

## MEIOTIC BEHAVIOUR IN TWO TRIPLOIDS: *ERIGERON ANNUUS* AND *EUPATORIUM ADENOPHORUM* (ASTERACEAE)

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### ABSTRACT

The present study shows the meiotic behaviour in two species of Asteraceae family. *Erigeron annuus* ( $2n=27$ ) and *Eupatorium adenophorum* ( $2n=51$ ) are triploid (i.e.  $3x$  based on  $x=9$  and  $x=17$ , respectively) appear to be apomictic in nature due to the presence of highly abnormal meiotic course and high pollen sterility but with normal seed setting. Meiosis in these species is characterized by the formation of many univalents and trivalents with a few bivalents. Anaphases and telophases are highly abnormal with the formation of many laggards and micronuclei during microsporogenesis. Cytomixis is also a common phenomenon noticed in all *Eupatorium adenophorum* populations. Pollen sterility varies from 40-71%. Both the species are widespread in nature and grow as weeds certainly reproduce through asexual means.

**Key Words:** Abnormal meiosis, apomixis, trivalents, univalents, pollen sterility.

### INTRODUCTION

Apomixis is a naturally occurring way of asexual reproduction through seeds (Winkler 1908). It is the replacement of normal sexual reproduction by asexual reproduction, without fertilization. Apomictic individuals contain a genome derived entirely from female parent that means bypassing female meiosis and syngamy. If fertile seeds are produced in the absence of pollens, apomixis is likely present. In addition to this, highly abnormal meiotic course and production of sterile pollen grains in species with normal seed setting are traditional and reliable indicators of apomixis. It is considered as short term solution to hybrid sterility and as an evolutionary dead end (Stebbins, 1950). This phenomenon is widespread in plants as documented in 44 different families and represented in most major Angiosperm clades

(Van Dijk and Vijverberg, 2005; Carneiro *et al.*, 2006). Earlier apomixis is found to occur in over 300 species of 35 different plant families (Bashaw and Hanna, 1990) while Carneiro *et al.* (2006) and Carmen (1997) suggested >400 species in 44 angiospermic families. Apomixis is the most prevalent phenomenon reported in Asteraceae, Poaceae, Ranunculaceae and Rosaceae families (Asker and Jerling, 1992; Bicknell and Koltunow, 2004). Most of the apomicts are either polyploids or hybrids suggesting that polyploidy coupled with hybridization could be key components of apomixis. Thus polyploidy is proposed as a trigger for apomixis (Grimanelli *et al.*, 2001). Gametophytic apomixis provides a convenient method to bypass meiosis in autopolyploids and prevents the production of cytologically imbalanced offsprings. Like polyploids,

apomicts compete successfully with their diploid parents for distribution in similar habitats or may occupy different habitats.

This is well known phenomenon in the members of Asteraceae family as presently observed in two species, namely, *Erigeron annuus* and *Eupatorium adenophorum*. Noyes and Rieseberg (2000) in *Erigeron annuus* and Lu *et al.* (2008) in *Eupatorium adenophorum* studied the apomixis. Noyes (2007) gave 22 genera of Asteraceae family considered to be apomictic. In spite of their high meiotic instability and pollen sterility, the seed formation is not apparently affected in presently studied species, suggesting that they are apomicts. The better adaptation of these apomicts is attributed to stabilization of selected genotypes which often allow for rapid colonization of established habitats (Lewis 1979). Both the species are noxious invasive weeds. Lu *et al.* (2008) studied and discussed the reproductive potential of the species by checking pollen viability, pollination, seed setting and germination. Noyes and Rieseberg (2000) showed that the two independent loci controlled agamospermy is operated in *Erigeron annuus*.

Both the species are extensively and widely growing in the district Kangra (Himachal Pradesh), North India. The geographical area of Kangra is 5739 km<sup>2</sup> lies in 30°.2'-32°.5' latitude and 75°-77°.45' longitude and supports rich diversity of flora and fauna. The present attempt is made to explore both of the species meiotically on population basis so as to give a view of meiotic behaviour in triploids.

## MATERIAL AND METHODS

The plant materials were collected from different altitudinal zones i.e. sub-tropical and temperate with 1221-3300m for *Erigeron annuus* and 490-1780m for *Eupatorium adenophorum*. Collections were made throughout the year in 2008-2010 from different localities of Kangra (Himachal Pradesh), North India. Voucher specimens are deposited in Herbarium, Department of Botany, Punjabi University, Patiala (PUN). Appropriate sized capitula are fixed in Carnoy's fixative (6:3:1= absolute

alcohol: chloroform: glacial acetic acid v/v/v) for 24 hrs and preserved in 70% alcohol at 4°C. For meiotic studies the appropriate sized anthers are squashed in 1% acetocarmine. From meiotic preparations, the chromosome numbers and meiotic behaviour in 300-500 pollen mother cells (PMCs) are carefully examined at different phases of meiosis (P-I, M-I, A-I, T-I, M-II, A-II, T-II). Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine (1:1). Normal well filled and deeply stained pollens are taken as fertile while shrivelled up and unstained pollens as sterile. Measurements of pollen size are taken using ocular micrometer. Photomicrographs of chromosome counts are made from freshly prepared slides using Leica Qwin and Nikon Microscope Eclipse 80i systems.

## RESULTS

### *Erigeron annuus* (L.) Pers.

Six populations were collected from different localities. Though morphologically these populations do not show any noticeable difference, but there are some differences in frequencies of meiotic abnormalities. All the populations are triploid with  $2n=27$  (based on  $x=9$ ) with the occurrence of varying number of trivalents and univalents, besides few bivalents. The frequencies of trivalents and univalents also vary among different populations (Table 1; Figs. 1-3). Further meiotic course during anaphases and telophases is also anomalous due to formation of 1-13 laggards and 1-2 chromatin bridges (Table 2; Figs. 4-8). The incident of cytomixis is observed in P-4 and P-5. The migration of chromatin material is either through direct broad or narrow cytoplasmic channels at P-I stage. In P-4, 31.92% PMCs are involved while in P-5, 23.0% PMCs are involved in cytomixis (Figs. 9, 10). As a result of chromatin migration to adjacent PMCs, cells with varied chromosome numbers than the actual chromatin complement are formed. Microsporogenesis is also found to be highly affected by abnormal meiosis and results into formation of dyads, triads, tetrads without or with 1-5 micronuclei (Table 3; Figs. 11-13). High percentage of pollen grain sterility is observed (37-71%) in different

**Table 1. Analysis of chromosomal associations at diakinesis/ M-I in *Erigeron annuus*.**

Populations/ PUN <sup>1</sup>	Locality with altitude in meters	Number of PMCs observed	Number of different chromosomal associations per PMC				
			III	II	I		
P-1/ PUN 53385	Palampur, 1,219	4	8	1	1		
		7	5	4	4		
		2	5	3	6		
		3	4	3	9		
		4	3	5	8		
		2	3	4	10		
		1	2	3	15		
	Total:	23	109	78	138		
	Average frequency /PMC		4.73	3.39	6		
	%age of chromosome involved		52.55	25.11	22.22		
P-2/ PUN 53380	Chhota Bhagal; Multhan, 2,000	9	7	3	0		
		11	5	4	4		
		6	2	0	21		
		1	4	3	9		
		4	3	4	10		
			Total:	31	146	90	219
			Average frequency /PMC		4.70	2.90	7.06
	%age of chromosome involved		52.22	21.48	26.14		
P-3/ PUN 53381	Chhota Bhagal; Badagaon, 3,300	7	8	1	1		
		15	6	2	5		
		1	3	8	2		
		1	2	5	11		
			Total:	24	151	50	95
			Average frequency /PMC		6.29	2.08	3.95
			%age of chromosome involved		69.88	15.40	14.62
P-4/ PUN 53382	Chhota Bhagal; Dyot, 2,037	6	5	3	6		
		8	4	5	5		
		3	4	3	9		
		13	4	5	5		
		1	4	4	7		
			Total:	31	130	136	175
			Average frequency /PMC		4.19	4.38	5.64
	%age of chromosome involved		46.55	32.44	20.88		
P-5/ PUN 53383	Chhota Bhagal; Swar, 2,500	4	7	2	2		
		7	5	3	6		
		2	5	2	8		
		3	4	5	5		
			Total:	16	85	48	81
			Average frequency /PMC		5.3	3.0	5.06
			%age of chromosome involved		58.88	22.22	18.74
P-6/ PUN 56420	Chhota Bhagal; Multhan, 2,000	7	8	1	1		
		6	7	2	2		
		1	5	4	4		
		1	5	3	6		
		2	5	2	8		
			Total:	17	118	30	45
			Average frequency /PMC		6.94	1.76	2.64
	%age of chromosome involved		77.11	13.03	9.77		

PUN<sup>1</sup> is the herbarium code of the Department of Botany, Punjabi University, Patiala as per "Index Herbariorum" by Holmgren and Holmgren (1998).

populations. Further three pollen grain size ranges: small (7.5-11.25  $\mu\text{m}$ ), medium (11.25-15.00  $\mu\text{m}$ ) and large (18.75-22.50  $\mu\text{m}$ ) are noticed (Table 4). Mostly the small sized pollen grains are sterile one.

#### ***Eupatorium adenophorum* Spreng.**

It is native to Mexico, but is known in many other parts of the world as an introduced species and often a noxious weed. Presently four populations collected from four different localities of altitude range from 490-1780 m. All the populations are found to be triploid with  $2n=51$  (based on  $x=17$ ). Meiotic course is

abnormal with some multivalents and univalents in all the four populations (Figs. 14, 15). Due to small size of PMCs and large number of chromosomes, it is rather difficult to identify and count the exact form and number of multivalents. All the populations are messed with huge meiotic irregularities in the form of non-synchronized chromosomes, laggards and cytomixis. Non-synchronized univalents (2-7) at M-I and M-II phases are observed in all the populations though the frequency of abnormal PMCs vary from population to population (Table 5). Further during anaphases and telophases, 2-9 laggards are noticed with variation in frequency

**Table 2. Data on abnormal meiosis in six populations of *Erigeron annuus*.**

Populations	Total PMCs observed	PMCs showing laggards		PMCs showing bridges	
		A-I/T-I	A-II/T-II	A-I/T-I	A-II/T-II
P-1	Number: 96	28	21	17	11
	%age	29.1	21.8	17.7	11.4
P-2	Number: 51	4	4	14	3
	%age	7.8	7.8	27.45	5.8
P-3	Number: 105	27	19	2	6
	%age	25.7	18.0	1.9	5.7
P-4	Number: 50	14	7	2	6
	%age	28.0	14.0	4.0	12.0
P-5	Number: 77	11	13	5	8
	%age	14.2	16.8	6.4	10.3
P-6	Number: 41	8	2	6	1
	%age	19.5	4.8	14.6	2.4

**Table 3. Data on abnormal microsporogenesis in six populations of *Erigeron annuus*.**

Populations	Total sporads observed	Dyad with micronuclei	Triad	Triad with micronuclei	Tetrad	Tetrad with micronuclei
P-1	Number: 42	5	-	6	22	11
	%age	11.9	-	14.2	52.3	26.1
P-2	Number: 46	-	1	9	8	9
	%age	-	2.1	19.5	17.3	19.5
P-3	Number: 61	-	-	2	51	8
	%age	-	-	3.27	83.6	13.1
P-4	Number: 66	-	-	-	33	33
	%age	-	-	-	50.0	50.0
P-5	Number: 103	-	-	-	25	78
	%age	-	-	-	24.2	75.7
P-6	Number: 78	-	-	-	52	26
	%age	-	-	-	66.6	33.3

**Table 4. Data on pollen grain size variation in six populations of *Erigeron annuus*.**

Populations	Total pollen grains observed	Number of pollen grains showing three size ranges in $\mu\text{m}$		
		small 7.5 – 11.25	medium >11.25 – 15.00	large 18.75 – 22.50
P-1	Number: 192	76	29	87
	%age	39.5	15.1	45.3
P-2	Number: 292	-	103	189
	%age	-	35.2	64.7
P-3	Number: 142	-	48	94
	%age	-	33.8	66.1
P-4	Number: 331	56	88	187
	%age	16.9	26.5	56.4
P-5	Number: 176	16	61	99
	%age	9.0	34.6	56.2
P-6	Number: 223	28	80	115
	%age	12.5	35.8	51.5

**Table 5. Data on non-synchronized chromosomes in four populations of *Eupatorium adenophorum*.**

Population/ PUN	Locality with altitude in meters	Total number of PMCs observed	PMCs showing non-synchronized univalents at M-I		PMCs showing non-synchronized chromosomes at M-II	
			Number	%age	Number	%age
P-1/ PUN 53425	Harchakian, 621	67	11	16.41	13	19.40
P-2/ PUN 53426	Bassa, 490	59	09	15.25	07	11.86
P-3/ PUN 53427	Mcleodganj, 1780	40	10	25.00	12	30.00
P-4/ PUN 53428	Palampur, 1219	51	11	21.56	05	09.80

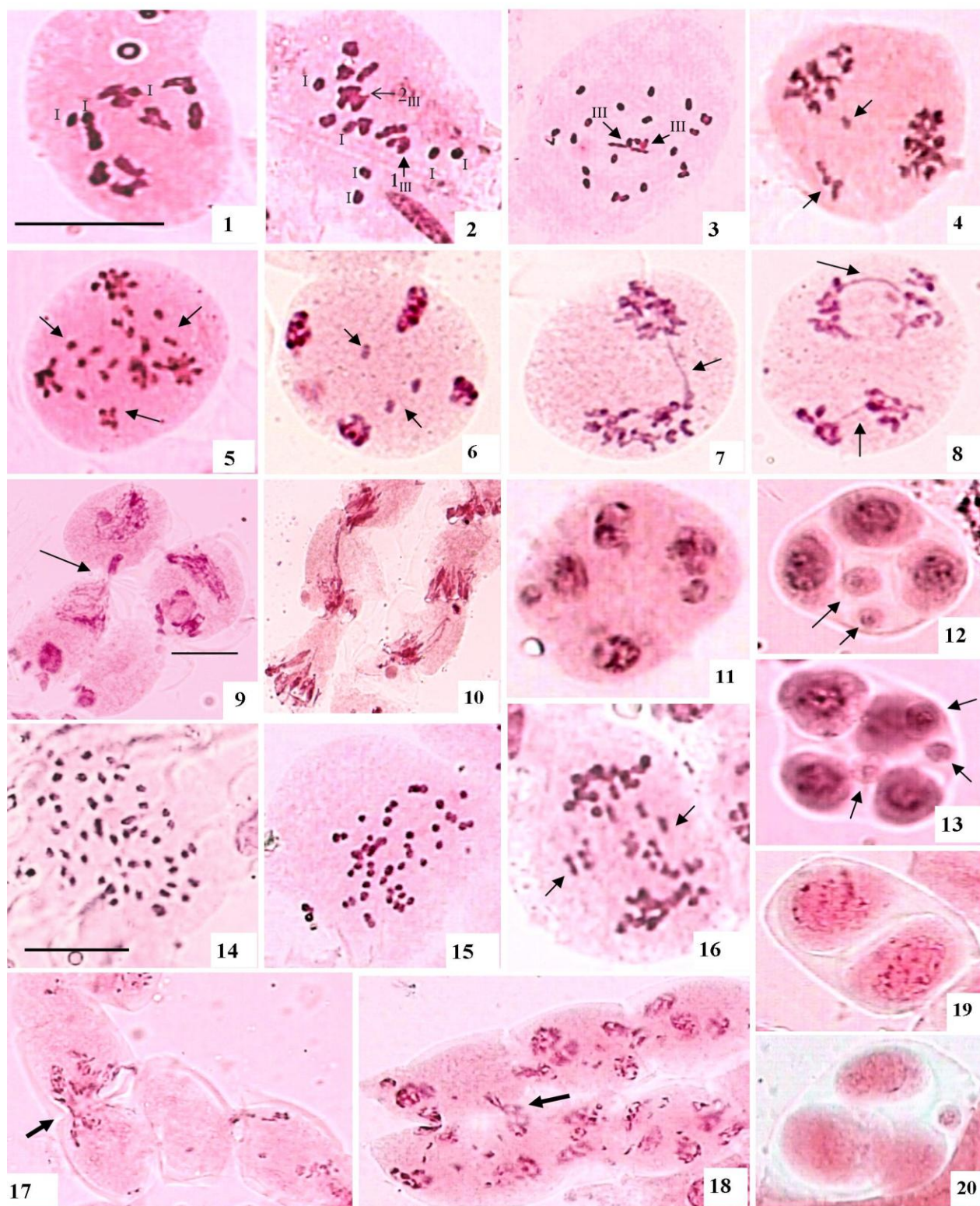
**Table 6. Data on laggards in four populations of *Eupatorium adenophorum*.**

Population	Total number of PMCs observed	Number of PMCs with laggards	%age of PMCs with laggards	Number of laggards		%age of PMCs with laggards		Number of laggards
				A-I/ T-I	A-II/ T-II	A-I/ T-I	A-II/ T-II	
P-1	64	7	10.93	2-4	18	28.12	2-4	
				5-9	4	6.25	5-9	
P-2	60	15	25.00	2-4	10	16.66	2-4	
				5-9	13	21.66	5-9	
P-3	77	7	09.09	2-4	17	22.07	2-5	
				5-7				
P-4	90	9	10.00	2-3	13	14.44	2-3	
				4-9	16	17.77	4-9	

of abnormal PMCs (Table 6; Fig. 16). Maximum percentage of PMCs is involved in transfer of chromatin material through either direct broad channels or narrow cytoplasmic channels (1-3) which results in PMCs with lower or higher the chromosome numbers with many vacant PMCs. This phenomenon is reported in almost all the

stages of meiosis from P-I up to T-II (Table 7; Figs. 17, 18). Microsporogenesis is also found to be abnormal with the formation of 1-2 micronuclei in tetrads which affected 15-18% microsporads in all the populations (Figs.19, 20). All these meiotic irregularities finally lead to pollen sterility that varies from 49-69%.



**Figure-1. Meiotic configurations in presently investigated two species (Scale bar=10µm).**

***Erigeron annuus*:** (1) PMC at M-I with  $2n=8_{III}+3_I$ ; (2) PMC at M-I with  $2n=3_{III}+6_{II}+6_I$ ; (3) PMC at M-I with  $2n=2_{III}+3_{II}+15_I$ ; (4) PMC at A-I showing laggards; (5, 6) PMCs at A-II and T-II showing laggards; (7, 8) PMCs at A-I and A-II with bridges; (9, 10) PMCs at P-I showing chromatin transfer; (11) PMC at T-II with micronuclei; (12) Triad with 2 micronuclei; (13) Tetrad with 3 micronuclei. ***Eupatorium adenophorum*:** (14) PMC at M-I showing 51 univalents; (15) PMC at M-I showing  $2n=51$ ; (16) PMC at A-I showing many laggards; (17, 18) PMCs involved in cytomixis at M-I and A-II stages; (19) Diad; (20) Tetrad with micronucleus.

**Table 7. Data on cytomixis in four populations of *Eupatorium adenophorum*.**

Population	Total PMCs observed	Stages of meiosis	Number of PMCs involved in cytomixis	%age of PMCs involved in cytomixis
P-1	55	Prophase-I	13	23.63
		Anaphase-I	03	05.45
		Telophase-I	14	25.45
P-2	185	Prophase-I	86	46.48
		Telophase-I	29	15.67
		Anaphase-I	19	10.27
		Telophase-II	16	8.64
P-3	96	Prophase-I	29	30.20
		Anaphase-I	16	16.66
		Telophase-I	25	26.04
P-4	104	Prophase-I	26	25.00
		Telophase-I	21	20.19
		Anaphase-I	19	18.26
		Telophase-II	18	17.30

Further size of pollen grains also varies among populations: 09.37-18.75  $\mu\text{m}$  in P-1, 12.37-18.00  $\mu\text{m}$  in P-2, 12.25-16.37  $\mu\text{m}$  in P-3 and 11.12-18.25  $\mu\text{m}$  in P-4.

## DISCUSSION

The triploid chromosome count for *Erigeron annuus* is well known in Indian populations (Mehra *et al.*, 1965). From outside India, besides this triploid cytotype, diploid, hexaploid and some aneuploid cytotypes are also known (Chojnacki *et al.*, 1980; Hong and Zhang, 1990; Nesom, 1978, Jhansi Rani, 2013). Further up to 9 B-chromosomes are reported in diploid cytotype (Rudyka, 1988). The high seed setting in spite of very low pollen fertility in this species is attributed to phenomenon of apomixis (Nesom, 1989). *Eupatorium adenophorum* is well known with  $2n=51$  chromosome number both from India (Mehra *et al.*, 1965; Khonglam, and Singh 1980) and abroad.

Presence of 2-8 trivalents, 1-21 univalents besides 1-8 bivalents formation in *E. annuus* and as many as 51 univalents or less in *E. adenophorum* clearly points out the irregular chromosomal segregation which produces laggards in high frequency. The percentage of chromosomes involved in trivalent formation varies from 46.55 in P-1 to 77.11 in P-6 in *E. annuus*. The multivalent formation leading to

unequal segregation, random chiasmata formation and cytologically imbalanced gametes are produced. Where uneven numbers of sets of genomes are present, as in a triploid such as 3 sets of chromosomes, the plants are frequently sterile. This is for the reason that with an uneven number of chromosomes completes pairing of homologous chromosomes is not achievable.

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