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BRIEF COMMUNICATION

Gametes with somatic chromosome number and their significance in interspecific hybridization in *Fuchsia*

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Abstract

Sexual polyploidization has both a theoretical as well as an applied significance. Morphological screening for large pollen grains and shape of pollen produced by the individual, cytological investigation of hybrid progeny, and unbalanced separation of chromosomes at anaphase I in pollen mother cells were used to detect the gametes with somatic chromosome number in *Fuchsia*. The interspecific hybrids of *F. fulgens* (sect. *Ellobium*) × *F. magellanica* (sect. *Quelusia*), *F. fulgens* (sect. *Ellobium*) × *F. splendens* (sect. *Ellobium*), and *F. triphylla* (sect. *Fuchsia*) × *F. splendens* (sect. *Ellobium*) produced at the University of Auckland, New Zealand, showed both large and normal pollen grains in the same anther indicating the presence of unreduced gametes. Cytological investigation carried out on the hybrid progeny of *F. fulgens* (diploid, 2n=22, sect. *Ellobium*) × *F. magellanica* (tetraploid, 2n=44, sect. *Quelusia*) and *F. triphylla* (diploid, sect. *Fuchsia*) × *F. arborescens* (diploid, sect. *Schufia*) revealed unexpected chromosome numbers of 2n=44 and 2n=33, respectively. In general, the hybrids showed low fertility caused by genetically unbalanced gametes resulted from random disjunction of chromosomes at anaphase I. Studies on meiosis together with the presence of different shapes and sizes of pollen grains in *Fuchsia* proved indirectly that unreduced gametes are the products of first division meiotic nuclear restitution. These unreduced gametes were viable irrespective of pollen shape, their predominance in the hybrids, nuclear DNA amount and species phylogenetic position.

Additional key words: diploids, 2n gametes, meiotic nuclear restitution, polyploids.

The production of gametes with somatic chromosome number, referred to subsequently as 2n gametes has been reported in 85 angiosperm genera from across the taxonomic spectrum including *Archillea* (Ramsey and Schemske 1998), *Boechera* (Taskin *et al.* 2009), *Chamerion* (Burton and Husband 2000, Husband and Schemske 2000, Husband 2004), *Fragaria* (Bringhurst and Senanayake 1966, Bringhurst and Gill 1970), *Gilia* (Grant 1952), *Lolium* (Jansen and Den Nijs 1993), *Rosa* (Crespel *et al.* 2006) and *Trifolium* (Parrott and Smith 1984). The frequency of 2n gamete production differs between plant species (Bretagnolle and Thompson 1995) and even within the individuals of a species as found in *Medicago sativa* among the flowers of the same plant (McCoy 1982) and even between anthers of a single bud as in *Solanum tuberosum* (Ramanna 1983).

2n gametes are produced as a consequence of meiotic errors that may occur at the first meiotic division, resulting in first division restitution (FDR) or in second division restitution (SDR). The formation and frequency of 2n gametes can be detected in a variety of ways. These include observations on pollen size (1.2 - 1.4 times larger

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Abbreviations: BG - *F. boliviana* × *F. glazioviana*; BM - *F. boliviana* × *F. magellanica*; FDR - first division restitution; FB_1 - *F. fulgens* × *F. boliviana* (plant 1); FS_1 - *F. fulgens* × *F. splendens* (plant 1); HbB - *F. hatschbachii* × *F. bolivian;* SDR - second division restitution; STr_1, STr_2 - *F. splendens* × *F. trumpetor* (plants 1 and 2); TA_1 - *F. triphylla* × *F. arborescens* (plant 1); TB_1, TB_2, TB_3 - *F. triphylla* × *F. boliviana* (plants 1, 2 and 3); TS_1 - *F. triphylla* × *F. splendens* (plant 1).

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than the reduced pollen size), measurement of nuclear DNA content by flow cytometry, examination of micro- and mega-sporogenesis and progeny analysis of chromosome number from crosses between parents of known chromosome number (Darlington 1937). In hybrids, the frequency of 2n gamete production may be many times greater than that of non-hybrid plants as irregular chromosome pairing between the genomes contributed by the parent species followed by non-disjunction is usually more frequent (Winge 1917).

The genus *Fuchsia* (family *Onagraceae*) contains approximately 110 species that are either diploid (2n=22) or tetraploid (2n=44) (Berry *et al.* 2004). Nowicke *et al.* (1984) have reported that the diploids and polyploids usually produce pollen grains with two and two-three apertures and this could provide a rapid method of screening for ploidy level in species, cultivars, and artificial hybrids. The present study aims to estimate the frequency of unreduced gametes in *Fuchsia*, to identify the hybrids produced with unusual chromosome numbers and random disjunction of chromosomes at anaphase as it plays a greater role in unilateral or bilateral polyploidization in the interspecific hybridization. In general, failure of chromosomes to synapse and segregate in a normal manner or reduced chromosome pairing at meiosis leads to F_1 sterility and can be overcome by somatic (mitotic) doubling of chromosomes thereby providing homologous pairing (Van Tuyl and De Jeu1997, Hermsen 1984).

The species and cultivars belonging to six sections of the genus based on molecular characterization superimposed with the cytomorphological and geographical data (Berry *et al.* 2004) were used for experimental hybridization. The unrooted cuttings of *Fuchsia* species were obtained from the University of Auckland and *Hammett Plant Breeding*, *Ltd.*, Auckland, New Zealand, An accession of *F. procumbens* was also obtained from Landcare Research, Lincoln, USA. These unrooted cuttings were rooted in the mist chamber in pumice and were later on transferred to potting mixture. These rooted cuttings were grown in a glasshouse at the University of Auckland. The plant species, cultivars and artificial hybrids used in this study are listed in Table 1. *F. reflexa* (Breedlove 1969) used in the present study was identified as *F.* × *bacillaris*, a natural hybrid between *F. thymifolia* ssp. *thymifolia* and *F. microphylla* ssp. *microphylla* that has been under cultivation under this name (Ashby 2001). A minimum of five flowers in each species was emasculated one day before anthesis and hand pollinated during the flowering period for interspecific hybridization. Out of 125 total cross combinations attempted by interspecific hybridization at the University of Auckland, 43 cross combinations set fruits and meiotic analysis of only the viable progeny was carried out. The morphology of species, cultivars and hybrids grown in a glasshouse was examined to study the heritability of the characters using the descriptors (Evert *et al.* 1992, Lawrence 1966) and the Horticultural colour chart, issued by the British colour council in collaboration with the Royal Horticultural Society (Wilson 1942).

To determine the pollen diameter, anthers from freshly opened flowers were collected and placed in a drop of cotton blue on a slide. After a minimum of 10 min, the pollen diameters of 20 pollen grains were measured using a calibrated ocular micrometer. The triporate pollen grains were spherical, so only a single measurement through one pore to the opposite side of the pollen grain was made. With the biporate pollen, the polar (long) and equatorial (short) diameters were measured and the mean was calculated. Small numbers of pollen grains with one, four or five apertures were also observed in many hybrids but their diameters were not measured as they are not the predominant types. The viability of pollen from freshly opened flowers was estimated using the fluorescein diacetate technique of Heslop-Harrison and Heslop-Harrison 1970. Slides were observed using an epifluorescence microscope and data from a total of 500 pollen grains/species or hybrid was collected. Controls were carried out using 10 % sucrose solution without the fluorescein diacetate. Chromosome numbers of all species/cultivars and of the progeny to screen for 2n gametes were determined using the Feulgen method and squashing the root tips in a drop of FLP orcein (Jackson 1973). For meiotic analysis**,** immature flower buds were collected and fixed in ethanol + chloroform + acetic acid (6:3:1) for 24 h. The buds were transferred to 70 % ethanol and stored at 4 °C until required and the anthers were squashed in FLP orcein. A minimum of five plants in each of species (clones), cultivars (clones) and hybrid progeny (seedlings) were maintained in the glasshouse. During the course of study, depending upon the availability of flower buds in species, cultivars and the extent of morphological segregation present in the hybrid progeny, observations on meiotic analysis were made on 20 cells per plant.

It is evident that *Fuchsia* species, cultivars and hybrids produce pollen of highly variable size and more than one type of pollen morph is present in pollen grains (Table 1). In all diploid species, the pollen was uniformly biporate whereas in the four polyploids, three showed a mixture of biporate and triporate grains. One of the polyploids, *F. magellanica* had only triporate grains. The size of viable pollen grains of diploid species that had two apertures ranged from 37.3 µm (*F. reflexa*) to 75.0 µm (*F. fulgens*) and that of tetraploid species with three apertures ranged from 45.2 µm (*F. hatschbachii*) to 92.6 µm (*F. glazioviana*). Amongst the cultivars, Gartenmeister Bonstedt showed only two aperturate pollen grains, whereas cv. Timothy Hammett had both types (two and three aperturate) of pollen grains. The range in the size of pollen grains in these two cultivars was from 81 to 51 µm in the former and later cultivar, respectively. In case of hybrids, the diploids showed two aperturate pollen morphs only. Interestingly, the number

Table 1. Chromosome number (2n) and pollen parameters in *Fuchsia* species, cultivars and artificial hybrids. (nad = no anther dehiscence, - = not present, * = not recorded, 1,2,3 subscripts used in the abbreviations given to hybrid cross indicate individual plant numbers of segregating population used in the interspecific hybridization studies).

Plant material	2n	Pollen with apertures [%]				Mean pollen diameter [µm]		Unreduced gametes [%]	Pollen viability [%]	Shriveled pollen [%]
		1	2	3	4	reduced	unreduced			
F. arborescens Sims. (sect. *Schufia)*	22	-	100	-	-	50.8±15	-	-	70	7
F. encliandra Steudel. (sect. *Encliandra*)	22	-	100	-	-	56.9±15	74.4±23	3.6	54	8
F. fulgens DC (sect. *Ellobium)*	22	-	100	-	-	75.0±21	-	-	80	3
F. microphylla H.B.&K. (sect. *Encliandra*)	22	-	100	-	-	48.2±16	58.6±13	3.1	53	12
F. procumbens R.Cunn. (sect. *Procumbentes*)	22	-	100	-	-	57.3± 9	-	-	72	5
F. reflexa (sect. *Encliandra*)	22	-	100	-	-	37.3±11	-	-	35	30
F. splendens Zucc. (sect. *Ellobium*)	22	-	100	-	-	71.7±20	-	-	85	2
F. triphylla L.(sect. *Fuchsia*)	22	-	100	-	-	71.6±20	-	-	90	4
F. trumpetor (sect. *Fuchsia*)	22	-	100	-	-	66.4±18	93.8± 6	13.0	52	31
F. boliviana Carr. (sect. *Fuchsia*)	44	-	30	70	-	52.9± 7	71.8± 9	1.4	90	5
F. glazioviana Taub. (sect. *Quelusia*)	44	-	10	90	-	92.6± 5	-	-	79	15
F. hatschbachii Sims.(sect. *Quelusia*)	44	-	9	91	-	45.2± 5	98.6± 8	1.0	88	10
F. magellanica Lam. (sect. *Quelusia*)	44	-	-	100	-	57.3± 6	-	-	29	35
cv. Dr. Hammett	99	*	*	*	*	*	*	-	0	98
cv. Gartenmeister Bonstedt	33	-	100	-	-	81.0±21	-	-	9	90
cv. Timothy Hammett	44	-	10	90	-	51.0± 6	-	nad	25	45
F. encliandra × *F. procumbens* (EP)	22	-	100	-	-	41.0±15	-	nad	5	92
F. fulgens × *F. boliviana* (FB$_2$)	33	-	22	45	33	83.0± 9	-	-	14	70
F. fulgens × *F. magellanica* (FM$_1$)	44	-	-	95	5	*	*	nad	0	87
F. fulgens × *F. magellanica* (FM$_2$)	66	2	22	76	-	39.0± 9	73.0± 2	2.3	31	67
F. fulgens × *F. splendens* (FS$_2$)	22	-	100	-	-	60.0±13	80.0±15	3.3	15	60
F. glazioviana × *F. magellanica* (GM)	44	-	-	100	-	73.0± 5	-	-	62	2
F. triphylla × *F. arborescens* (TA$_2$)	33	-	4	83	13	50.0± 6	-	-	22	48
F. triphylla × *F. splendens* (TS$_2$)	22	-	100	-	-	57.0±12	85.0±11	1.1	39	61
F. triphylla × *F. splendens* (TS$_3$)	22	5	95	-	-	*	*	*	20	79
Timothy Hammett × *F. boliviana* (THB$_1$)	44	-	10	87	3	50.0± 7	*	-	15	70
Timothy Hammett × *F. glazioviana* (THG$_1$)	44	-	100	-	-	60.0±12	-	nad	0	100

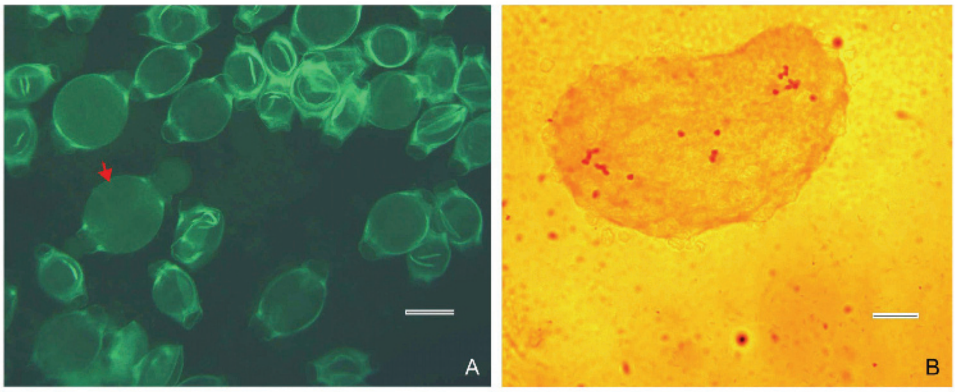

Fig. 1. *A* - Fresh pollen under photomicroscope (fluorescein di-acetate method): Please note 2n viable pollen shown by *arrow* among viable, non-viable and shriveled 1n pollen in *F. triphylla* × *F. splendens* hybrid (*scale bar* 25 µm). *B* - Anaphase I showing univalents lagging behind on the equatorial plate in *F. triphylla* × *F. splendens* (*scale bar* 10 µm)

of apertures in polyploidy hybrids varied widely, *i.e.*, one, three to five apertures. This variation can also be observed even within the individual progeny of a particular cross. The hybrids *F. fulgens* × *F. magellanica*, *F. fulgens* × *F. splendens*, and *F. triphylla* × *F. splendens* (Fig. 1*A*) showed large and normal pollen grains in the same anther indicating the presence of unreduced gametes.

In the present study, unexpected chromosome number in some hybrids as for a consequence of unreduced gamete formation was not only confirmed by the observations on pollen size but also by the presence of unusual chromosomes in the progeny of diploid-tetraploid and diploid-diploid crosses. Examples of the contribution of 2n gametes by one of the parents include *F. fulgens* (diploid) × *F. magellanica* (tetraploid) and *F. triphylla* (diploid) × *F. arborescens* (diploid) that revealed unexpected chromosome numbers of 2n=44 and 2n=33 (Table 1) in the segregating population than the expected chromosome numbers 2n=33 and 2n=22, respectively. The ploidy level of other plants in particular cross combination was as expected that included diploids (FS_1, TS_1, STr_1, STr_2); triploids (FB_1, TA1, TB_1, TB_2, TB_3), and tetraploids (BG, BM, HbB). Ramsey and Schemske (1998) reported that allotriploids in the F_1 generation are produced by reduced and unreduced gametes from the parent genotypes; these are used in the F_2 generation either by backcrossing or selfing to produce tetraploids. As a result of 2n gamete production, gene flow between diploid (*F. fulgens*) and tetraploid (*F. magellanica*) levels is possible and may act to increase the heterozygosity of the progeny. Similar lines of work with other species may be useful in bridging the ploidy levels in future *Fuchsia* breeding. Triploids in some inter- and intra- specific hybrids are partially male sterile but may produce unreduced eggs and could be useful for further manipulation of ploidy level in *Fuchsia*. The 2n gametes used to produce 4x progeny by unilateral or bilateral polyploidization (unilateral polyploidization in this study) may increase heterosis in the progeny, in addition to representing an important pathway for gene transfer between ploidy levels. This heterosis may be manifested in the form of a prolonged flowering period as in the hybrid between *F. fulgens* and *F. magellanica.* Shriveled or malformed pollen grains were observed in all the species and cultivars irrespective of their ploidy level. These shriveled pollen grains were more common in cultivars than in the species. The results of shriveled pollen grains of different species, cultivars or hybrids (Table 1) show that the frequency ranged from 2 % in *F. splendens* (diploid) to 35 % in *F. magellanica* (polyploid). In the cultivars, it ranged from 98 % in Dr. Hammett to 45 % in Timothy Hammett. The hybrids contained very high percentage of shriveled pollen grains (100 % in Timothy Hammett × *F. glazioviana*) (Table 1) with the exception of the hybrid between *F. glazioviana* and *F. magellanica* that had only 2 %.

There appeared to be a notable difference with regard to pollen fertility of the hybrids depending upon their parents that varied from completely sterile to almost fully fertile. To explain further, the pollen viability of all the diploid hybrids was lower than that of its parents. The polyploid F_1 hybrids of *F. glazioviana* × *F. magellanica* had the maximum pollen fertility of 62 %. Other crosses such as, *F. fulgens* × *F. magellanica,* and Timothy Hammett × *F. glazioviana* were totally sterile (Table 1). The anthers did not dehisce in these sterile to partially sterile hybrids. Overall, the fertility in many hybrids is less than that of their parents and this is a reflection of the meiotic irregularities as seen in *F. fulgens* × *F. boliviana*, Timothy Hammett × *F. boliviana*, *F. triphylla* × *F. arborescens*, and *F. triphylla* × *F. splendens* (Fig. 1*B*) was caused by genetically unbalanced gametes that resulted from random disjunction of different chromosomes contributed by different genomes. Infertility arises because chromosomes fail to pair at metaphase I in hybrids and at anaphase I due to the lagging of univalents at the equatorial region or lack of orientation that may result in unbalanced gametes with variable chromosome numbers or unreduced gametes. In view of the fairly large number of fertile or partially fertile hybrids produced in this investigation (*F. fulgens* × *F. magellanica*, *F. fulgens* × *F. splendens*, and *F. triphylla* × *F. splendens*), and the association with the production of viable unreduced gametes, it appears that these hybrids may be a good source for use as parents in a further breeding programme. Regardless of the mode of inheritance of meiotic nuclear restitution, it may be possible to select genotypes with an increased rate of restitution through a progressive selection for fertility in desynaptic hybrids that have potential in the production of polyploid progeny. However, this could have limitations in the production of diploid sexual progeny.

In the present study, many types of pollen grains with varying shapes occur that result from the irregular meiosis. The present study can be linked to the findings of Beer (1907) who observed normal second division of the pollen mother cell meiosis. The pollen grains with varying numbers of apertures (ranging from one to five) and sizes found in the F_1 hybrids produced in the current study are the consequences of irregularities in the first division (FDR meiotic nuclear restitution). These unreduced gametes were viable irrespective of their shape and/or their predominance in the hybrids or species. The variation in pollen aperture number in the *Onagraceae* family has been studied quite extensively and different workers have correlated these numbers with ploidy level (Mosquin 1966, Nowicke *et al.* 1984). Nonetheless, pore number is not an absolute indicator of polyploidy and instead pore number may also be the consequence of meiotic irregularities, which occur so frequently in the *Onagraceae* and more specifically in this context in *Fuchsia*. The presence of one-pored grain seemed to be aborted pollen, the product of irregular segregation (Beer 1907, Brown 1967). Further, it is clear that there is no definite relationship between pollen size, nuclear DNA amount (Talluri and Murray 2009) and the phylogenetic position (Berry *et al.* 2004).

References

Ashby, M.S.: An investigation of natural and artificial interspecific hybridization in the genus *Fuchsia L.* - M.Sc Thesis, The University of Auckland, Aucland 2001.

Beer, R.: The supernumerary pollen grains of *Fuchsia*. - Ann. Bot. **21**: 305-307, 1907.

Berry, P.E., Hahn, W.J., Sytsma, K.J., Hall, J.C., Mast, A.: Phylogenetic relationships and biogeography of *Fuchsia* (*Onagraceae*) based on non-coding nuclear and chloroplast DNA data. - Amer. J. Bot. **91**: 601-614, 2004.

Breedlove, D.E.: The systematics of *Fuchsia* sect. *Encliandra* (*Onagraceae*). - Univ. Calif. Publ. Bot**.** **53**: 1-69, 1969.

Bretagnolle, F., Thompson, J.D.: Gametes with somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. - New Phytol. **129**: 1-22, 1995.

Bringhurst, R.S., Gill, T.: Origin of *Frageria* polyploids. II. Unreduced and double unreduced gametes. - Amer. J. Bot. **57**: 969-979, 1970.

Bringhurst, R.S., Senanayake, Y.D.A.: The evolutionary significance of natural *Fragaria chiloensis* × *F. vesca* hybrids resulting from unreduced gametes. - Amer. J. Bot. **53**: 1000-1006, 1966.

Brown, C.A.: Pollen morphology of the *Onagraceae*. - Rev. Palaeobot. Palynol. **3**: 163-180, 1967.

Burton, T.L., Husband, B.C.: Fitness differences among diploids, triploids, and their triploid progeny in *Chamerion angustifolium*: mechanisms of inviability and implications for polyploidy evolution. - Evolution **54**: 1182-1191, 2000.

Crespel, L., Ricci, S. C., Gudin, S.: The production of 2n pollen in rose. - Euphytica **151**: 155-164, 2006.

Darlington, C.D.: Recent Advances in Cytology. - Blakiston's, Philadelphia 1937.

Evert, R.F., Eichhorn, S.E., Raven, P.H.: Laboratory Topics in Botany. - Worth Publishers, New York 1992.

Grant, V.: Cytogenetics of the hybrid *Gilia millefoliata* ×*achilleaefolia* 1. Variations in meiosis and polyploidy rate as affected by nutritional and genetic conditions. - Chromosoma **5**: 372-390, 1952.

Hermsen, J.G.T.: Nature, evolution, and breeding of polyploids. - Iowa State J. Res. **58**: 411-420, 1984.

Heslop-Harrison, J., Heslop-Harrison, Y.: Estimation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. - Stain Technol. **45**: 115-120, 1970.

Husband, B.C.: The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. - Biol. J. Linn. Soc. **82**: 537-546, 2004.

Husband, B.C., Schemske, D.W.: Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. - J. Ecol. **88**: 689-701, 2000.

Jackson, R.C.: Chromosome evolution in *Haplopappus gracilis*: a centric transposition. - Evolution **27**: 243-245, 1973.

Jansen, R.C., Den Nijs, A.P.M.: A statistical mixture model for estimating the proportion of unreduced pollen grains in perennial ryegrass (*Lolium perenne* L.) *via* the size of pollen grains. - Euphytica **70**: 205-215, 1993.

Lawrence, G.H.M.: Taxonomy of Vascular Plants. - MacMillian Company, New York 1966.

McCoy, T.J.: The inheritance of 2n pollen formation in diploid alfalfa, *Medicago sativa*. - Can. J. Genet. Cytol. **24**: 315-323, 1982.

Mosquin, T.: A new taxonomy for *Epilobium angustifolium* L. (*Onagraceae*). - Brittonia **18**: 167-188, 1966.

Nowicke, J.W., Skvarla, J.J., Raven, P.H., Berry, P.E.: A palynological study of the genus *Fuchsia* (Onagraceae). - Ann. Mo. Bot. Garden **71**: 35-91, 1984.

Parrott, W.A., Smith, R.R.: Production of 2n pollen in red clover. - Crop. Sci. **24**: 469-472, 1984.

Ramanna, M.S.: First division restitution gametes through fertile desynaptic mutants of potato. - Euphytica **32**: 337-350, 1983.

Ramsey, J., Schemske, D.W.: Pathways, mechanisms, and rates of polyploid formation in flowering plants. - Annu. Rev. Ecol. Syst. **29**: 467-501, 1998.

Taskin, K.M., Turgut, K., Scott, R. J.: Apomeiotic pollen mother cell development in the apomictic *Boechera* species. - Biol. Plant. **53**: 468-474, 2009.

Talluri, R.S., Murray, B.G.: DNA C-values and chromosome numbers in *Fuchsia* L. (Onagraceae) species and artificial hybrids. - New Zeal. J. Bot. **47**: 33-37, 2009.

Van Tuyl, J.M., De Jeu, M.J.: Methods for overcoming interspecific crossing barriers. - In: Sawhney, V.K., Shivanna, K.R. (ed.): Pollen Biotechnology for Crop Production and Improvement. Pp. 273-292. Cambridge University Press, Cambridge 1997.

Wilson, R.F.: Horticultural colour chart. - British Colour Council in collaboration with the Royal Horticultural Society, London 1942.

Winge, O.: The chromosomes, their numbers and general importance. - Compt. Rend. Trav. Lab. Carlsberg **13**: 131-275, 1917.