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ORIGINAL ARTICLE

Meiotic behavior and pollen fertility in triploid and tetraploid natural populations of *Campuloclinium macrocephalum* (Eupatorieae, Asteraceae)

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Abstract *Campuloclinium macrocephalum* DC. is a perennial herb widely distributed in the New World and introduced in South Africa, where it is commonly called "pompom weed". This species is considered one of the most important weeds of Brazil and one of the problematic invasive plants of South Africa. The meiotic system can be studied to assess the ability of a weed to spread, but only few studies on *C. macrocephalum* have been realized. In this study, we examined the meiotic behavior and pollen fertility of 14 natural populations of *C. macrocephalum* from Argentina and Uruguay. Meiotic analysis revealed 2 triploid (2n = 3x = 30), 11 tetraploid (2n = 4x = 40) and 1 mixed population (2n = 2x = 20, 2n = 4x = 40). Both, triploid and tetraploid specimens showed a widely variable meiotic behavior with irregular chromosome pairing showing univalents, bivalents, trivalents (in triploids) and tetravalents (in tetraploids) at diacinesis of first meiotic division. Different abnormalities were observed, such as: laggard chromosomes, chromatin bridges, and out of plate chromosomes at metaphase I. During meiosis I (prophase), some cells showed the phenomenon of cytomixis or chromatin transfer between pollen mother cells. The meiotic indexes suggest that only four populations were normally fertile (over 90 % of fertile pollen), indicating meiotically stable plants. The remaining populations share variable pollen fertility, with triploids ranging from 46.64 to 54.83 % and tetraploids varying from 3.54 to 45.30 %. We suggest that polyploidy seems to be recurrent in *C. macrocephalum*, promoting partial sterility of pollen grains, generating large numbers of individuals by apomixis promoting invasion of crop fields. This study presents the meiotic behavior of this weed, these could be useful for future studies of biological control in areas with no natural enemies.

Keywords Chromosome number · Cytotype · Cytomixis · Meiotic abnormalities · Polyploidy

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Introduction

Campuloclinium macrocephalum DC. (Asteraceae) is a perennial herb widely distributed in the New World, from Mexico to Argentina (Cabrera 1974), including Brazil, Bolivia, Paraguay, Uruguay and north of Argentina (Freire 2008). In the 60s decade, this species was introduced in South Africa, where it is now commonly called "pompom weed". Among 1990 and 2000, it entered a dramatic expansion phase, and a biological control program was initiated (McConnachie et al. 2011).

In Argentina, meiotic studies realized by Turner et al. (1979) found n = ca. 20II for specimens from the province of Santa Fe, while Galiano and Hunziker (1987) found 19II + 1I or 20II + 1I in a population of Buenos Aires. A most recent mitotic analysis found 2n = 20 and 2n = 40 in specimens of different samples from Paraguay, suggesting that the species includes populations with different ploidy levels (Dematteis et al. 2007). A wide meiotic analysis in several populations from Corrientes showed 2n = 30 with different meiotic configurations and an unusual high number of irregularities at different meiotic stages, including apomixis in at least one population (Farco et al. 2012).

Apomixis has been observed in 18 genera of Asteraceae and aposporous apomixis occurs in five genera (Noyes 2007). *C. macrocephalum* is an aposporous apomictic

species with microsporogenesis commonly including univalent formation and poor pollen quality (Farco et al. 2012). However, embryological studies in most genera are limited, meaning that the number of apomictic species in this family is in fact much larger (Noyes 2007). The first case of apomixis found in the tribe Eupatorieae was reported by Holmgren (1919) in *Eupatorium glandulosum* Kunth. Apomixis was also reported in six species of the tribe Eupatorieae from South America: *Ageratina riparia* (Regel) R.M. King & H. Rob. (Sparvoli 1960), *Chromolaena callilepis* (Sch. Bip. ex Baker) R.M. King & H. Rob. (Coleman and Coleman 1984), *C. squalida* (DC.) R. M. King & H. Rob. (Coleman and Coleman 1988), *C. odorata* (Lam.) R. M. King & H. Rob. (Coleman 1989), *Gyptis tanacetifolia* (Gill. ex Hook. & Arn.) D.J.N.Hind & Flann (Rozenblum et al. 1988) and *Praxelis pauciflora* (Kunth) R. M. King & H. Rob. (Bertasso Borges and Coleman 1998). In consequence, *C. macrocephalum* was the first species in the genus *Campuloclinium* reported to display apomixis (Farco et al. 2012).

In this paper, we carried out a meiotic analysis in different populations of *C. macrocephalum* from Argentina and Uruguay to evaluate the influence of different meiotic events on pollen fertility of triploid and tetraploid cytotypes. The main purpose was providing new insights that could prove helpful for biological control programs of *C. macrocephalum* in South Africa.

Table 1 Voucher information of populations of *Campuloclinium macrocephalum* analyzed in this study

A Argentina. Corrientes: Dept. Empedrado. 500 m S from Derqui, next to the road. *G. E. Farco* et al. *8* (CTES)
B Argentina. Corrientes: Dept. Saladas. Route 12 to San Lorenzo, next to the road. *G. E. Farco* et al. *9* (CTES)
C Argentina. Corrientes: Dept. San Martin. 23 km W from La Cruz, next to the road. *G. E. Farco 19* (CTES)
D Argentina. Corrientes: Dept. General Alvear. 5.5 km N of General Alvear, next to the road. *G. E. Farco* et al. *20* (CTES)
E Argentina. Corrientes: Dept. Ituzaingó. 1.8 km S of San Carlos, next to the road. *G. E. Farco 28* (CTES)
F Argentina. Corrientes: Dept. Ituzaingó. 16 km N of Virasoro, next to the road. *G. E. Farco 22* (CTES)
G Argentina. Corrientes: Dept. Santo Tomé. 7 km W of Santo Tomé, next to the road. *G. E. Farco 21* (CTES)
H Argentina. Corrientes: Dept. Paso de los Libres. 65 km SW of Paso de los Libres, next to the road. *G. E. Farco 17* (CTES)
I Argentina. Misiones: Dept. L. N. Alem. Route 14, 5.1 km NE of L. N. Alem, next to the road. *G. E. Farco 26* (CTES)
J Argentina. Misiones: Dept. Candelaria. Route 103, 18 km E of Santa Ana, next to the road. *G. E. Farco 25* (CTES)
K Argentina. Misiones: Dept. Concepción. 2 km NE of Concepción de Sierra, next to the road. *G. E. Farco 27* (CTES)
L Argentina. Misiones: Dept. Capital. Camino al bañado del arroyo Zaiman, next to the road. *G. E. Farco 24* (CTES)
M Argentina. Entre Ríos: Dept. Federación. Route 14, 6.4 km NW of Chajarí, next to the road. *G. E. Farco 16* (CTES)
N Uruguay. Dept. Rivera. 12 km SE of Masoller, stream edge. *M. Dematteis* et al. *3739* (CTES)

Materials and methods

Plant material

Fourteen populations and five individuals per populations of *C. macrocephalum* were collected in Argentina and Uruguay. A population was defined as a group of plants separated by more than 40 km. Voucher specimens were deposited at the herbarium CTES of the Instituto de Botanica del Nordeste. The collection information is summarized in Table 1.

Meiotic analysis

For meiotic analysis, floral buds were fixed in a 5:1 lactic acid–ethanol solution by 48–72 h, transferred to 70 % ethanol and then stored at 4 °C. Anthers were stained and squashed in 2 % lacto-propionic orcein. The meiotic behavior in pollen mother cells (PMC) was analyzed at early prophase I, metaphase I (MI), anaphase I (AI), and tetrad stage. Permanent preparations were carried out using Euparal as mounting media. Slides were examined with a Zeiss microscope and images were obtained using a Canon Coolpix digital camera. Analysis included counting of chromosomes, determination of the ploidy level and description of the meiotic behavior of each sample. The meiotic index was calculated as the ratio between the number of normal sporads divided by the total number of sporads analyzed per population and then multiplied by 100. Tetrads were considered normal when they had four cells of equal size.

Pollen fertility

Pollen grains obtained from anther squash preparations were stained and evaluated using the acetic carmine-glycerol. Pollen grains were stained for about 12 h and then 1,000–3,500 grains for each sample were analyzed. Fully stained pollen grains were considered fertile (Marks 1954).

Results

Meiotic behavior

Results of the meiotic analysis in the 14 populations (A–N) of *C. macrocephalum* are presented in Table 2. Two

Table 2 Meiotic configurations and ploidy level in *Campuloclinium macrocephalum*

Population	2n	Ploidy level	Meiotic configurations	Percentage	Number of cells
A	30	3x	8I + 10II + 1III	12.5	8
			6I + 2II + 2III + 2IV	12.5	
			7I + 10II + 1III[A]	12.5	
			10I + 7II + 2III	12.5	
			4I + 7II + 4III[B]	12.5	
			15I + 6II + 1III	12.5	
			8I + 8II + 2III[C]	12.5	
			12I + 9II	12.5	
B	30	3x	9I + 9II + 1III	50	4
			8I + 8II + 2III[C]	25	
			7I + 10II + 1II	25	
C	40	4x	2I + 11II + 4III + 1IV	33.33	3
			18II + 1IV[D]	33.33	
			2I + 14II + 2III	33.33	
D	40	4x	18II + 1IV[D]	14.28	7
			7I + 10II + 3III + 1IV	14.28	
			3I + 12II + 3III + 1IV	14.28	
			1I + 7II + 3III + 4IV	14.28	
			2I + 14II + 2III + 1IV	14.28	
			1I + 11II + 3III + 2IV	14.28	
			2I + 7II + 7III + 3IV	14.28	
E	40	4x	20II[E]	42.85	7
			2I + 17II + 1IV	14.28	
			2I + 19II[F]	14.28	
			1I + 15II + 3III	14.28	
			16II + 2IV[G]	14.28	
F	40	4x	6I + 17II	28.57	7
			3I + 17II + 1III	14.28	
			1I + 18II + 1III	14.28	
			16II + 2IV[G]	14.28	
			5I + 16II + 1III	14.28	
			2I + 15II + 2IV	14.28	
G	40	4x	10I + 9II + 4III	33.33	3
			4I + 16II + 1IV	33.33	
			8I + 13II + 2III[C]	33.33	
H	30	3x	8I + 11II	20	5
			4I + 8II + 4III (32)[B]	20	
			7I + 4II + 5III	20	
			7I + 10II + 1III[A]	20	
			9I + 8II + 2III (31)	20	
	40	4x	9I + 12II + 1III + 1IV	25	4
			4I + 14II + 2III + 1IV (42)	25	
			10I + 13II + 1IV	25	
			8I + 9II + 2III + 2IV	25	
I	40	4x	1I + 8II + 3III + 4IV(41)	16.66	6
			2I + 12II + 2III + 2IV	16.66	
			1I + 14II + 1III + 2IV	16.66	
			12II + 4IV	16.66	
			2I + 19II[F]	16.66	

Table 2 continued

Population	2n	Ploidy level	Meiotic configurations	Percentage	Number of cells
			10II + 5IV	16.66	
J	40	4x	17II + 2III	33.33	3
			15II + 3IV (42)^{H}	33.33	
			2I + 15II + 2IV	33.33	
K	40	4x	20II^{E}	14.28	7
			18II + 1IV	14.28	
			1I + 16II + 1III + 1IV	14.28	
			1I + 12II + 1III + 3IV	14.28	
			2I + 16II + 2III	14.28	
			14II + 3IV^{H}	14.28	
			16II + 2IV^{G}	14.28	
L	40	4x	2I + 8II + 5IV	33.33	3
			4I + 14II + 2IV	33.33	
			8I + 14II + 1III + 1IV (43)^{C}	33.33	
M	40	4x	4I + 4III + 8II + 2IV	33.33	3
			4II + 6III + 4IV	33.33	
			1I + 13II + 3III + 1IV	33.33	
N	40	4x	18II + 1IV^{D}	60	5
			11II + 2III + 3IV	20	
			20II^{E}	20	

Numbers in parentheses indicate total number of chromosomes. Similar chromosome configurations are indicated with superscript

populations (A, B) were triploid with chromosome number 2n = 30, while 11 populations were tetraploid with 2n = 40 (C–N), and only 1 population was mixed having some specimens with 2n = 30 and others bearing 2n = 40 chromosomes (H).

The triploid populations (A, B) showed a wide variation of meiotic configurations (Fig. 1a, b), which are detailed in Table 2. The meiotic configuration 8I + 8II + 2III was the most common in the two populations (Fig. 1b). Population H was mixed, having triploid and tetraploid specimens. The meiotic configuration of the H triploid individuals was similar to the triploid individuals of A population with 7I + 10II + 1III (Fig. 1a), but showed other cells with 4I + 7II + 4III. The most frequent configurations were 18I + 1IV (populations C, D, K, and N), 20II (E, K, and N) and 16II + 2IV (E, F, and K) (see Fig. 1c–e, respectively). The C–N tetraploid populations displayed different meiotic configurations, but some pairing configurations were similar among them (Table 2). A few PMC of the triploid and tetraploid cytotypes analyzed showed until three additional chromosomes (see Table 2, in parentheses are the numbers additional chromosomes).

The percentage of normal tetrads in triploid populations ranged between 80.26 and 91.42 %, while in tetraploid populations varied from 59.83 to 98.25 %. Meiosis resulted in a high frequency of irregular meiotic products such as dyads, triads (Fig. 1i) and tetrads; some tetrads showed a variable number of microcytes and, occasionally, of micronuclei. Meiotic indexes ranged between 44.69 and 94.64 % (Table 3). According to this index, populations B, C, F, H 4x, and K were considered meiotically normal (values above 90 %).

Cytomixis and chromosome stickiness were commonly observed meiotic abnormalities. Cytomixis was found in populations A, B, I, K, and L (Fig. 1j, k), but partial or complete chromosome stickiness was observed in all samples. Cytomixis involving inter PMC transfer of chromatin material was observed in triploid and tetraploid populations. The transfer of chromatin was mostly observed at early prophase stage through one to many chromatin bridges and a partial chromatin transfer. In most cases, cytomixis produced other meiotic abnormalities, such as unorganized chromatin threads in the cytoplasm during early prophase I.

Pollen fertility

Pollen fertility ranged between 44.74 and 52.69 % in triploid populations B and A, respectively, and between 54.69 and 95.76 % in tetraploid populations C–N. Variation was also observed in the size of pollen grains, which ranged from very small (micrograins) to twice the normal size (20–25 μm; Fig. 1l). We were unable to provide the percentage of fertile pollen grains in the mixed population H of cytotype 4x, because only non-flowering material was available for this study.

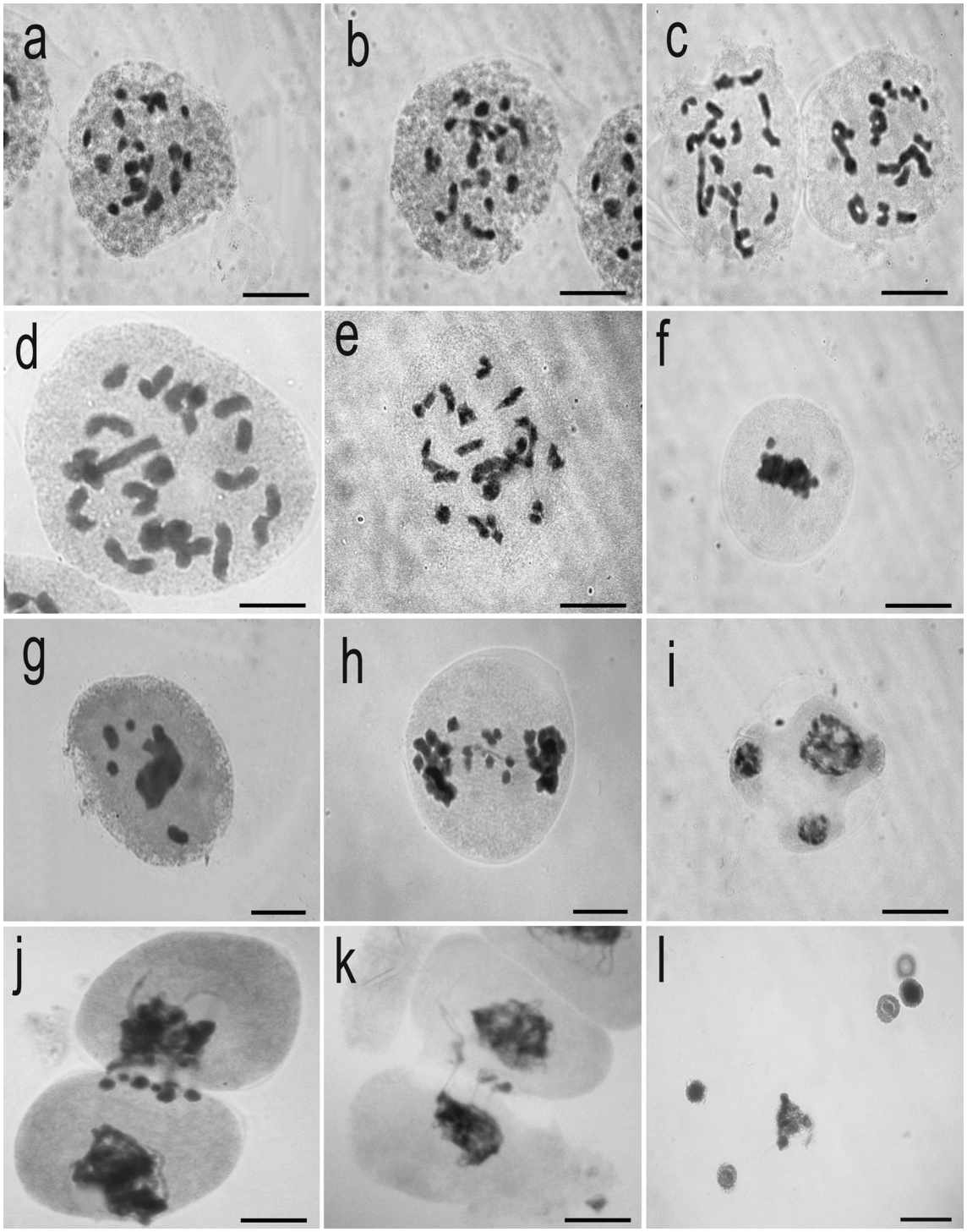

Fig. 1 Microsporogenesis in triploid and tetraploid specimens of *Campuloclinium macrocephalum*. **a** Diakinesis shared in two populations A and H 3x, 7I + 10II + 1III; **b** diakinesis shared in two populations 3x A and B, 8I + 8II + 2III; **c** diakinesis cell of the left showing 18II + 1IV, configuration shared in four populations 4x analyzed (C, D, K, N) and cell on the right showing 1I + 16II + 1III + 1IV; **d** diakinesis with 20II shared in three populations 4x (E, K, N); **e** diakinesis with 16II + 2IV shared in three populations 4x; **f** M I with a chromosome outside the plate; **g** M I of population mixed of cytotypes triploid with 2I + 2II chromosome outside the plate; **h** anaphase I showing a bridge and eight chromosomes lagging; **i** triad; **j** cytomixis, chromatin transfer through multiple chromatin strands; **k** three chromatin bridges; **l** pollen grains fertile and sterile of different sizes. *Scale bar* 7.5 μm (**d**), 10 μm (**g**, **h**), 15 μm (**a**–**c**, **e**, **f**, **i**, **j**), 20 μm (**k**), 30 μm (**l**)

Table 3 Proportions of meiotic abnormalities in the meiotic phases metaphase I and anaphase I and of states of regular and irregular tetrads in *Campuloclinium macrocephalum*

Population	A	B	C	D	E	F	G	H (3x)	H (4x)	I	J	K	L	M	N
Metaphase I															
Regular	0	14	75.51	75.37	70	79.56	57.89	0.95	76.88	74.30	89.30	87.30	55.46	87.80	93.08
Chromosomes outside plate	100	86	24.48	24.62	30	20.43	42.10	99.04	23.11	25.70	10.67	12.69	44.53	12.19	6.91
Number of cells analyzed	25	50	49	134	100	93	57	105	199	175	159	63	119	123	217
Anaphase I															
Regular	81.90	76.05	94.28	79.77	78.60	89.44	85.32	80	81.62	62.41	84.48	90.06	74.35	72.77	58.82
Laggards	17.14	24.28	5.71	20.22	19.53	9.31	14.22	19.09	18.37	37.11	14.94	9.27	25.32	26.17	21.76
Bridges with fragment	0	0	0	0	0	0.62	0	0	0	0.23	0	0	0	0	4.70
Bridges without fragment	0.95	0	0	0	0.46	0	0.45	0.90	0	0	0	0.66	0	0.52	0
Bridges + laggards	0	0	0	0	1.39	0.62	0	0	0	0	0	0	0.32	0	5.88
Laggards + bridges + fragments	0	0	0	0	0	0	0	0	0	0.23	0.57	0	0	0.52	0.58
Number of cells analyzed	210	71	105	89	215	161	218	110	234	423	174	151	308	191	170
Tetrads															
Regular	80.26	91.42	93.54	78.63	88.93	98.25	81.67	44.69	94.25	80.61	81.99	94.64	79.13	59.83	87.95
Irregular	19.73	8.57	6.45	21.36	11.06	1.74	18.32	55.30	5.74	19.38	18	5.35	20.86	40.16	12.04
Number of cells analyzed	451	175	279	117	262	343	562	179	609	227	511	616	278	244	498
Meiotic index	80.27	91.43	93.55	78.63	88.93	98.25	81.67	44.69	94.25	80.62	82.00	94.64	79.14	59.84	87.95

Discussion

The meiotic analysis in the weedy *C. macrocephalum* identifies aberrations in chromosome pairing during diakinesis and M I, with the formation of univalents, bivalents, trivalents, and higher multivalents. The meiotic behavior was similar to other triploid Eupatorieae species, such as *Campovassouria bupleurifolia* and *Chromolaena callilepis* (Bertasso Borges and Coleman 2005). Due to these different configurations, the triploid populations A and B showed some meiotic irregularities at M I and A I (Table 3; Fig. 1f–h). Metaphase I cells had chromosomes outside the plate in 86–100 % of analyzed cells (i.e., 1–10 chromosomes outside of plate). The majority of irregularities was found in the triploid cytotype of the mixed population H, which showed 99.04 % of cells with chromosomes out of plate (Table 4). However, in the tetraploid specimens of this population, M I cells were normal in 76.88 % of cases. In anaphase I, cells of the two triploid populations showed a normal meiotic behavior in 76.05 to 81.90 % of the cases. Meiosis resulted in a high frequency not only of tetrads but also dyads, both with or without supernumerary microspores and low meiotic indexes and pollen fertility (Table 5).

The chromosome behavior analysis during the meiotic division in the 14 populations of *C. macrocephalum* revealed a wide diversity of abnormalities from prophase to the tetrads stage, especially in triploid individuals. Previous

Table 4 Frequency of chromosomes outside the plate in metaphase I in population H cytotype 3x

Chrom. outside plate	Frequency	Chrom. outside plate	Frequency
1I	3	1I + 1II	2
2I	5	2I + 1II	5
3I	6	2I + 2II	10
4I	9	2II + 1III	1
5I	5	2I + 2III	2
6I	1	2I + 1III	2
1II	1	2I + 3II	4
2II	2	3I + 1II	12
3II	2	3I + 3II	1
1III	1	3I + 1III	5
1I + 2II + 1IV	1	3I + 2II	1
1I + 4II	2	4I + 2II	1
1I + 2III	1	4I + 1III	1
1I + 1II + 1III	2	4I + 1II	3
1I + 2II	3	4I + 1III	1
1I + 1II + 1III	1	4I + 1II	1
1I + 2II	1	5I + 1II	4
1II + 1IV	1	6I + 1III	1
Total of cell analyzed			104

Table 5 Comparison of fertility of pollen grains in populations of *Campuloclinium macrocephalum*

Population	A	B	C	D	E	F	G	H (3x)	H (4x)	I	J	K	L	M	N
Fertile	52.69	44.74	87.67	91.03	83.86	95.76	86.38	27.65	–	81.76	83.49	88.20	87.08	90.40	54.69
Sterile	46.64	54.83	9.93	8.45	15.64	3.54	13.18	72.18	–	16.85	15.40	11.04	12.38	9.20	45.30
Unreduced	0.66	0.41	2.38	0.51	0.49	0.68	0.43	0.15	–	1.38	1.10	30	0.52	0.38	0
Number of cells analyzed	3.469	3.585	2.930	2.744	2.442	2.907	2.761	3.182	–	1.442	2.635	3.994	2.648	2.313	2.002

meiotic analyses of a population from Buenos Aires (Argentina) recorded 19II + 1I or 20II + 1I (Galiano and Hunziker 1987). We observed up to three additional chromosomes in some cells of the triploid and tetraploid cytotypes. Such additional chromosome(s) could be B chromosomes derived from the autosomal complement (Camacho et al. 2000). These data were supported in mitotic study in *C. macrocephalum* (Farco and Dematteis 2011).

B chromosomes are unnecessary extra chromosomes which are found only in some individuals of a population, which are not produced by duplication of the A chromosomes in diploids or polyploids (Jones 1995; Cabrero and Camacho 2009). Also, they differ from aneuploids, where the extra chromosomes are duplicates of one or more of the basic A set and reveal their homologies at meiosis. Furthermore, B chromosomes fail to pair or recombine with any of the A chromosomes at meiosis, are often morphologically distinct, usually smaller than the A chromosomes, and they can vary in number within and between individuals (Jones 1995; Bougourd and Jones 1997). Their main characteristic, however, is that they do not recombine with the standard chromosomes and thus follow their own evolutionary pathway (Camacho and Parker 1993).

The occurrence of cytomixis in *C. macrocephalum* is reported here for the first time. Cytomixis has been considered to be an anomaly, either pathological (Weiling 1965; Morisset 1978), induced by fixation or by traumatic injury phenomenon (Takats 1959; Zhen and Li 2009). Other authors considered also environmental effects, such as temperature and pollution (Basavaiah and Murthy 1987; Malallah and Attia 2003; Haroun et al. 2004; Kumar and Tripathi 2008) and nutritional deficiency (Miljajev 1967). Recently, it has been suggested that the cytomixis is under gene(s) control (Mandal and Datta 2012; Kaur and Singhal 2012).

Cytomictic channels and chromatin transfer, which have a deep effect on the meiotic process and its end-products, are more prevalent in genetically, physiologically, and biochemically unbalanced plants such as triploids, haploids, hybrids, and aneuploids (de Nettancourt and Grant 1964; Haroun 1995; Nirmala and Rao 1996). There are also reports of cytomixis prevailing among polyploids (Semyarkhina and Kuptsou 1974; Singhal et al. 2007) than their diploid counterparts. Compared to other species, in *C. macrocephalum* chromatin transfer was observed only in M I, while in *Clematis orientalis* L. (Kumar et al. 2010) and *Meconopsis aculeata* Royle (Singhal and Kumar 2008), cytomixis was significant influencing all meiotic phases.

Chromatin stickiness was the most frequent meiotic anomaly in early prophase in *C. macrocephalum*. Singhal et al. (2010) suggest that chromatin stickiness is a result of cytomixis. However, the possible causes, cytological

consequences and genetic significance of cytomixis, are still debated (Li et al. 2009). Mantu and Sharma (1983) suggest that cytomixis is a natural phenomenon under direct genetic control, although physiological factors certainly could influence its manifestation (Bellucci et al. 2003).

An important difference between triploid populations and the mixed population was found in the meiotic behavior in MI of the triploid cytotype. In the mixed population, 99.04 % of the analyzed chromosomes were outside the plate, with univalents, bivalents, trivalents and tetravalents. Khush (1973) suggests that outside the plate or lagging chromosomes may separate during A I and, if not included in telophase I nuclei, will be eliminated in gametes. Thus, in outside the plate chromosomes not included in telophase II nuclei may also be eliminated through microcyte formation (Satina and Blakeslee 1937). However, the population H triploid (mixed population) exhibited 72.18 % of sterile pollen, indicating that apomixis would have greater importance in the spread.

Coexistence of different cytotypes within a single species without recognition of infraspecific taxa is rather frequent (Suda 1998; Keeler and Davis 1999; Suda and Lysák 2001; Hodálová et al. 2007). In *C. macrocephalum,* the existence of populations including individuals with different ploidy levels had not been documented before our study, in which we found one mixed population (population H). Intensive intra-population screening is needed to reveal other such cases. However, according to theoretical predictions, the co-occurrence of different cytotypes in a population should be unstable, with minority cytotypes exposed to the frequency dependent elimination process unless niche differentiation or strong pre-zygotic isolation between the cytotypes exists (Levin 1975; Van Dijk and Bakx-Schotman 1997; Husband and Schemske 2000; but see Halverson et al. 2008).

All types of meiotic irregularities found in of *C. macrocephalum* might associate with reduced pollen fertility, but also might associate to cytomixis (Singhal and Kaur 2011). In this sense, the major cause of sterility in the triploid populations could be due to the origin of polyploids that are unstable at meiosis and have little probabilities of producing offspring. However, it is known that apomictic reproduction is an escape from sterility, as observed in triploid populations studied previously (Farco et al. 2012). Indeed, triploid 2n gametes could produce viable gametes resulting in tetraploid individuals within an originally triploid population, which could explain the mixed nature of population (H) reported in this study. Pollen fertility has been related to the abnormal meiosis caused by deficient pairing, non-separation or asymmetrical chromosome distribution (Singh 1992). These suggestions are supported by our findings of high frequency of abnormal chromosome behavior throughout the whole meiotic process. However, cytomixis could be affecting pollen fertility (Baptista-Giacomelli et al. 2000; Saggoo et al. 2011; Srivastava and Kumar 2011). Few studies have focused on polyploid weedy species: e.g., *Chondrilla juncea* (Burdon J et al. 1981), *Cortaderia jubata* (Costas-Lippmann 1979; Drewitz and DiTomaso 2004), *Eupatorium adenophorum* (Baker H et al. 1965; Khonglam and Singh 1980), *E. riparium* (Khonglam and Singh 1980), *E. odoratum* (Khonglam and Singh 1980), *Hypericum perforatum* (Pritchard 1960), *Taraxacum officinale* (Richards 1970), *Hieracium sp.* (Fornasari 1996), and *Pilosella officinarum* (Chapman et al. 2000).

C. macrocephalum does not reproduce vegetatively, has a perennial rootstock that produces annual stems in summer (December/January). Stems senesce in winter (June/July) and the plant survives as a rootstock during winter. Winter is the dry season and thus pompom is not adversely affected by grass fires, frost and negligible rainfall at this time of year (Goodall et al. 2012).

Mandal et al. (2013), highlight the importance of formation of polyploid/aneuploid PMCs due to cytomixis as of evolutionary significance but this event need to be justified.

This study brings new insights about the polyploid pompom weed, which is becoming a serious problem for management of infested crop fields (McConnachie et al. 2011; Goodall et al. 2012). We suggest that polyploidy seems to be recurrent in this species, promoting partial sterility of pollen grains, generating large numbers of individuals by apomixis, and, in this way, facilitate invasion of crop fields. Improving our knowledge of the meiotic behavior in *C. macrocephalum* may allow us to provide useful knowhow for the biological control of this weed, especially in infested areas lacking natural enemies, such as herbivores.

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