5. GENERAL DISCUSSION
From the foregoing text and Table 1, at present it is evident that meiotic studies on 360 populations pertaining to 174 species of 82 genera and 23 families of polypetalous group of flowering plants have been carried out from district Kangra of Himachal Pradesh. As these limited numbers of species are spread over different families, the inferences drawn for general conclusions are to be taken with caution. The generalized salient features of these studies are as follows:

5.1 Chromosome numbers:
The chromosome numbers in Angiosperms are known to show considerable variation (Stebbins 1958). The lowest number of 2n=4 is recorded in *Haplopappus gracilus* and *Brachycome dichromosomatica* (Asteraceae) and *Ornitogalum tenuifolium* (Hyacinthaceae), as well as *Zingeria biebersteiniana* and *Colpodium versicolor* (Poaceae) and the highest of 2n=ca.640 in *Sedum suaveolens* (Crassulaceae). The most common chromosome number in Dicots is reported to be 2n=18, followed by 2n=16, 22, 24, 26, 14, 28, 36, etc., whereas in Monocots the most common number is 2n=14 followed by other numbers as 2n=28, 24, 20, 36, 18, 22, 16, etc. (Grant 1963).

In the presently studied 174 species/194 cytotypes, the lowest chromosome number is recorded to be 2n=12 in 12 species (*Impatiens arguta, I. glandulifera, I. scabrida, Lotus corniculatus, Vicia hirsuta, V. tetrasperma, V. sativa, Nigella sativa, Viola betonicifolia, V. biflora, V. canescens and V. serpens*) and highest of 2n=130 noticed in *Abelmoschus manihot* along with various intermediate chromosome numbers (Fig. 176). Total of 18 different chromosome numbers are noticed in 174 species/194 cytotypes of which 2n=14 is the most common chromosome number found in 23.85 % species followed by 2n=16 (18.27%), 2n=28 (13.70%) 2n=22 & 32 (7.61% each), 2n=20 (4.56%), 2n=26 (4.56%), 2n=36 (3.55%), 2n=18 (3.04%), 2n=24 (2.03%), 2n=56 (2.03%), 2n=42 (1.01%), while, 2n=30, 34, 40, 52 and 130 are the chromosome numbers with the least frequency (0.5%).
In 20 species, chromosome numbers variability has been recorded at intraspecific level, e.g. *Agrimonia eupatoria* (2n=28, 56), *Bupleurum lanceolatum* (2n=16, 32), *Capsella bursa-pastoris* (2n=16, 32), *Impatiens arguta* (2n=12, 14), *I. bicolor* (2n=14, 16), *I. brachycentra* (2n=14, 16), *I. glandulifera* (2n=12, 14), *I. scabrida* (2n=12, 14), *Medicago polymorpha* (2n=14, 32), *Oxalis corymbosa* (2n=14, 28), *Potentilla fulgens* (2n=14, 28), *P. desertorum* (2n=14, 28), *P. sundaica* (2n=14, 28), *Ranunculus laetus* (2n=28, 32), *Rosa macrophylla* (2n=14, 28), *Silene conoidea* (2n=20, 40), *Thalictrum foetidum* (2n=14, 16), *Vicia hirsuta* (2n=12, 14), *V. sativa* (2n=12, 14) and *V. tetrasperma* (2n=12, 14). Hence, these species definitely exhibit significant amount of chromosome numbers diversity.

Fig. 176: Frequency of different chromosome numbers in the presently investigated 194 cytotypes of 174 species.

### 5.2 B-chromosomes:

In plants much of the early work on B-chromosomes was undertaken in maize, beginning with Kuwada in 1915, but it was Longley (1927), and later Randolph (1941) who first distinguished these extras in maize as being supernumerary and who presented the first detailed study in plants on their behaviour and characteristics. B-chromosomes are now known in at least 1,372 flowering plants, of which 12 are conifers and 1,360 are Angiosperms (Camacho *et al.* 2000; Jones *et al.* 2008). B-chromosomes have been
reported to be more frequent in some plant families *viz.*, Poaceae, Liliaceae, Asteraceae and Ranunculaceae (Battaglia 1964). These are supposed to be dispensable extra chromosomes, found in only some individuals of a population, and which are not duplicates of any member of the basic A-chromosome set in diploids or polyploids. Perhaps, thus explains that B’s are not only extra, but also different and fail to pair with any of the A-chromosomes at meiosis. They are often morphologically distinct, usually smaller than the A-chromosomes, and they can show numerical variation within the PMCs of the same anther, flower buds of the same individual and ever between individuals (Jones 1995). B-chromosomes probably arise from A-chromosomes, but have followed their own evolutionary pathways (Beukeboom 1994). Furthermore, B-chromosomes have irregular and non-Mendelian modes of inheritance and are not believed to undergo recombination with any members of the basic A-chromosome set (Jones 1995). They are mostly heterochromatic (Jones & Rees 1982) and may have originated through chromosomal breakage of A-chromosome.

Some of the important review articles on B-chromosomes have been published by Roman & Ullstrup (1951), Jones & Rees (1982), Jones (1985), Beukeboom (1994), Langdon *et al.* (2000), etc. The occurrence of B-chromosomes is earlier known in some ferns (Jones 1995), but in fungi it has been reported for the first time by Miao *et al.* (1991) in *Nectria haematococca* which is a pathogen of *Pisum sativum*.

It is widely accepted that B-chromosomes could be derived from the A-chromosomes e.g. *Crepis capillaries* (Jamilena *et al.* 1994), *Z. mays* (Stark *et al.* 1996) and/or from sex chromosomes in animals e.g. *Eyprepocnemis plorans* (Lopez-Leon *et al.* 1994). However, there is also evidence suggesting that B-chromosomes can be generated spontaneously in response to the new genome conditions following interspecific hybridization e.g., in *Coix aquaticus* and *Coix gigantea* (Sapre & Deshpande (1987). B-chromosomes are a major source of intraspecific variation in nuclear DNA amounts in numerous species of plants. They favour large genomes, and create polymorphisms for DNA variation in natural populations (Jones *et al.*
General Discussion

2007). Owing to their particular properties, B-chromosomes have been used to elucidate the function of post-translational histone modifications, such as histone H3 phosphorylation (Manzanero et al. 2000) and methylation (Houben et al. 2003). They are of particular interest in maize (Zea mays), in which they have been extensively used in genetic analysis involving A–B translocations for mapping (Birchler 1991) and for the identification of centromere structure and size (Kaszas & Birchler 1998).

At present, 0-3B-chromosomes are recorded in 4 species such as Anemone obtusiloba (2n=14+0-3B), Clematis grata (2n=16+0-1B), Impatiens balsamina (2n=14+0-1B) and Ranunculus diffusus (2n=28+0-1B) with the difference in percentage of pollen fertility within various populations of these species marked with or without B-chromosomes. Previously, Jones & Rees (1982) noted the decrease in pollen fertility with increase in number of B-chromosomes. Besides, the B-chromosomes have also been suggested to change certain morphological characteristics of the individuals as well (Jones & Rees 1982; Jones & Houben 2003) or cause some selective advantageous effects (Jones & Rees 1982; Plowman & Bougourd 1994). Nothing of this type has been observed in the presently studied material.

5.3 Basic chromosome numbers:

It is generally defined as the lowest known ancestral gametophytic chromosome number present in the genus. However in some of the genera, high chromosome numbers are presumed to be multiples of the lower numbers which do not actually exist (Stebbins 1958). Grant (1982a) discussed the ‘palopolyploid’ and ‘aneuploid polyploid’ hypothesis to explain higher levels of basic numbers and the later one finding more acceptability as adopted earlier by (Stebbins 1958). To help further, sometimes other criteria are also used, i.e. the number of nucleolar chromosomes in a complement (Gates 1942), number of chromosomes with secondary constrictions per complement or secondary associations of the chromosomes during meiosis-I (Moffett 1931; Lawrence 1931). But, these methods have their own limitation. Raven (1975) has suggested a wide
knowledge of its phylogeny as a pre requirement for calculating the original basic number of any group. In similar way, enough data pertaining to chromosome numbers is a prerequisite for inferring the basic chromosome numbers of a genus and weightage has to be given to the maximum number of species showing the particular gametic number. The due importance is given to those chromosome numbers on which intraspecific euploid series are formed (Grant 1982 a, b).

Although, extreme levels of basic numbers in Angiosperms are reported to be as low as $x=2$ in *Haplopappus* of Asteraceae and highest to be as $x=43$ in a member of Winteraceae (cf. Kumari & Bir 1987), yet the basic numbers for total Angiosperm has been considered to be $x=10$ or lower than this number (Stebbins 1950). There is another postulations given by Raven & Kyhos (1965); Ehrendorfer *et al.* (1968); Raven (1975), believing the primary basic number of Angiosperms to be $x=7$ and all others to be secondarily derived ones. One more possibility based on common occurrence has been proposed by taking $x=8$ as an ancestral basic number of Angiosperms by Grant (1982a).

Fernandes & Franca (1975) cited basic chromosome numbers of the genera pertaining to different formal studies from Mozambique and in 1978 gave similar information regarding legumes alone from Portugal. It is pertinent to mention here that later on Grant (1982b) published monographic work on ‘Periodicities’ in the Chromosome numbers of the Angiosperms’ on the basis of extensive information available from chromosome numbers compilations appearing up to 1974. Similar attempt was made earlier by Kumari & Bir (1987) to compile the chromosome numbers of total legumes and then work out the basic chromosome numbers of all the 337 genera cytologically known till then.

At present the basic numbers of the genera to which these cytologically worked out species belongs, have been carefully calculated afresh, keeping in mind not only mathematical method but also interrelations of inter and intraspecific levels at species as elaborated for each genus in the Chapter ‘Results & Discussion’. These are further summed up for 82 genera:
a) Monobasic: These are 24 genera as Acacia (x=13), Agrimonia (x=14), Arge\-mone (x=7), Barbarea (x=8), Berberis (x=14), Circaea (x=11), Clematis (x=8), Coronopus (x=16), Dalber\-gia x=(10), Delphinium (x=8), Desmodium (x=11), Fragaria (x=7), Ge\-um (x=7), Grewia (x=9), Lych\-nis (x=12), Melilotus (x=8), Murraya (x=9), Oenothera (x=7), Potentilla (x=7), Rubus (x=7), Sibbaldia (x=7), Thlaspi (x=7), Triumfetta x=8,(10) and Urena x=7.

b) Dibasic: These are 14 genera as Abelmoschus (x=18, 20), Boenninghausenia (x=9, 10), Capsella (x=6, 8), Cas\-ealpinia (x=11, 12), Cotoneaster (x=8, 17), Filipendula (x=7, 8), Heracleum (x=10, 11), Lathy\-rus (x=6, 7), Nasturtium (x=8, 11), Nigella (x=6, 7), Prinsepia (x=14, 16), Ranunculus (x=7, 8), Rosa (x=6, 7) and Spiraea (x=8, 9).

c) Tribasic: These are 18 genera and include Abutilon (x=7, 8, 9), Aquilegia (x=7, 8, 9), Bupleurum (x=6, 7, 8), Caragana (x=8, 9, 10), Chaerophyllum (x=6, 7, 11), Corchorus (x=7, 8, 9), Epilobium (x=9, 10, 13), Fumaria (x=6, 7, 8), Indigofera (x=6, 7, 8), Lespedeza (x=9, 10, 11), Lotus (x=5, 6, 7), Medicago (x=7, 8, 9), Momordica (x=8, 11, 14), Pimpinella (x=9, 10, 11), Sesbania (x=6, 7, 8), Silene (x=9, 10, 12), Thalictrum (x=6, 7, 8) and Vicia (x=5, 6, 7).

d) Polybasic: It includes 26 genera as Aconitum (x=8, 10, 12, 13, 17), Anemone (x=5, 7, 8, 12), Arenaria (x=7, 8, 9, 10, 11, 12, 13), Astragalus (x=6,7,8,11, 12,13), Bauhinia (x=8,12 ,13,14), Caltha (x=8, 10, 12, 14), Cassia (x=6,7,8,10,11,13), Corydalis (x=5, 6, 7, 8), Geranium (x=9, 10, 11, 12, 13, 14, 15, 16, 17, 23), Gypsophila (x=6, 10, 12, 13,15, 17), Hypericum (x=4, 7, 8, 9, 10, 12, 19), Impatiens (x=3, 4, 5, 6, 7, 8, 9,10), Malva (x=12, 18, 20, 21), Malvastrum (x=12, 15, 16, 17, 18, 21, 22), Mimosa (x=12, 13, 14, 16, 20), Oxalis (x=5, 6, 7, 8, 9), Papaver (x=6, 7, 9, 11), Pelargonium (x=4, 7, 8, 9, 10, 11, 12,15), Pueraria (x=10, 11, 12,16), Saxifraga (x=5, 6,7, 8, 9, 10,13), Sedum (x=4, 5, 6, 7, 8, 9, 10, 11, 13), Sida (x=6,7,8,9), Sisymbrium (x=7, 8, 9, 10, 11, 13), Stellaria (x=9, 10, 11, 12, 13, 14), Trifolium (x=5, 6, 7, 8) and Viola (x=2, 4, 5, 6, 7, 8, 9, 11, 13, 17). Some of
the genera show clear cases of dysploidy, thus supporting polyploidy-aneuploid hypothesis of Grant (1982a).

5.4 Polyploidy:

Polyploidy is an evolutionary process in which two or more genomes are brought together into the same nucleus by hybridisation followed by chromosome doubling or by duplication of same genome. Winkler (1916) introduced the term polyploidy while working on vegetative grafts and chimeras of Solanum nigrum. It is one of the important processes for evolution of plants (Stebbins 1971, 1975; Wendel & Doyle 2005; Cui et al. 2006; Otto 2007; Wood et al. 2009). The phenomenon of polyploidy is considered to help the plants by protecting them against immediate deleterious effects of mutations (Aase 1935). It is an important process in the evolutionary history of plants and has intense impact on biodiversity dynamics and ecosystem functioning (Ainouche & Jenczewski 2010). Polyploidy and its occurrence within one species is a common phenomenon among plant groups (Soltis & Soltis 1993; Wendel 2000; Soltis et al. 2004; Hodálová et al. 2007; Ojiewo et al. 2007). Polyploidy has also been regarded as a major force in evolution and speciation (Soltis & Soltis 1995). It is estimated that between 47% and 70% of Angiosperm species are polyploid (Ramsey & Schemske 1998) and this shoots up to as high as 95% in Pteridophytes (Leitch & Bennett 1997, Ramsey & Schemske 1998). Polyploidy is rather common in some families like Rubiaceae, Asteraceae, Iridaceae, Gramineae, etc. It is uncommon but present in other families such as Caesalpinaceae, Passifloraceae and Fagaceae (Grant 1981). In bryophytes, mosses seem to be a classical example for the occurrence of natural and artificial polyploids (Stebbins 1950), but is rather uncommon in liverworts (Grant 1981). Stebbins (1950) even pointed out to inter-generic differences in the frequency of polyploids and cited the example of Saliaceae where polyploidy was common in Salix but rare in Populus. In Gymnosperms it is rare.

Inspite of the prevalence of high rates of polyploidy in many of the plant species, there have been opposing views on the relative contribution of
polyploidy towards the process of speciation (Otto & Whitton 2000). A major route of polyploid formation involves gametic ‘nonreduction,’ or ‘meiotic nuclear restitution,’ during micro- and megasporogenesis. This process generates unreduced gametes, also referred to as ‘2n gametes,’ which contain the full somatic chromosome number (see reviews in Harlan & de Wet 1975). The union of reduced and unreduced gametes, or of two 2n gametes, can generate polyploid embryos. Polyspermy, the fertilization of an egg by more than one sperm nucleus, is known in many plant species (Vigfusson 1970), and has been observed to induce polyploidy in some orchids (Hagerup 1947). However, it is generally regarded as an uncommon mechanism of polyploid formation (Grant 1981). According to Winge (1917) and Harlan & de Wet (1975) production of unreduced gametes has been the primary mechanism for polyploidization in plants. Otto & Whitton (2000) described the two main modes of origin of polyploidy, as sexual by non-reduction in meiosis and asexual by somatic doubling in mitosis. Endomitosis, which implies that the chromosomes undergo a condensation and division cycle as in mitosis is also a type of polyploidy (Ramsey & Schemske 1998). According to Paterson et al. (2003) other mechanisms of polyploidy induction include nuclear fusion in binucleated cells and polyspermy.

During present study, only 48 species (28.18%), show polyploid nature. It is seen to be predominant in members belonging to genera Caltha, Cassia, Ranunculus, and Epilobium and families Caesalpinaceae, Ranunculaceae and Rosaceae. The highest level of ploidy is witnessed in Sibbaldia micropetala (8x) whereas, 4x remains to be the most common prevailing polyploid level (Fig.177). Similar observations regarding the predominance of tetraploids has been made earlier by de Wet (1980) and Kumar & Singhal (2011).
5.4.1 Polyploidy in relation to life form:

Role of polyploidy in speciation has been well recognised. The frequency of polyploidy is reported to be higher in perennials as compared to the annuals by many workers (Müntzing 1936; Baquar 1976). de Wet (1980) suggested that origin and success of polyploids depend upon the habit, habitat and breeding system. Gustafsson (1947) and Favarger (1967) are of the opinion that polyploidy is more common among perennial herbs than annuals and woody species. Baquar (1976) on the basis of his analysis of polyploidy in six major families of flowering plants from Pakistan also concluded that perennials exhibit a higher frequency of polyploidy in comparison to annuals. Levin & Wilson (1976) proposed the intermediate position for woody species, being flanked by perennial herbs and annuals. On the other hand, Wright (1976) rejected the concept of polyploidy-habit correlation.

In the presently studied 48 polyploid species, the phenomenon is highest in perennials in comparison to biennials and minimum in annuals (Fig.178). These observations are in line with those made by Stebbins (1938), Bandel (1974), Baquar (1976), Fernandes & Queiros (1978), Gupta & Gill (1987) and Kumari & Bir (1987). Further, analysis of these 48 species reveals that 36 species form the predominant group showing single polyploid
level each and only 12 species show euploid levels through both diploid and polyploidy category (Table 44).

![Frequency of various ploidy levels on the basis of habit in the presently investigated 174 species/194 cytotypes.](image)

**Table 44: Ploidy levels in cytologically worked out polyploidy species at present.**

<table>
<thead>
<tr>
<th>Habit</th>
<th>Ploidy levels</th>
<th>Species with single ploidy level</th>
<th>Species with complex ploidy levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4x</td>
<td>6x</td>
</tr>
<tr>
<td>Annual</td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Biennial</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Perennial</td>
<td></td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>

**5.4.2 Intraspecific polyploidy:**

Among the presently studied species, intraspecific euploidy is known in 83 species (Table 45). Among these 27 species are represented by more than 2 cytotypes (Table 45). Out of 83 species only 8 species as *Abelmoschus manihot* (2n=40,130), *Cassia occidentalis* (2n=24, 26, 28, 56), *Epilobium cylindricum* (2n=36), *E. royleanum* (2n=36, 72), *Geum roylei* (2n=42, 70), *Mimosa pudica* (2n=48, 52, 72), *Potentilla argyrophylla* (2n=28, 42, 56, 70, 77, 84, 91) and *Ranunculus muricatus* (2n=32, 40, 42, 48, 64) are the
examples for which, diploids have not been reported yet. Hence, it is clear that
diploidy has invariably been retained along with the overwhelming number,
i.e. 83 species marked with euploidy.

From the present studies, there are certain species which depict
different euploid series based on more than one basic number. These species include *Abutilon indicum* (x=7: 2n=14, 28, 42; x=9: 2n=36, 72), *Anemone rivularis* (x=7: 2n=14, 28; x=8: 2n=16, 24, 48), *Arenaria serpyllifolia* (x=10: 2n=20, 30, 40; x=11: 2n=22, 44), *Caltha palustris* (x=8: 2n=16, 32, 48, 56, 64, 72; x=10: 2n=50, 60, 70), *Cassia tora* (x=13: 2n=26, 52; x=14= 2n=28, 56), *Geranium lucidum* (x=10: 2n=20, 40, 60; x=14: 2n=28, 42), *Impatiens balsamina* (x=6: 2n=12, 24; x=7: 2n=14, 28, 56), *Nasturtium officinale* (x=8: 2n=16, 32, 48, 64; x=11: 2n=33, 66), *Ranunculus laetus* (x=7: 2n=28, 42; x=8=2n=16, 32, 56), *R. scleratus* (x=7: 2n=28, 56; x=8: 2n=16, 32, 56, 64), *Saxifraga filicaulis* (x=6:2n=24,66; x=8: 2n= 16, 32), *Thalictrum minus* (x=7: 2n=14, 28, 42; x=8: 2n=40, 80) and *Trifolium pratense* (x=7: 2n=14, 28; x=8: 2n=16, 32, 48). It is inferred that polyploidy is not evenly distributed in
the presently studied species and is observed to be of common occurrence in
families like, Onagraceae, Ranunculaceae, Rosaceae, Brassicaceae and
Caesalpiniaceae. Similarly, all species of the genus *Caltha, Cassia, Epilobium, and Ranunculus* showed polyploid nature. On the other hand, presently investigated species of *Aconitum, Aquilegia, Astragalus, Barbaraea, Bauhinia, Berberis, Boenninghausenia, Caesalpinia, Caragana, Chaerophyllum, Circaea, Coronopus Corydalis, Dalbergia, Desmodium, Filipendula, Grewia, Gypsophila, Heracleum, Lespedeza, Lychnis, Malvastrum, Murraya, Thlaspi and Vicia existed at diploid level.
General Discussion

Table 45: Intraspecific euploid series observed in the presently investigated species on the basis of world-wide cumulative data.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ploidy levels</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2x, 3x</td>
<td><em>Heracleum brunonis</em></td>
</tr>
<tr>
<td>2.</td>
<td>2x, 3x, 4x</td>
<td><em>Arenaria serphyllifolia</em>, <em>Melilotus alba</em>, <em>Momordica dioica</em>, <em>Potentilla sundaica</em>, <em>Ranunculus hirtellus</em>, <em>Saxifraga filicaulis</em></td>
</tr>
<tr>
<td>3.</td>
<td>2x, 3x, 4x, 5x, 6x, 11x</td>
<td><em>Astragalus hamosus</em></td>
</tr>
<tr>
<td>4.</td>
<td>2x, 3x, 4x, 6x</td>
<td><em>Anemone rivularis</em></td>
</tr>
<tr>
<td>5.</td>
<td>2x, 3x, 4x, 6x, 8x</td>
<td><em>Nasturtium officinale</em></td>
</tr>
<tr>
<td>6.</td>
<td>2x, 3x, 4x, 6x, 8x, 11x</td>
<td><em>Geranium lucidum</em>, <em>Hypericum perforatum</em>, <em>Lotus corniculatus</em>, <em>Trifolium pratense</em></td>
</tr>
<tr>
<td>7.</td>
<td>2x, 3x, 4x, 6x</td>
<td><em>Potentilla fulgens</em></td>
</tr>
<tr>
<td>8.</td>
<td>2x, 3x, 4x, 8x</td>
<td><em>Viola serpens</em></td>
</tr>
<tr>
<td>10.</td>
<td>2x, 4x</td>
<td><em>Agrimonia eupatoria</em></td>
</tr>
<tr>
<td>11.</td>
<td>2x, 4x, 5x, 6x</td>
<td><em>Caltha palustris</em></td>
</tr>
<tr>
<td>12.</td>
<td>2x, 4x, 5x, 6x, 7x, 8x, 9x</td>
<td><em>Thalictrum minus</em></td>
</tr>
<tr>
<td>13.</td>
<td>2x, 4x, 5x, 6x, 8x, 10x</td>
<td><em>Fragaria indica</em></td>
</tr>
<tr>
<td>14.</td>
<td>2x, 4x, 5x, 8x, 10x, 12x</td>
<td></td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Ploidy levels</td>
<td>Species</td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>15.</td>
<td>2x, 4x, 6x</td>
<td><em>Clematis montana, C. orientalis, Papaver dubium, Potentilla nepalensis, Thalictrum foetidum</em></td>
</tr>
<tr>
<td>16.</td>
<td>2x, 4x, 6x, 7x</td>
<td><em>Ranunculus laetus</em></td>
</tr>
<tr>
<td>17.</td>
<td>2x, 4x, 6x, 8x</td>
<td><em>Abutilon indicum</em></td>
</tr>
<tr>
<td>18.</td>
<td>2x, 4x, 7x, 8x</td>
<td><em>Ranunculus sceleratus</em></td>
</tr>
<tr>
<td>19.</td>
<td>2x, 4x, 8x</td>
<td><em>Cassia tora, Impatiens balsamina, Sibbaldia micropetala, Urena lobata</em></td>
</tr>
<tr>
<td>20.</td>
<td>2x, 4x, 8x, 12x</td>
<td>Viola betonicifolia</td>
</tr>
<tr>
<td>21.</td>
<td>2x, 4x, 16x</td>
<td>Argemone mexicana</td>
</tr>
<tr>
<td>22.</td>
<td>2x, 6x</td>
<td><em>Fumaria indica, Indigofera gerardia</em></td>
</tr>
<tr>
<td>23.</td>
<td>2x, 6x, 8x</td>
<td><em>Cotoneaster microphyllus</em></td>
</tr>
<tr>
<td>24.</td>
<td>2x, 6x, 8x, 9x, 12x</td>
<td><em>Potentilla atrosanguinea</em></td>
</tr>
<tr>
<td>25.</td>
<td>2x, 6x, 8x, 10x, 16x</td>
<td><em>Nigella sativa</em></td>
</tr>
<tr>
<td>26.</td>
<td>2x, 8x</td>
<td><em>Viola serpens</em></td>
</tr>
<tr>
<td>27.</td>
<td>3x, 4x, 8x</td>
<td><em>Ranunculus hyperboreus</em></td>
</tr>
<tr>
<td>28.</td>
<td>3x, 6x, 10x</td>
<td><em>Geum roylei</em></td>
</tr>
<tr>
<td>29.</td>
<td>4x, 6x</td>
<td><em>Mimosa pudica</em></td>
</tr>
<tr>
<td>30.</td>
<td>4x, 6x, 8x</td>
<td><em>Ranunculus muricatus</em></td>
</tr>
<tr>
<td>31.</td>
<td>4x, 6x, 8x, 10x, 11x, 12x, 13x, 14x</td>
<td><em>Potentilla argyrophylla</em></td>
</tr>
<tr>
<td>32.</td>
<td>4x, 8x</td>
<td><em>Cassia occidentalis</em></td>
</tr>
</tbody>
</table>

Fifteen species in bold show more than one type of euploid series, based on different basic numbers
5.5 Cytomorphological variation:
The phenotypic or morphological variations within the species make an important factor in the process of evolution. Fosberg (1942) recognized these morphovariants to be incipient species at various stages of the development of a new taxon. Geographic variation in plant morphology is a function of phenotypic changes in response to local environmental conditions, genetic variation and evolution among populations, and the biogeographic history of an individual species. Characteristics such as leaf shape are constrained genetically, yet they also can be affected greatly by the local environment in which they develop (Thompson 1991). Morphological variation and geographical separation among populations are also prerequisite to the formation of subspecies and species (Losos & Glor 2003). Phylogeographic analysis can be used to illuminate the interplay of climate, geographical history, and evolutionary dynamics in generating new taxa (Avise et al. 1987; Arbogast & Kenagy 2001). According to Ellison et al. (2004) variation in morphology reflects phenotypic responses to environmental conditions and evolutionary history of populations of species, and may indicate the local or regional changes in environmental conditions. Such genetic and morphological variations are more predominant among the individuals of different populations than among the members of a same population (Svensson 1983; Španiel et al. 2008).

During present study, 8 species exhibit intraspecific diversity in the form of different cytotypes accompanied by variations in some of the morphological features. These include *Agrimonia eupatoria* (2n=56), *Bupleurum lanceolatum* (2n=32), *Capsella bursa-pastoris* (2n=32), *Potentilla desertorum* (2n=28), *P. nepalensis* (2n=28) and *Silene conoidea* (2n=40). The morphological comparison of these cytotypes marked with diploid and polyploidy levels in these species revealed few significant variations as increase in size of stomata, stomatal index, and pollen size in polyploids compared to diploids besides plant height, number and size of leaf, number of flowers per plant or inflorescence, size of flower,
length of stipule. Comparatively, the polyploids show enhancement of certain characters. Such comparable results have been previously reported for many Angiosperms such as in *Capsella bursa-pastoris* (Svensson, 1983), *Cardamine pratensis* (Lihová *et al.* 2003), *Senecio jacobaea* (Hodálová *et al.* 2007), *Centaurea stoebe* (Španel *et al.* 2008) and *Ocimum basilicum* (Omidbaigi *et al.* 2010). The morphological variations may be attributed to the variation in chromosome number as has been reported earlier in many Angiosperms (de Oliveira *et al.* 2004, Nakagawa 2006, Španel *et al.* 2008, Cires *et al.* 2009). On the other hand diploid cytotypes of *Impatiens brachycentra* (2n=14, 16), *I. scabra* (2n=14, 16), also presented some variations in the form of plant height, leaf size, flower size, spur size, capsule size and flower colour, however, these variations are less significant. For 11 species, population based studies show the presence of more than one cytotype, but individuals carrying such variations do not show gross morphological changes, hence remain indistinct in the field. The examples are *Impatiens arguta* (2n=12, 14), *I. bicolor* (2n=14, 16), *I. glandulifera* (2n=12,14), *Oxalis corymbosa* (2n=14, 28), *Ranunculus laetus* (2n=28, 32), *Thalictrum foetidum* (2n=14, 16), *Potentilla fulgens* (2n=14, 28), *P. sundaica* (2n=14, 28), *Vicia hirsuta* (2n=12, 14), *V. tetrasperma* (2n=12,14) and *V. sativa* (2n=12, 14). On the other hand, distinct morphovariants with white and blue coloured flowers have been collected for *Anemone obtusiloba* with same chromosome number 2n=14. The morphovariants in the species showed the normal chromosome segregation and microsporogenesis. Earlier, same has been noticed in *Agrimonia eupatoria* and *Potentilla atrisanguinea* var. *argyrophylla* (Kumar 2011).

### 5.6 Meiotic Abnormalities:

The meiosis forms an integral part of the sexuality reproducing taxa and is one of the most sensitive stages in the life cycle of seed plants (Namuco & O’Toole 1986; Saini 1997; Porch & Jahn 2001; Romanova & Tret’yakova 2005; Fuzinatto *et al.* 2008). Any disturbance during the meiotic course results into reduced viability of gametes. Meiosis is a specialized cell
division involved in gametogenesis, which governs the transmission of genetic material throughout sexual life cycles are controlled by a large number of genes (Pagliarini 2000; Villeneuve & Hillers 2001). Mutation in any of these genes which govern micro- or megasporogenesis from pre-meiotic to post meiotic events, may result into serious irregularities, changed rhythm of cell cycle and ultimately leading to the genetically aberrant end products having adverse impact on fertility and overall reproductive efficiency of the species (Kumar et al., 2010; Singhal & Kumar 2010).

At present, majority of the plant species investigated follows normal meiotic course. However, in certain cases, meiosis reveals abnormalities in the pairing or segregation of chromosomes or during cytokinesis. Such disturbances may be environmental, physiological, genetical or cytological. It is well known that meiotic abnormalities affecting the chromosomes, are of great evolutionary significance (cf. Sharma 1976). Darlington (1973) emphasized that the changes brought through meiotic anomalies ultimately affect the genetic system and thus, produce isolation between the species. Therefore, detailed studies in certain taxa with irregular meiosis have been helpful in throwing light on their genetical make up and reproductive biology.

During the present study, a number of meiotic abnormalities have been recorded which include cytomixis, interbivalent connections, unoriented bivalents, chromatin stickiness, chromatin bridges, chromosomal laggards, multipolarity and abnormal microsporogenesis in 69 species. Consequently variable sized, apparently fertile pollen grains and considerable amount of sterile pollen grains are observed.

5.6.1 Cytomixis:
Though the movement of chromatin from one PMC to another was observed as early as in 1901 by Kornicke, yet the term ‘Cytomixis’ was coined for the first time by Gates (1911) in Oenthera. A perusal of the literature revels that cytomixis has been equally common in normal sexual plants, hybrid, and apomicts belonging to diverse families of Angiosperms.
Normally, PMCs are known to be involved in this phenomenon but cytomixis may also occur in epidermal cells (cf. Sapre 1978), tapetal cells (Tarkowska 1960), root tip cells (Bobak & Herich 1978), ovary cells (Koul 1990) and shoot apical cells (Guzicka & Wozny 2005). Presently, cytomixis among the PMCs has been noticed in 53 species. High frequency of cytomixis is noticed in 6 species as *Capsella bursa-pastoris*, *Clematis grata*, *C. montana*, *Geum roylei*, *Pueraria tuberosa* and *Ranunculus diffusus*. Comparatively low frequency of cytomixis is observed in 47 species as *Aconitum heterophyllum*, *Anemone obtusiloba*, *A. rivularis*, *A. vitifolia*, *Argemone mexicana*, *Berberis asiatica*, *B. vulgaris*, *Bupleurum lanceolatum*, *Chaerophyllum villosum*, *Clematis connata*, *C. orientalis*, *Coronopus didymus*, *Corchorus capsularis*, *Desmodium microphyllum*, *Filipendula vestita*, *Impatiens brachycentra*, *I. scabrida*, *Indigofera hamiltonii*, *Lotus corniculatus*, *Malva neglecta*, *Medicago polymorpha*, *Papaver dubium*, *Pimpinella achilleifolia*, *P. diversifolia*, *Potentilla atrosanguinea*, *P. desertorum*, *P. fulgens*, *P. nepalensis*, *P. sundraica*, *Prinsepia utilis*, *Ranunculus arvensis*, *R. hirtellus*, *R. hyperboreus*, *R. sceleratus*, *Rosa brunonii*, *R. indica*, *R. macrophylla*, *Silene conoidea*, *S. vulgaris*, *Sisymbrium irio*, *Stellaria media*, *S. monosperma*, *S. semivestita*, *Thalictrum foetidum*, *Trifolium alexandrinum*, *T. pratense* and *Triumfetta pilosa*. Out of 53 species, only 9 species, namely, *Geum roylei*, *Potentilla nepalensis*, *Ranunculus arvensis*, *R. diffusus*, *R. hirtellus*, *R. hyperboreus*, *R. sceleratus*, *Rosa macrophylla* and *Triumfetta pilosa* are polyploids. Previously, also many cytologists have noticed the presence of cytomixis to be most common in diploid species (Pagliarini & Pereira 1992; Malallah & Attia 2003; Kumar & Singhal 2011), however, Basavaiah & Murthy (1987) and Sheidai & Attaei (2005) noticed the cytomixis being more prevalent in polyploid species of genera *Stipa* and *Urochloa* of family Poaceae. One thing is certain about the species showing cytomixis, that pollen fertility is considerably reduced at the end of the meiosis. Cytomixis is a mechanism to explain the origin of aneuploid gametes and resultant some genetic
consequences (Sarvella 1958). The consequences of cytomixis are the formation of hypo-, hyperploids and enucleated PMCs, abnormal microspore tetrads and pollen sterility (Koul 1990; Malallah & Attia 2003; Sheidai et al. 2003; Sheidai & Fadael 2005; Singhal & Kumar 2008a, b; Singhal et al. 2009a, b, 2010).

During present study, majority of investigated species show cytomixis are observed to be coupled with various meiotic anomalies such as interbivalent connections, chromatin stickiness, chromosomal laggards and bridges. Finally these irregularities end up with the formation of heterogeneous sized pollen grains and significant levels of pollen sterility. Similar observations have been made earlier by various authors in many species (Mary & Suvarnalatha 1981; Lakshmi et al. 1989; Singhal et al. 2009a, b, 2010; Fadaie et al. 2010). Villeux (1985) noticed that production of unreduced gametes is of evolutionary significance so that it can lead to the production of plants with higher ploidy through polyploidization. According to Kim et al. (2009) large sized apparently fertile pollen grains which are certainly of ‘2n’ constitution may play a crucial role in the sexual polyploidization of species. The phenomenon of cytomixis is attributed to various reasons such as an artefact of fixation (Heslop-Harrison 1966), pathological changes (Bobak & Herich 1978), physiological control (Bell1964; Bahl & Tyagi 1988), chemicals and herbicides (Ajay & Sarbhoy 1987; Haroun 1995), pollution (Haroun et al. 2004), temperature (Narain 1976), stress factors and genetic control (Haroun et al. 2004), pressure differences (Tarkowska 1965) and dumped chromatin bridges during premeiotic anaphase (Mendes & Rijo 1951), etc. Even though environmental factors, especially cold stress, certainly influences the process but cytomixis seems to be a natural phenomenon under the genetic control in the presently investigated species, as has been proposed by many authors (Omara1976; Falistocco et al. 1995; Haroun1995; Bellucci et al. 2003; Malallah & Attia 2003; Haroun et al. 2004).
5.6.2 Interbivalent connections:
These are loose associations of heterochromatic regions between non-homologous chromosomes without any chiasmata formation. Alone this type of connection do not study but in combination with other meiotic abnormalities, it effects the meiosis as seen in *Capsella bursa-pastoris, Delphinum dendudatum, Ranunculus laetus* and *Thalictrum foetidum* in the present study. In the presently investigated species, interbivalent connections are observed in the PMCs at Diakinesis and M-I in *Capsella bursa-pastoris, Ranunculus laetus* and *Thalictrum foetidum* but in case of *Delphinum dendudatum* these connections are seen at Diakinesis stage only. These interbivalent connections are already known during meiosis in some plants (Viinikka & Nokkala 1981; Akpabio 1990; Falusi 2006). Majority of the workers noticed that with the advancement of meiosis, there is a reduction in the frequency of interbivalent connections and the connections disappear before the end of first metaphase stage. Viinikka & Nokkala (1981) told that the main function of interbivalent connections is to keep the bivalents in contact to each other before binding to the spindle fibers as they are formed by the fusion of heterochromatic regions of some of the chromosomes together early in the first meiotic division, probably resulting in the formation of chromatic knots, which lead to the connections. Chromatin stickiness and cytomixis are also supposed to be generally responsible for such connections (Thomas & Revell 1946, Habib & Chennaveeraiah 1976; Singhal & Gill 1985). Further, Thomas & Revell (1946) and Sanjappa & Bhat (1978) reported interbivalent connections in *Cicer arietinum* and *Alysicarpus*, respectively without cytomixis and chromatin stickiness but, these have been held responsible for occurrence of secondary associations. However, no secondary association has been noticed in the presently studied species.

5.6.3 Unoriented bivalents:
According to Nicklas & Ward (1994), unoriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers, hence one or two
bivalents lying outside equatorial plate at M-I, which may present as such in the
formation of laggards at later stages. These have been seen in many plant species
earlier (Carter 1978; Martin et al. 1997; Tepperberg et al. 1999; Higgins et al.
2004). Ordinarily, it is expected that bivalents should disjunct simultaneously at
the beginning of A-I when spindle apparatus is fully organised, but many a times,
non-synchronised (precocious and / or late) disjunction of bivalents takes place
in hybrids, unbalanced polyploids or taxa having different sized chromosomes.
The various reasons given for this abnormality are: (i) different rates of
terminalisation of the various chromosomes of the complement (Darlington
1937), (ii) changed homology of the chromosomes (Koul 1971), or (iii) absence
of co-ordination between chromosomes and spindle (Sharma 1976). Any
distortion or breakage in the spindle may result random sub-grouping of

Amongst the presently studied taxa, precocious disjunction of 1-2
bivalents is quite a common feature. According to Darlington (1937), a
bivalent with terminal chiasmata will disjunct precociously as compared to
the bivalents with interstitial chiasmata. The phenomenon is so widely
distributed in the plants that no cytological significance can be attached to it
except that sometimes it may lead to erroneous counting of the
chromosomes. At present, this phenomena is noticed in 20 species, namely,
Aconitum heterophyllum, Anemone obtusiloba, A. rivularis, A. vitifolia,
Bupleurum lanceolatum, Clematis connata, C. grata, C. orientalis, Impatiens
brachycentra, Oxalis corniculata, Papaver dubium, Pimpinella diversifolia,
Pueraria tuberosa, Ranunculus arvensis, R. hirtellus, R. hyperboreus, R.
scleratus, Saxifraga filicaulis, S. sibirica and Trimfetta pilosa.

5.6.4 Chromatin stickiness:
The phenomenon of chromatin stickiness was discovered by Beadle (1932)
in Zea mays, which it was recognized as an effect associated with a recessive
suggested that genetic as well as environmental factors are the main causes
of chromatin stickiness, but the biochemical basis of chromosome stickiness

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are still unknown. Other factors such as radiation treatments (Steffensen 1956), low temperature (Eriksson 1968), chemical agents (Caetano-Pereira et al. 1995; Bhat et al. 2007; Khan et al. 2009) and partial association of nucleoproteins (Kaufman 1956) have also been found to be responsible for this anomaly. In the presently studied species, however, it seems to be governed by genetic control and has been observed in 36 species. Out of these, 8 species are tetraploids (Barbarea intermedia, P. nepalensis, P. sundaica, Ranunculus arvensis, R. hirtellus, R. hyperboreus, R. sceleratus and Sesbania bispinosa) and rest are diploids (Aconitum heterophyllum, Anemone obtusiloba, A. rivularis, A. vitifolia, Argemone mexicana, Bupleurum lanceolatum, Clematis connata, C. orientalis, Coronopus didymus, Filipendula vestita, Impatiens brachycentra, I. scabrida, Medicago polymorpha, Papaver dubium, Pimpinella achilleifolia, P. diversifolia, Potentilla atrosanguinea, P. desertorum, P. fulgens, Silene conoidea, S. vulgaris, Stellaria media, S. monosperma, S. semivestita, Thalictrum foetidum, Nasturtium officinale, Saxifraga diversifolia and S. flagellaris). The chromatin stickiness is observed at different stages of meiosis but more frequent in the PMCs at M-I involving partial or often complete clumping of the bivalents. Most of these species also show cytomixis and other abnormalities and such observations have also been earlier made (Mary & Suvarnalatha 1981; Singhal & Kumar 2008a, b; Kumar et al. 2010). As the result of chromatin stickiness, delayed separation of bivalents/chromatids at A-I/II also sometimes adds to formation of laggards at anaphases/telophases. The chromatin stickiness also causes pollen grain sterility in some of the species (Golubovskaya 1989; Rao et al. 1990; Consolaro & Pagliarini 1996) and may hold good in some of the species with similar behaviour.

5.6.5 Chromatin Bridges:
For the first time, the bridge formation was detected by Greets in 1911 (cf. Haga 1946). Since then, this phenomenon has been widely observed in plants (cf. John & Lewis 1965). The origin of dicentric bridges through
heterozygosity was cytologically investigated by McClintook (1931, 1933) in Zea mays showing all the possible cytological manifestations of inversion bridges at A-I and A-II. According to Rothfels & Mason (1975) bridges may originate from chiasma formation in heterozygous inversions. When cell division occurs, a broken chromosome with two centromeres is pulled to the opposite poles of the cell, forming a long chromosome bridge called chromatid bridge (Zhang et al. 1997). Bridges and fragments are the results of spontaneous breakage and fusion of the chromosomes. The inversion crossing over hypothesis is a highly improbable phenomenon. In the presently studied plants, such bridges have not been seen at all. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness in different plant species (Nirmala & Rao 1996).

Early disjunction of bivalents normally does not affect the normal distribution of chromosomes at A-I, but late separation of bivalents which normally exists in hybrids and cytologically abnormal diploids causes some meiotic disturbances (chromatin bridges and laggards) and consequently pollen malformation (Kumar & Singhal 2008 Singhal & Kumar 2008a, b, 2010, Kumar et al. 2010). During present investigations, multiple bridges have been recorded in *Aconitum heterophyllum*, *Anemone rivularis*, *Clematis montana*, *C. orientalis* and *Ranunculus sceleratus*.

### 5.6.6 Chromosomal Laggards:

Bivalents and chromosomes that lag behind and are unable to reach at poles during anaphase-I, telophase-I, anaphase-II and telophase-II stages of meiosis, form laggards. In the present study, chromosomal laggards are noticed in 52 species. There are different explanations for the formation of chromosomal laggards such as interlocking of bivalents and paracentric inversions (Bhattacharjee 1953; Sinha & Godwar 1972; Tarar & Dyansagar 1980). One of the most acceptable reason for the formation of chromosomal laggards are lack of synapsis at early prophase stages or precocious separation and delayed terminalization of chismata (Pagliarini 1990; Kumar & Tripathi 2007). According to Gupta & Priyadarshan (1982) asynapsis,
General Discussion

desynapsis, failure of chiasma formation and premature disjunction of bivalents are the possible causes for the formation of chromosomal laggards. The existence of chromosomes as laggards has been found to be highly dependent on genotypes (Pagliarini et al. 2000; Fuzinatto et al. 2008). At present, most of these laggards failed to reach the poles and often constituted micronuclei during microsporogenesis. Pagliarini (1990) reported that laggards might have degenerated or may have resulted in the formation of polyads (Basi et al. 2006). A number of authors are of the opinion that formation of laggards and bridges are associated with the phenomenon of cytomixis (Mary & Suvarnalatha 1981; Lakshmi et al. 1989; Sheidai et al. 2008, 2009).

5.6.7 Multipolarity:
Sometimes the chromosomes are not segregated properly at poles during anaphases and telophases and this phenomenon is noticed in the presently worked out 10 species, namely, Clematis connata, C. grata, C. montana, C. orientalis, Filipendula vestita, Geum roylei, Ranunculus diffusus, R. hirtellus, R. hyperborus and R. leatus. All these species also show association with cytomixis in conformity with the observations made earlier by Lydia & Rao (1982), Sheidai et al. (2008, 2009) and Fadaie et al. (2010). Multipolar cells are observed at telophase-II. The main causes for the formation of multipolar cells are laggards and unoriented chromatin material that fail to get integrated at poles during anaphases to telophases. These often form unreduced pollen grains as well as responsible for pollen sterility. Similar type of observations are made earlier for Silene (Sheidai et al. 2008) and Arenaria gypsophiloides (Fadaie et al. 2010).

5.6.8 Abnormal microsporogenesis, pollen grains size variability and pollen grain sterility:
Anomalous microsporogenesis, pollen grains size variability and low pollen fertility is seen in all the meiotically abnormal plant species indicating these phenomena to be the integral part of the species showing abnormal meiosis. Formations of monads, dyads, triads or polyads with or without micronuclei
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are noticed in many species. Some of the species which show unoriented bivalents or laggards also show formation of micronuclei during microsporogenesis. Heterogeneous pollen grain formation and reduced pollen fertility are invariably seen at the end of meiosis in all these species (Table 1). Previously, similar observations have also been made in several angiospermic plants (Sheidai et al. 2008; Kumar et al. 2008a, b; Gupta et al. 2009). Pollen sterility is a common consequence of spindle anomaly (Mendes-Bonato et al. 2002) and also the formation of heterogeneous sized pollen grains (Sala et al. 1989; Singhal & Kaur, 2009). Recently, d'Erfurth et al. (2008) have isolated and characterized AtPS1 (Arabidopsis thaliana Parallel Spindle 1) gene involved in controlling the diploid (2n) gamete formation in Arabidopsis thaliana due to abnormal spindle orientation at male meiosis II.

5.7 Concluding remarks:

As a result of present cytological studies on 174 polypetalous plant species and after evaluating the chromosomal information in totality in the light of previous world-wide cytological literature following points of interest deserve to be mentioned:

(i) The results in the form of new/varied chromosome number reports of nearly 51% species on world and India level, respectively, have been made available to enrich the chromosome numbers database of India.

(ii) Presence of genetic diversity in many taxa stresses the need for cytological analysis on population basis from different geographical areas.

(iii) Intraspecific variability in many species (with complete information about field identification, distribution, localities etc.) has been brought to the fore, to be conserved and/or utilized for further plants improvement programme. Even some of the medicinally valuable variants can be subjected to cultivate for commercial cultivation.

(iv) The genetic diversity in medicinal plants points to the need of studies for chemical characterization and biological activity of these variants to mark out the better chemotypes for further conservation and exploitation.