Occurrence of Syncytes: A Possible Mechanism Owing to the Origin of Polyploid Cytotypes in *Achillea millefolium* L. within Indian Himalayas

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Summary Present study records the existence of tetraploid and hexaploid meiocytes and subsequently the formation of large and very large-sized pollen grains for the first time in the diploid individuals of *Achillea millefolium*. In majority of the diploid accessions, meiocytes exhibited nine bivalents, equal segregation of chromosomes during anaphases, regular tetrads and normal-sized 'n' pollen grain formation. In the accessions scored from Palchan (2400 m) and Dhundhi (3050 m) regions of Solang Valley, two to three proximate PMCs fused during early stages of Prophase-I and Metaphase-I resulting into tetraploid (4x) and hexaploid (6x) meiocytes with 18 and 27 bivalents, respectively. Although the frequency of polyploid PMCs was rather low these are detectable due to their larger size compared to diploid PMCs. In both the accessions, the diploid and polyploid PMCs exhibited the multiple chromosomal associations of four to eight chromosomes indicating the existence of structural heterozygosity. Analysis reveals that there is an increase in the chiasma frequency with structural heterozygosity for reciprocal translocations. Syncyte meiocytes followed a regular meiotic course resulting into the formation of normal tetrads but the products of such sporads yielded atypical (large and very large-sized) pollen grains compared to normal sized typical pollen grains. Although the exact cytological status of aforesaid pollen grains could not be ascertained but such fertile larger-sized pollen grains with possible '2n' or '3n' genetic constitution might be involved in fertilization to generate polyploid offsprings and the origin of intraspecific polyploid cytotypes (4x, 6x) which are known to exist in some populations of Himalayas in India.

Key words Meiosis, Syncytes, Quadrivalents, Tetraploid, Hexaploid, Pollen grains.

Achillea millefolium which is a highly polymorphic and widely distributed species in India and other parts of the world is one of the best studied species with regard to chromosome counts. Probably, the earliest chromosome counts from Europe and other parts of the world were those of Clausen *et al.* (1941), Löve and Löve (1944, 1948), Felföldy (1947), Polya (1948, 1949, 1950), and Harling (1950). Gervais (1977) who made an extensive cytological investigations from Quebec, Canada reported tetraploid ($2n=36$), pentaploid ($2n=45$), hexaploid ($2n=54$) and some aneuploid cytotypes and suggested it to be a species complex. Rehberger (2000) reported an octoploid ($2n=72$) cytotype along with tetraploid and hexaploid cytotypes from Austria, Central Europe. Chromosome numbers of *Achillea millefolium* complexes have been put into six categories ranges from diploid to octoploid (Danihelka and Rotreklová 2001). In Indian Himalayas also, the species is known to exist at different ploidy levels, ranging from diploid, $2n=18$ (Shetty 1964, Mehra *et al.* 1965, Mehra and Remanandan 1974, Kaur and Singhal 2015, Rana *et al.* 2015); tetraploid, $2n=36$ (Mehra and Remanandan 1974); and hexaploid, $2n=54$

(Mehra and Remanandan 1974). A cursory look at the chromosomal data on the species reveals that worldwide it shows a considerable amount of heterogeneity ($2n=18, 27, 35, 36, 44, 45, 53, 54, 63, 72, 74, 81\pm5$) involving both aneuploidy and polyploidy. Ehrendorfer (1953, 1959) suggested that *A. millefolium* is a polyploid complex consisting of six taxa; two at diploid level (*A. asplenifolia* and *A. setacea*), one at diploid and tetraploid level (*A. roseoalba*), one at the tetraploid level only (*A. collina*), another one a hexaploid (*A. millefolium*) and sixth being an octoploid (*A. pannonica*). Spontaneous hybridization across different ploidy levels is also existent which is evident from the existence of triploid ($2n=27$), pentaploid ($2n=45$) and heptaploid ($2n=63$) plants as reported by workers in the different parts of the world (Danihelka and Rotreklová 2001). Ehrendorfer (1957, 1960a, 1960b) opined that evolution in the species seems to be more complex involving some other cytological processes rather than the ploidy level alone. Accessory or B-chromosomes have also been reported in the species in tetraploid ($2n=36$) and hexaploid ($2n=56$) cytotypes (Ehrendorfer 1960a, Lavrenko and Serditov 1991, Androshchuk *et al.* 1979, Saukel and Langer 1992, Danihelka and Rotreklová 2001). Another phenomenon which has played an important role in the evolution of

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the species is the existence of structural heterozygosity for reciprocal translocations in diploid individuals of N-W Himalayas (Singhal *et al.* 2014). Such chromosomal interchanges are likely to increase the amount of genetic variability in the gametes forming new genetic linkage groups which may be used for adaptation to adverse climatic conditions. Though the species has been worked out cytologically quite extensively from Indian Himalayas by workers from this laboratory (Malik 2012, Bala and Gupta 2011, Singhal *et al.* 2014, Kaur and Singhal 2015, Rana *et al.* 2015), majority of the populations exist at diploid level with $2n=18$. However, a comprehensive study of this species complex is still lacking from some of the geographically isolated and unexplored regions of N-W Himalayas in India. The purpose of the present study was (1) to critically evaluate the chromosome counts on individual plant basis, (2) to locate the structural hybrids, and (3) to study any other cytological evolutionary process involved in the origin of intraspecific polyploid cytotypes.

Materials and methods

Materials for cytological studies were collected from

the wild plants growing at various altitudinal ranges of Solang valley and Parvati valley of Kullu District, Himachal Pradesh during the months of April–September, 2010–2013 from different localities. Voucher specimens of the cytological worked out accessions were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala. For male meiotic studies, capitula of suitable sizes were fixed in Carnoy's fixative (mixture of ethanol, chloroform and glacial acetic acid in a volume ratio of 6:3:1) for 24 h, transferred to 70% alcohol and stored in a refrigerator until analysis. Pollen mother cells (PMCs) were prepared by squash technique and stained with 1% acetocarmine. Freshly prepared slides were carefully examined to determine the chromosome number. PMCs were also analyzed for detailed meiotic behavior at different meiotic stages, diakinesis, metaphase I (MI), anaphase-I/II (AI/II), telophase-I/II (TI/II) and sporad stage. In accessions with normal meiotic course, a total of 100–150 PMCs were examined for determining the chromosome counts, while in case of accessions showing larger-sized PMCs, a total of 1678 PMCs prepared from different anthers/florets were analyzed. Pollen fertility was estimated through stainability tests by squashing the anthers from mature capitula in

Table 1. Province, locality, altitudes, accession number, meiotic chromosome number (n), meiotic course, and pollen fertility % in *Achillea millefolium*. ST=Structural heterozygote.

Province, locality, altitudes	Accession number (PUN)	Chromosome number (n)	Meiotic course	No. of PMCs analysed at M-I		% of PMCs with lag-gards at A-I	% of PMCs with lag-gards at A-II	% of PMCs with lag-gards at T-II	Pollen fertility (%)	Pollen grains size (μm)
				(without multivalents)	(with multivalents)					
Kullu district										
Solang Valley										
Palchan, 2450 m	58571	9	ST	42	19	5.45	6.66	—	68.00	Typical sized: 23.09×21.63 μm Large sized: 25.70×24.77 μm Very large sized: 31.19×27.31 μm
Solang Nullah, 2700 m	56570	9	Normal	78	—	—	—	—	99.00	Typical sized: 21.55×22.02 μm
Solang Village, 2750 m	56885	9	Normal	69	—	—	—	—	98.00	Typical sized: 22.67×21.15 μm
Dhundhi, 3050 m	58572	9	ST	54	63	8.69	—	4.34	71.49	Typical sized: 20.32×22.39 μm Large sized: 25.49×23.37 μm Very large sized: 28.65×29.32 μm
Parvati Valley										
Shaarni, 2300 m	56092	9	Normal	93	—	—	—	—	100	Typical sized: 22.61×18.98 μm
Pulga, 2300 m	56119	9	Normal	78	—	—	—	—	100	Typical sized: 22.41×20.49 μm
Braagha, 2600 m	56111	9	Normal	62	—	—	—	—	100	Typical sized: 21.63×21.10 μm
Nareng, 2700 m	56119	9	Normal	47	—	—	—	—	100	Typical sized: 22.11×21.00 μm
Mallana, 2650 m	57165	9	Normal	102	—	—	—	—	100	Typical sized: 20.43×18.88 μm

Fig. 1. (a) A PMC with nine equal sized bivalents at diakinesis. (b) A diploid PMC shows seven bivalents and one quadrivalent (arrowed). (c) A PMC with 6 bivalents, 1 quadrivalent (arrowhead), and 2 univalent chromosomes (arrowed) at M-I. (d) A PMC with a laggard at A-II (arrowed). (e) A PMC with 9:9 equal chromosomal distribution at A-I. (f) A normal diploid PMC exhibits 9:9:9:9 distribution pattern of chromosomes at A-II. (g) A PMC depicts four nuclei at T-II. (h) Identical sized typical pollen grains. (i) Two proximate PMCs undergo fusion prior to syncyte formation. (j) A large sized PMC with two chromatin mass (arrowed). (k) A large sized syncyte PMC showing double chromosome number (arrowhead) and a normal sized $2n$ PMC (arrowed). (l) A large sized syncyte PMC with three distinct chromatin masses (arrowed). (m) A large sized tetraploid PMC (arrowhead) along with two normal diploid PMCs at prophase-I (arrowed). (n) Two diploid PMCs showed fusion at similar phases. Scale bar= $10\mu\text{m}$.

glycero-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile while shriveled and unstained ones were counted as sterile. Chromosome spreads were analyzed with an Olympus light microscope, and the best plates of chromosome counts, meiotic abnormalities, and pollen

grains were photographed from temporary mounts with a Nikon Eclipse 80i microscope. Pollen size was measured through micrometry.

Fig. 2. (a) Three diploid PMCs undergo fusion. (b) Two diploid PMCs depict fusion at the different phases of meiosis. (c) A tetraploid PMC depicts 18 bivalents at metaphase-I. (d) A tetraploid PMC with 14 bivalents and two quadrivalents (arrowhead). (e) A hexaploid PMC with 27 bivalents at metaphase-I. (f) Heterogenous sized pollen grains as typical (arrowed), large sized pollen grains (spear-arrowhead) and very-large sized pollen grains (arrowhead). Scale bar=10 μ m.

Results

Chromosome counts

Present study includes a detailed male meiotic analysis and pollen grain formation in nine different accessions collected from Solang Valley and Parvati valley in Kullu district, Himachal Pradesh, India. The information on site of collection, accession number, meiotic chromosome number, meiotic course including presence of multivalents due to structural heterozygosity for re-

ciprocal translocations, pollen fertility, and pollen grain size is provided in Table 1. Majority of the accessions depicted meiocytes with diploid chromosome counts of $n=9$ while two accessions showed the presence of syn-cytes/polyploid meiocytes with a meiotic chromosome count of $n=18$ and $n=27$.

Accessions with normal meiotic course

Of the nine accessions scored presently for male meiotic studies, the individuals of seven accessions uniformly depicted a diploid chromosome count of $n=9$ as

revealed by the presence of nine equal-sized bivalents at M-I (Fig. 1a). Further meiotic course including microsporogenesis was found to be regular resulting into high pollen sterility (98–100%).

Accessions with structural heterozygosity and cell fusion

Two accessions scored from Palchan 2450 m (PUN 58571) and Dhundhi 3050 m (PUN 58572) which also existed at $2x$ level depicted the presence of multiple chromosomal associations of four to eight chromosomes due to structural heterozygosity for reciprocal translocations (Figs. 1b, 1c) and fusion among two to three proximate PMCs at different stages of prophase-I and M-I (Figs. 1i–m).

a. Diploid PMCs with multiple chromosomal associations

Analysis of 180 normal diploid meiocytes in the two accessions revealed that 31.15% (Palchan accession) to 52.94% of the meiocytes (Dhundhi accession) showed the presence of multiple associations of four to eight chromosomes (Fig. 1b, Table 4). The multivalents in such diploid meiocytes are either ring, zigzag or typical or chain type. In Dhundhi accession, 1.68% of the PMCs were also noticed with two univalent chromosomes (Fig. 1c). In spite of the presence of laggards at A-I/T-I and A-II/T-II (Fig. 1d), majority of the PMCs showed 9:9 (Fig. 1e) and 9:9:9:9 (Fig. 1f) equal chromosomal segregation resulted into PMCs with equal sized nuclei at T-II (Fig. 1g), normal sporads and fertile equal sized pollen grains (Fig. 1h).

b. Syncyte (4x, 6x) PMCs

These accessions (Palchan and Dhundhi) showed the fused PMCs consisting of chromatin material either at the same stage of meiotic division (Figs. 1n, 2a) or in dissimilar phases (Fig. 2b). Such syncyte PMCs depicted tetraploid (Figs. 2c, 2d) and hexaploid (Fig. 2e) chromosome counts of $2n=36$ (18_{II} or $2_{IV}+14_{II}$) and $2n=54$ (27_{II}), respectively. Although the frequency of syncytes/polyploid PMCs was very low, at 43 out of 1889 (2.28%) (Table 3), these could be differentiated by their larger size in a pool of typical sized ' $2n$ ' PMCs. The tetraploid ' $4n$ ' PMCs measure 46.52×31.91 – $48.62 \times 45.33 \mu\text{m}$ while ' $6n$ ' hexaploid PMCs were 53.68×51.29 – $54.87 \times 52.19 \mu\text{m}$ compared to the normal diploid ' $2n$ ' PMCs (28.63×23.26 – $31.33 \times 19.68 \mu\text{m}$). The $4x$ and $6x$ PMCs depicted regular constitution of 18 and 27 bivalents during diakinesis and M-I leading to normal sporads. In spite of the fact that majority of the polyploid PMCs showed regular bivalents formation, 31.15 (Palchan)–52.94% PMCs (Dhundhi) revealed the existence of structural heterozygosity for reciprocal translocations as is evident from the presence of multiple chromosome associations (Fig. 2d).

Formation of heterogenous sized pollen grains

Although the meiocytes (diploid and polyploid) followed a regular meiotic course with equal distribution of chromosomes, the two accessions depicted the existence of heterogenous-sized pollen grains. On size basis, the fertile pollen grains were categorized into three types, as normal sized or typical (20.32×22.39 – $23.09 \times 21.63 \mu\text{m}$, 88.48–92.21%), large-

Table 2. Analysis of chromosomal associations at diakinesis/M-I and chiasma frequency in the diploid meiocytes of *Achillea millefolium*.

Accessions studied	Chromosomal associations							Chiasma per PMC (mean±standard deviation)	Chiasma per bivalent
	Bivalents		Quadrivalents			Octavalent	Univalents		
	Ring	Rod	Ring		Chain				
			Zigzag ring	Typical ring					
Palchan, 2450 m									
PMCs with nine bivalents	42	219/378 (57.93%)	159/378 (42.06%)					11–17 (13.71±1.72)	1.52
PMCs with bivalents and multivalents	19	59/107 (55.14%)	48/107 (44.86%)	6/30 (20%)	15/30 (50%)	9/30 (30%)	1/1 (100%)	13–17 (15.33±1.28)	1.70
Total PMCs analysed % of chromosomes involved in chromosomal associations	61	556/1098 (50.64%)	414/1098 (37.70%)	24/1098 (2.19%)	60/1098 (5.46%)	36/1098 (3.28%)	8/1098 (0.73%)		
Dhundhi, 3050 m									
PMCs with nine bivalents	54	216/486 (44.44%)	270/486 (55.56%)	—	—	—	—	11–16 (13.5±1.50)	1.45
PMCs with eight bivalents and two univalents	2	5/16 (31.25%)	11/16 (68.75%)	—	—	—	4	12–13 (12.5±0.75)	1.38
PMCs with bivalents and multivalents	63	270/567 (47.62%)	153/567 (26.98%)	6/567 (1.06%)	63/567 (11.11%)	3/567 (0.53%)	—	11–19 (15.76±2.08)	1.75
Total PMCs analysed % of chromosomes involved in associations	119	992/2142 (46.31%)	854/2142 (39.86%)	24/2142 (1.12%)	252/2142 (11.76%)	12/2142 (0.56%)	8/2142 (0.37%)		

sized (25.49×23.37 – $25.70 \times 24.77 \mu\text{m}$, 4.04–11.11%) and very-large sized (28.65×29.32 – $31.19 \times 27.31 \mu\text{m}$, 1.73%) (Fig. 2f, Tables 1, 4). Such large and very-large sized pollen grains might be the products of '4x' and '6x' PMCs and have '2n' and '3n' genetic constitution compared to normal-sized which have 'n' chromosome number.

Chiasma frequency

Analysis of chiasma frequency in the diploid and syncyte meiocytes on the basis of data collected on the accessions from Palchan and Dhundhi (Table 2) reveals that the mean chiasma frequency per PMC in the normal diploid PMCs with nine bivalents works out to be 13.71 ± 1.72 and 13.50 ± 1.50 with an average chiasma

Table 3. Cytological status of PMCs at different stages of meiosis in two accessions of *Achillea millefolium*.

Province, locality, altitudes	Accession number (PUN)	Chromosome number (n)	Meiotic course	No. of PMCs analysed at M-I		% of PMCs with lag-gards at A-I	% of PMCs with lag-gards at A-II	% of PMCs with lag-gards at T-II	Pollen fertility (%)	Pollen grains size (μm)
				(without multivalents)	(with multivalents)					
Kullu district Solang Valley Palchan, 2450m	58571	9	ST	42	19	5.45	6.66	—	68.00	Typical sized: $23.09 \times 21.63 \mu\text{m}$ Large sized: $25.70 \times 24.77 \mu\text{m}$ Very large sized: $31.19 \times 27.31 \mu\text{m}$
Solang Nullah, 2700m	56570	9	Normal	78	—	—	—	—	99.00	Typical sized: $21.55 \times 22.02 \mu\text{m}$
Solang Village, 2750m	56885	9	Normal	69	—	—	—	—	98.00	Typical sized: $22.67 \times 21.15 \mu\text{m}$
Dhundhi, 3050m	58572	9	ST	54	63	8.69	—	4.34	71.49	Typical sized: $20.32 \times 22.39 \mu\text{m}$ Large sized: $25.49 \times 23.37 \mu\text{m}$ Very large sized: $28.65 \times 29.32 \mu\text{m}$
Parvati Valley Shaarni, 2300m	56092	9	Normal	93	—	—	—	—	100	Typical sized: $22.61 \times 18.98 \mu\text{m}$
Pulga, 2300m	56119	9	Normal	78	—	—	—	—	100	Typical sized: $22.41 \times 20.49 \mu\text{m}$
Braagha, 2600m	56111	9	Normal	62	—	—	—	—	100	Typical sized: $21.63 \times 21.10 \mu\text{m}$
Nareng, 2700m	56119	9	Normal	47	—	—	—	—	100	Typical sized: $22.11 \times 21.00 \mu\text{m}$
Mallana, 2650m	57165	9	Normal	102	—	—	—	—	100	Typical sized: $20.43 \times 18.88 \mu\text{m}$

Table 4. Province, locality, altitudes, accession number, % of syncytes, pollen size and their relative frequency in structurally heterozygous populations of *Achillea millefolium*.

Solang Valley		
Locality, altitudes	Palchan, 2450m	Dhundhi, 3050m
Accession number (PUN*)	58571	58572
Meiotic irregularities		
Syncytes (4x, 6x) PMCs	1.73% (22/1269)	1.45% (9/620)
Diploid PMCs with multivalents	31.15% (19/61)	52.94% (63/119)
Syncytes with quadrivalents	25.00% (4/16)	30.00% (3/10)
Diploid PMCs with univalents	—	1.68% (2/119)
PMCs with sticky chromatin	11.45% (11/96)	15.17% (22/145)
Apparent pollen sterility	31.45% (78/248)	28.50% (61/214)
Average pollen size (R.F.)		
n	23.09×21.63 {94.21%}	20.32×22.39 {88.88%}
2n	25.70×24.77 {4.04%}	25.49×23.37 {11.11%}
3n	31.19×27.31 {1.73%}	—/—

R.F.: relative frequency. Data given in the parentheses represent observed number of cytologically irregular PMCs or sterile pollen grains in the numerator and total number of PMCs or pollen grains in the denominator. *PUN is the abbreviation for herbarium of Department of Botany, Punjabi University, Patiala.

per bivalent of 1.52 and 1.45, respectively. On the other hand, the mean chiasma frequency per PMC and per bivalent was recorded to be significantly higher in the diploid PMCs showing structural heterozygosity for reciprocal translocations in both the accessions (15.33 ± 1.28 , 1.70 and 15.76 ± 2.08 , 1.75, respectively). Meioocytes with bivalents and univalents showed a relatively low chiasma frequency and chiasma per bivalent of 12.5 ± 0.75 and 1.38, respectively. Same trend in chiasma frequency/PMC and per bivalent has been noticed in the syncyte 4x and 6x PMCs (Table 5).

Discussion

Present count of diploid chromosome number ($2n=18$) in the studied accessions supports the earlier chromosome reports by Shetty (1964), Mehra *et al.* (1965), Mehra and Remanandan (1974), Mathew and Mathew (1988), and Gupta and Gill (1989), Kaur and Singhal (2015), and Rana *et al.* (2015) from different parts of India and by a number of workers from outside of India. *Achillea millefolium* which constitutes a species complex having 2x, 3x 4x, 5x, 6x and 8x cytotypes (based on $x=9$) is one of the most extensively studied species from N-W Himalayas. Recently, Singhal *et al.* (2014) have reported the existence of reciprocal translocations in the 2x plants collected from the districts of Kullu and Kinnaur in Himachal Pradesh. On the basis of intensive and extensive study carried out presently on individual plant basis from Solang Valley and Parvati Valley, we here report the existence of reciprocal translocations in the diploid

individuals. Besides, such individuals also showed the presence of cell fusion among two to three adjacent meiocytes to generate '4x' and '6x' meiocytes.

Multiple associations of chromosomes in the diploid plants depicted the occurrence of structural heterozygosity for reciprocal translocations. Reciprocal translocations, also known as chromosomal interchanges, arise due to the exchange of broken segments of non-homologous chromosomes (Mahama and Palmer 2003, Talukdar 2013). Ever since the first report of reciprocal translocations by Belling (1914), their occurrence and possible consequences have been reported in a number of flowering plants from N-W Himalayas by various workers (Gohil and Koul 1978, Sharma and Gohil 2003, 2008, 2011, Gupta *et al.* 2010, Kohli and Gohil 2011, Rana *et al.* 2012, Kumar and Singhal 2013, Kumar *et al.* 2015). Presently such reciprocal translocations have been noticed in both the 2x and syncyte (4x) PMCs.

Analysis of 2x, and 4x PMCs in such 2x individuals with reciprocal translocations also throw light on the level of genetic recombination in the species. On an average, the values of chiasma frequency in the meiocytes with multivalents are recorded to be higher compared to the meiocytes without multiple chromosomal associations, indicating the positive effect of reciprocal translocations on chiasma frequency. Similar increase in chiasma frequency due to multivalent formations has been observed in the 2x and (4x) syncyte PMCs. However, the PMCs depicted with univalent chromosomes tend to have decreased value of chiasma frequency. Such an increase in chiasma frequency due to structural hetero-

Table 5. Analysis of chromosomal associations and chiasma frequency in syncyte PMCs at diakinesis/M-I in *Achillea millefolium*.

	Chromosomal associations								Chiasma per PMC (mean±standard deviation)	Chiasma per bivalent
	No. of PMCs analysed	Bivalents		Quadrivalents			Octavalents	Univalents		
		Ring	Rod	Ring		Chain				
				Zigzag ring	Typical ring					
Palchan (2450 m) accession										
Syncyte (4x) PMCs with 18 bivalents	10	101/180 (56.11%)	79/180 (43.89%)	—	—	—	—	—	23–33 (28.3±1.9)	1.55
Syncyte (6x) PMCs with 27 bivalents	2	30/54 (55.56%)	24/54 (44.44%)	—	—	—	—	—	40–44 (42±2.82)	1.55
Syncyte (4x) PMCs with bivalents, multivalent and univalents	4	40/59 (67.80%)	19/59 (32.20%)	—	4/6 (66.67%)	2/6 (33.33%)	—	2	28–32 (30.25±1.70)	1.68
Total PMCs analysed % of chromosomes involved in associations	16	342/612 (55.88%)	240/612 (39.21%)		16/612 (2.61%)	8/612 (1.30%)		6/612 (0.98%)		
Dhundhi (3050 m) accession										
Syncyte (4x) PMCs with 18 bivalents	7	69/126 (54.76%)	57/126 (45.24%)						24–30 (27.71±2.49)	1.53
Syncyte (4x) PMCs with bivalents, multivalent and univalents	3	31/43 (72.09%)	12/43 (27.91%)	—	4/5 (80%)	1/5 (20%)			30–32 (31±1.00)	1.72
Total PMCs analysed % of chromosomes involved in associations	10	200/360 (55.55%)	69/360 (19.17%)	—	16/360 (4.44%)	4/360 (1.11%)		2/360 (0.55%)		

zygosity has also been reported in *Saxifraga diversifolia* by Kumar and Singhal (2013), *Achillea millefolium* (Singhal *et al.* 2014), and *Anemone rivularis* (Kumar *et al.* 2015).

Spontaneous occurrence of syncytes due to the fusion of adjacent meiocytes had been reported for the first time by Gates and Rees (1921) in *Lactuca sativa*. Since then, the phenomenon has been reported in a number of flowering plants by Levan (1941), Price (1956), Katayama (1964), Mehra and Kalia (1973), Rao and Koduru (1978), Sarbhoy (1980), Padmaja (1988), Caetano-Pereira *et al.* (1999), Mendes-Bonato *et al.* (2001, 2003), Boldrini *et al.* (2006), Risso-Pascotto *et al.* (2006), Singhal and Kumar (2008, 2010), Kim *et al.* (2009), Singhal *et al.* (2010, 2011), Kumar *et al.* (2010, 2011, 2012), Kumar and Singhal 2012, and Malik *et al.* (2014). In the present study, we report for the first time the formation of syncytes through fusion of two to three adjacent PMCs in the diploid populations of *Achillea millefolium*. The resultant polyploid PMCs with double or triple the genetic material could be recognized by their much larger size and have tetraploid and hexaploid chromosome numbers. Furthermore, such PMCs follow perfectly regular meiotic course with 18 and 27 bivalents respectively, equal chromosome segregation during A-I and normal sporads formation. However, both the diploid accessions possessing normal ($2n$) PMCs and syncyte meiocytes ($4n$, $6n$), yielded heterogenous sized viable pollen grains, as typical, large and very large-sized. The typical pollen grains which are the products of normal ' $2n$ ' PMCs possess ' n ' genetic constitution. Although the syncytes occur at higher frequency (16.77–29.16%), the fusion among proximate PMCs occurred at early stages of meiosis I and the products of such PMCs yielded large and very large-sized atypical pollen grains occurring at rather low frequencies of 4.04–11.11% and 1.73% with ' $2n$ ' and ' $3n$ ' genetic constitution, respectively.

The exact cytological status of such large and very-large sized viable pollen grains could not be ascertained during the present study but their higher cytological status (' $2n$ ' and ' $3n$ ') is clearly depicted from their almost double and treble size compared to typical ' n ' pollen grains as increasing DNA content may in turn influence the pollen diameter (Vorsa and Bingham 1979, Pundir *et al.* 1983, Bamberg and Hanneman 1991, Jansen and Den Nijs 1993, Bretagnolle and Thompson 1995, Ortiz 1997). The origin of such polyploid PMCs due to cell fusion or whole chromatin transfer during cytomixis and the production of large-sized pollen grains with unreduced ($2n$) genetic constitution has also been reported in *Chrysanthemum* (Kim *et al.* 2009), *Lindelofia longiflora* (Singhal *et al.* 2011), inter-specific hybrids of *Brassica* (Mason *et al.* 2011), and *Mertensia echiodides* (Malik *et al.* 2014). Origin of gametes with unreduced genetic constitution in the diploid plants also referred as diplogametes (De Storme and Geelen 2013) as a product of cell

fusion might have played an important role in the origin of intraspecific polyploid cytotypes ($3x$, $4x$, $5x$, $6x$, $7x$, $8x$) in the presently studied species. Kim *et al.* (2009) in *Chrysanthemum*, Singhal *et al.* (2011) in *Lindelofia longiflora*, Malik *et al.* (2014) in *Mertensia echiodides* have also advocated the role of cell fusion mediated syncyte formation and subsequently the participation of ' $2n$ ' and ' $3n$ ' pollen grains in fertilization to generate polyploid offspring and thereby constitute the basis for the establishment of intraspecific polyploid cytotypes.

Syncyte formation as a result of cell fusion has been recorded in a wide variety of flowering plants, both dicots and monocots. But the possible factor/s responsible is yet to be ascertained. Divergent views have been put forward by different workers as the effect of chemicals, X-rays, temperature, moisture stress, culture conditions or genetic factors (Stern 1946, Merwine and Bennett 1966, Pantulu and Manga 1971, Rao and Koduru 1978, Rao *et al.* 1991, Nirmala and Rao 1996, Kumar and Srivastava 2009, Mason *et al.* 2011, Pecrix *et al.* 2011, De Storme *et al.* 2012, Singhal *et al.* 2011, Malik *et al.* 2014). In the presently studied diploid populations of *Achillea millefolium*, the origin of syncytes due to the fusion of two to three PMCs and structural heterozygosity for reciprocal translocations could be attributed to low temperature stress conditions coupled with ecologically disturbed habitats due to various anthropogenic activities like grazing, construction of roads, and tunnels in conjunction with natural calamities of cloud bursts and landslides prevailing in Solang valley where temperature dips to below freezing point during May–June when the plants enter the flowering stage as suggested by Singhal *et al.* (2011) in *Lindelofia longiflora*.

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