical/atypical *C. indicum*.

Chromosome segregation at anaphase I and II and late telophase II

Chromosomes in most PMCs regularly segregated to opposite poles at anaphase I (Fig. 4a) and anaphase II (Fig. 4d), resulting in four distinct nuclei in late telophase II (Fig. 4g) in all plants. However, parts of chromosomes were often irregularly segregated. As a result, chromosome bridges or laggards were observed at anaphase I (Figs. 4b, c) and/or anaphase II (Figs. 4e, f). One or more micronuclei were also rarely or occasionally observed at late telophase II (Fig. 4h). Frequencies of cells exhibiting such irregular chromosome segregation were relatively low and fairly similar in *Chrysanthemum zawadskii* and *C. boreale*. The mean frequencies of cells with irregular chromosome segregation were 5.83% and 5.43% at anaphase I, 4.47% and 4.31% at anaphase II and 2.01% and 1.57% at late telophase II in *C. zawadskii* and *C. boreale*, respectively (Table 1). The frequencies were much higher in both typical and atypical *C. indicum* individuals: 13.35% and 19.93% at anaphase I, 14.52% and 10.35% at anaphase II and 10.00% and 6.11% at telophase II in typical and atypical *C. indicum*, respectively (Table 1).

Notably, as meiosis proceeded, the frequencies of cells exhibiting irregular chromosome segregation gradually decreased in all species. Nevertheless, at any meiotic stage, the frequency of cells exhibiting irregular chromosome segregation was approximately three to four times higher in both typical and atypical *Chrysanthemum indicum* than in *C. zawadskii* and *C. boreale*. ANOVA showed that the differences in irregular chromosome segregation at any meiotic stage are significant among the species ($pP<0.05$). Scheffe's test further showed that a significant difference exists at anaphase I between *C. zawadskii* and atypical *C. indicum* as well as between *C. boreale* and atypical *C. indicum*; at anaphase II and late telophase II between *C. zawadskii* and typical/atypical *C. indicum*, and *C. boreale* and typical *C. indicum*.

Pollen fertility

Each individual contained both stained and unstained pollen grains at varying frequencies (Figs. 5a–c). Frequencies of stainable pollen grains were the highest (mean=91.58%) in *C. boreale* (Fig. 5c), followed by *C. zawadskii* (mean=84.46%, Table 1, Fig. 5a). Stainable pollen grains were less frequent in typical *C. indicum* individuals (mean=54.74%, Fig. 5b), while atypical *C. indicum* individuals had the lowest frequency of stainable pollen grains (mean=36.56%, Table 1). ANOVA showed that these differences are all significant ($p<0.05$). Scheffe's test also gave a significant difference between any two of all the species except between *C. zawadskii* and *C. boreale*.

In typical *Chrysanthemum indicum*, unstainable pollen grains were $17.5\text{--}27.5\text{ }\mu\text{m}$ (mean $\pm$ standard deviation= 21.79 ± 1.76) in diameter, and stainable grains were $20.0\text{--}32.5\text{ }\mu\text{m}$ (24.18 ± 1.90). Likewise, in atypical *C. indicum*, unstainable pollen grains were $8.75\text{--}30.0\text{ }\mu\text{m}$ (22.63 ± 2.17) and stainable grains were $22.5\text{--}37.5\text{ }\mu\text{m}$ (26.32 ± 1.97). The smaller size of unstainable pollen grains was significant (t -test: $p<0.01$).

Discussion

Chromosome rearrangement and diverse karyotypes

Some PMCs (approximately 17–37%, see Table 1) in all 33 plants of *C. zawadskii*, *C. indicum*

(including atypical individuals with white ray-flowers) and *C. boreale* contained bivalents and multivalents (mostly quadrivalents and occasionally trivalents) at late prophase I and metaphase I, indicating that reciprocal translocation between two non-homologous chromosomes had occurred. Also, the bivalents included at least one or two distinct bivalents, with the two chromosomes differing in length and/or centromere position. As the presence of such bivalents does not hinder chromosome segregation in later meiotic stages, they were likely formed by an unequal crossing-over between homologous chromosomes, as reported in other angiosperm taxa, including *Viscum* (Soman and Bhavanandan 1993). Thus, chromosome rearrangements, such as reciprocal translocations and unequal crossing-over, appear to be prevalent in the three *Chrysanthemum* species, resulting in gametes with different chromosome complements.

Chromosomal rearrangements are likely to reduce pollen fertility (Levin 2002), because of the failure of regular chromosome segregation in later meiotic stages. However, in the three *Chrysanthemum* species, the frequencies of cells exhibiting irregular chromosome segregation gradually decreased as the meiotic stages proceeded (Table 1). Particularly in *C. zawadskii* and *C. boreale*, the frequencies of cells exhibiting irregular chromosome segregation were far less than those of cells with multivalents (quadrivalents and trivalents; Table 1), indicating that even cells with multivalents generally undergo regular chromosome segregation at anaphase I and II to produce stainable pollen. Among other angiosperm species, *Convallaria keiskei* (Ruscaceae) exhibits 52.2% pollen fertility, despite the high frequency of cells with quadrivalents (>94%; Noda and Zhu 1996). In *C. keiskei*, almost half of the cells with quadrivalents undergo normal chromosome disjunction to produce fertile pollen. Thus, with respect to chromosome segregation, *Chrysanthemum* species appear to go through meiotic division similarly to *C. keiskei*, producing fertile pollen with high genetic diversity in wild populations. Fertilization by these gametes should result in diverse somatic karyotypes, as found in our previous study (Kim *et al.* 2003).

Hybridization events in Chrysanthemum

Among the three species examined, the frequency of laggard chromosomes, chromosome bridges, and/or micronuclei was highest in *C. indicum*, which resulted in irregular chromosome segregation at much higher frequencies at anaphase I and II and late telophase II than in the other species (Table 1). *C. indicum* also showed a much higher rate of unstainable pollen than did *C. zawadskii* or *C. boreale* (Table 1), and the unstainable pollen grains were smaller than stainable pollen grains. Although we did not pursue the fate of laggard chromosomes in these plants, in other angiosperm species, including *Tribulus* spp. (Husain 1986) and *Avena sativa* L. (Baptista-Giacomelli *et al.* 2000), laggard chromosomes form micronuclei, which develop into nonfunctional dwarf pollen. This may be the case in *C. indicum*. Laggards and micronuclei, which were found at the highest frequencies during meiosis, appeared to develop into small-sized unstainable pollen grains, resulting in a high rate of unstainable pollen in *C. indicum*.

Why do both typical and atypical *C. indicum* have such high rates of unstainable pollen? *C. indicum* always occurs with *C. zawadskii* and *C. boreale* in populations. In angiosperms interspecific hybridization causes irregular meiosis and/or a high rate of pollen sterility (Saitwal *et al.* 2003). *C. indicum* has been regarded as a distinct taxon (Lee 1996), but it may have originated as a hybrid between *C. zawadskii* and *C. boreale*. However, diploid *C. indicum* individuals may be old and long established. They appear to set some seeds, although we have not confirmed their viability. Atypical *C. indicum* individuals have white ray-flowers, as does *C. zawadskii*, and may represent a recent cross with *C. zawadskii*. They are morphologically intermediate between *C. zawadskii* and *C. indicum*.

Some wild populations are composed only of diploid *C. zawadskii* and *C. boreale* individuals. These species also exhibit lower but considerably high rates of PMCs with multivalents and irregular chromosome segregation. There is the possibility that older hybridization events have led to such

Fig. 5. Micrographs of pollen grains from diploid *Chrysanthemum* individuals. Stained grains by aniline blue are estimated to be fertile. a. *C. zawadskii* (Kim 309). b. *C. indicum* (Kim 319). c. *C. boreale* (Kim 280). Scale bar=50 μ m.

chromosome behaviours in *C. zawadskii* and *C. boreale*. We suspect that in those populations, *C. indicum* may be generated in the future by hybridization between *C. zawadskii* and *C. boreale*. On the other hand, it may already have been extirpated, owing to high pollen sterility. Fertility in hybrids is restored by polyploidization (e.g., Isshiki and Taura 2003, Pushpam and Raveendran 2006). In other East Asian areas, *C. indicum* now grows as a tetraploid or hexaploid (Taniguchi 1987). Recently, recurrent origins of tetraploid *C. indicum* in China were suggested by molecular evidence (Yang *et al.* 2006). The aforementioned homoploid hybridization events, which were suspected to have occurred in diploid *Chrysanthemum* species, may also be confirmed by a molecular study using DNA sequences as recently attempted by Kim *et al.* (2008) in *Sonchus* species (Asteraceae) or species-specific markers (for review of current researches see Hegarty and Hiscock 2005).

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