CHAPTER 5

GENERAL DISCUSSION
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Meiosis deals with the most important aspect of cell cycle and is a key step of sexual reproduction. It includes two successive divisions of the nucleus, one the reductional division (meiosis-I) and other the equational division (meiosis-II), leading to the formation of 4 gametes each with half of the chromosomes of the mother cell during sexual reproduction. It is a dynamic process involving a number of complex molecular and cellular events, such as DNA and chromosome replication, chromosome pairing and genetic recombination, chromosome segregation and cytokinesis, and hence maintains genome stability and integrity over sexual life cycles, besides producing intraspecific genetic variations. It also generates genome variations such as deletions, duplications, and other rearrangements within the genic and non-genic genomic regions and has been considered a major driving force for gene and genome evolution in nature. Meiotic events have been found to be under genetic control and a number of genes have been found to steer these events in eukaryotes (Rabitsch et al. 2001; Hamant et al. 2006). Meiotic abnormalities lead to chromosomally imbalanced gametes e.g. diploid and aneuploid gamets which lead to polyploidization and genome expansion. Knowledge of various meiosis-driven genome variations provides insight into genome evolution and genetic variability in plants and facilitates plant genome research (Cai & Xu, 2007).

Presently, meiotic studies are undertaken in 134 species, on population basis, covering 80 genera and 17 families under subclass Gamopetalae from Kashmir Himalaya, India. The various observations reported during the male meiotic course by analyzing sufficient number of PMCs in each population are discussed here.

5.1. Chromosome number: Chromosomes were first observed in plants by Nageli in 1842 and the term ‘chromosome’ was coined by Waldeyer in 1888. Chromosomes are packaged/organized structures of DNA and proteins located in the nucleus of an organism. They carry the genetic information and transmit it to the offspring through sexual life cycle.

Each chromosome has a constriction point called the centromere, which divides the chromosome into two sections, or “arms.” During the cell cycle, chromosomes at interphase cannot be seen even through microscope. However, after DNA duplication, chromosome become condensed and tightly packed during cell
division and is then visible under a microscope. The condensed chromosomes are inaccessible for transcription, however, become ideal structures for cytological examinations. Specific number of chromosomes characteristic for a particular species implies its chromosome number which can be taken as a haploid number (n) as in gametes or a diploid number (2n) as in somatic cells.

In the present study, a total of 24 different chromosome numbers are noted in 134 species cytologically worked out. The diploid chromosome number (2n) recorded for each species is presented in Table 1. Seven species have been given the chromosome number for the first time whereas 7 species have been reported with new ploidy levels and 2 with aneuploid varied chromosome numbers. Also 15 species are recorded with chromosome numbers for the first time from India and 3 species have been reported with B-chromosomes for the first time. The overall chromosome number in the presently studied species varies from as low as 2n=10 (Crepis sancta, Draccephalum nutans and Picris hieracioides) to as high as 2n=72 (Atropa acuminata, Bidens biternata, B. pilosa & Solanum nigrum). These observed chromosome numbers are presently compared with their frequencies of occurrence in different species and presented in bar diagramme (Graph 2).

Graph 2. Different chromosome numbers observed in 134 species worked out presently

The Graph shows 2n=18 as the most common number occurring in 19 per cent or 26 species, followed by 2n=16 in 11.2 per cent. Whereas 2n=46, 52 and 64 are with the least frequency found in 0.74 per cent species.

Same comparison is made in three presently studied large families- Asteraceae (Graph 3), Lamiaceae (Graph 4) and Scrophulariaceae (Graph 5) revealing 2n=18 as
the most frequent number in Asteraceae and Lamiaceae whereas in Scrophulariaceae 2n=16 predominates.

**Graph 3. Presently observed chromosome numbers in 58 species of Asteraceae**

**Graph 4. Presently observed chromosome numbers in 26 species of Lamiaceae**

**Graph 5. Presently observed chromosome numbers in 19 species of Scrophulariaceae**
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5.2. Base number: The lowest haploid chromosome numbers for a genus are assumed to represent the nonpolyploidized state, termed the base chromosome number (x). In other words, it is simply the lowest known ancestral gametophytic chromosome number in a genus. In eukaryotic evolution chromosome number is considered as a remarkably dynamic feature. Chromosome numbers have repeatedly increased by doubling (polyploidy), increased by a single chromosome (ascending dysploidy) via chromosome fission and decreased by a single chromosome (descending dysploidy) via chromosome fusion.

Estimations regarding the determination of base numbers and the thresholds employed thereof vary (see: Otto & Whitton 2000). Stebbins (1938) considered a species polyploid if it has a haploid chromosome number, which is a multiple (or near multiple) of the lowest one found in the genus. In this way only recent polyploidization events may be covered but not the chromosome number changes arising due to dysploidy. In other somewhat recent methods called maximum parsimony principle (Hansen et al. 2006; Timme et al. 2007) a certain lineage is inferred to be polyploid if its chromosome number is larger by a chosen factor than the base chromosome number. Although this is widely used approach but has also limitations and is therefore substituted by using a more recent probabilistic approach (Mayrose et al. 2010). This method takes into account such changes in chromosome number over time that are the result of a combination of polyploidy and dysploidy events during phylogeny.

In the present study, base numbers of 80 genera (covering present 134 cytologically worked out species) are discussed. It is done through an exhaustive review of chromosome data, covering all the cytologically known species in a genus including the present results. As stated earlier, there may be ambiguity in assigning the base number for a genus, particularly where there is complexity in understanding of the chromosomal evolution. For example, x=14, is assigned to genus Anaphalis (c.f. Khatoon & Ali 1993) which is previously known to have x=7 as the primary base number (Darlington & Wylie 1955). In this genus, no species at present shows 2n=14. Similar confusions exist in Digitalis with x=7 as the primary base number and x=14 and x=28 as the derived ones through paleopolyploidy. In case of Senecio, x=5 is considered as the primary base number which gave rise to x=10 through polyploidy but some consider x=5 as the derived one from x=10 through phylogenetic reduction.
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With some reports of 2n=42 in *Anaphalis* species showing normal meiosis, x=7 is supported and x=14 is secondarily originated indicating paleopolyploid nature of the genus. The base number in the Campanulaceae has been suggested to be x = 8 (Contandriopoulos 1984), but Raven (1975) and Zhang *et al.* (2011) suggested x = 7 as the ancestral number. An ancestral base number of x = 7 is supported by counts for *Cyananthus* (Hong & Ma 1991) which is considered as the most primitive genus within the family (Cronquist 1988) based on its superior ovary. Chromosome breakage may result into x = 8 and x = 9, as in *Codonopsis*. The other possibility to form x=18 is autopolyploidization of x = 9, that formed paleopolyploids and then gradually modern secondary diploids or polyploids. Zhang *et al.* (2011) mentioned six possible ways for explaining origin of different base numbers, including hybridization, fission, polyploidization and chromosome loss. In case of *Gentiana*, Pringle (1990) took x=10 and x=13 as the prevailing base numbers, whereas Hong (1990) assigned x=7 as the original base number.

In the presently studied genera, base numbers are determined through a meticulous and exhaustive review of the chromosome number data and the genera are accordingly categorized into 5 headings- monobasic, doubtful dibasic, dibasic, doubtful polybasic and polybasic. For such categorization, some basic criteria are presently kept in mind, which include the availability of the chromosome numbers in the genus with due weightage to gametic number shared by maximum number of species and exhibiting polyploidy series. The dominating lowest chromosome number (in a polyploid series) is taken as the base number on the basis of published chromosome data of the genus after comparing it with the proposed base numbers by different workers. In some cases, if a chromosome number deviating from the base number/s (dominating numbers) is exclusively present or occurs in combination with the dominant chromosome number in very small percentage of species, it is ignored on the grounds of aneuploidy through cytomixis and/or wrong identification. Such chromosome numbers with a bit more increasing frequency of occurrence contribute to doubtful cases. Such derivations are assumptions based on available chromosome data and personally assumed criteria. Chromosomal evolution is very vast and derivation of original base numbers in many cases is not an easy task. Successful attempts for original base number derivation require wide knowledge and experience, besides large and accurate chromosomal data. The five categories of presently studied genera on the basis of basic chromosome numbers are as follows:
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5.2. Monobasic: These include genera with single base number omitting the variation of paleopolyploid cases. Achillea (x=9), Anaphalis (x=7), Anthemis (x=9), Atropa (x=12), Bellis (x=9), Chrysanthemum (x=9), Codonopsis (x=8), Cynoglossum (x=12), Datura (x=12), Dubyaeæ (x=8), Cichorium (x=9), Eclipta (x=11), Galinsoga (x=8), Hieracium (x=9), Lycopus (x=11), Mertensia (x=12), Myriactis (x=18), Onopordum (x=17), Origanum (x=15), Perilla (x=10), Picris (x=5), Polemonium (x=9), Sigesbeckia (x=15), Solidago (x=9) and Xanthium (x=18).

5.2.2. Doubtful dibasic: These include those monobasic genera with one other chromosome number represented exclusively/separately at least once and/or a few times in combination with the main chromosome number. The main base number is here written in bold e.g. Dipsacus (x=8, 9), Elsholtzia (x=8, 9), Euphrasia (x=10, 11), Lamium (x=8, 9), Mazus (x=10, 19), Prunella (x=14, 16) Taraxacum (x=8, 9) and Tragopogon (x=6, 7).

5.2.3. Dibasic: It includes genera with clearly two base numbers. Here the deviating chromosome numbers if present are with very less frequency and never represented independently in more than 1 species. These include Carpesium (x=9, 10), Cicerbita (x=8, 9), Erigeron (x=8, 9), Hyoscyamus (x=14, 17), Leonurus (x=9, 10), Prenanthes (x=8, 9) and Sambucus (x=18, 19).

5.2.4. Doubtful polybasic: Here the one or two base numbers are easily marked out. Doubtful numbers are more than of one type if single base number is clear. The rest criteria is the same as in doubtful dibasic. The main base numbers are written in bold. It includes Digitalis (x=12, 14, 15), Jaeschkea¹ (x=8, 9, 10, 11), Phlomis (x=6, 10, 11), Sonchus (x=7, 8, 9, 10), Valeriana (x=7, 8, 9, 11) and Verbena (x=5, 6, 7).

5.2.5. Polybasic: Here the genera with more than two base numbers exist. The dominant base numbers are written in bold. However, the other base numbers not bolded are represented by significant frequency of species. These include Androsace (x=9, 10, 19), Artemisia (x=8, 9, 17), Aster (x=5, 8, 9, 13), Bidens (x=10, 11, 12, 18), Calamintha (x=9, 10, 12), Campanula (x=7, 8, 9, 10, 12, 13, 15, 17), Carduus (x=8, 9, 10, 11, 13, 17), Carthamus (x=10, 11, 12), Centaurea (x=8, 9, 10, 11, 12, 13, 14, 15), Cirsium (x=10, 15, 16, 17), Convolvulus (x=9, 10, 11, 12, 15), Crepis (x=3, 4, 5, 6, 7, 8, 9, 11), Dracophyllum (x=5, 6, 7), Echinops (x=14, 15, 16), Galium (x=9, 10,

¹ Genus with few cytologically known species each having a different chromosome number
11, 12), Gentiana (x=6, 7, 8, 9, 10, 13), Lactuca (x=5, 8, 9, 17), Linaria (x=6, 7, 9), Marrubium (x=9?, 10, 14, 17), Mentha (x=9, 10, 12), Nepeta (x=8, 9, 17), Pedicularis (x=6, 7, 8), Plantago (x=4, 5, 6, 9), Salvia (x=6, 7, 8, 9, 10, 11, 13, 17, 19), Saussurea (x=12, 13, 14, 16, 17, 18), Scrophularia (x=9, 10, 11, 12, 13, 15, 29), Senecio (x=5, 9, 10, 11, 12, 23), Stachys (x=8, 9, 10, 12, 15, 17), Strobilanthes (x=10, 15, 16), Swertia (x=7, 9, 10, 12, 13), Thymus (x=12, 13, 14, 15, 29), Verbacum (x= 13, 14, 15, 16, 17, 18) and Veronica (x= 6, 7, 8, 9, 17, 20).

The following Graph (Graph 6) shows that out of the 80 presently covered genera, polybasic ones contribute the highest frequency (41.25%) with 33 genera followed by monobasic (32.5%) with 26 genera. The category of doubtful dibasic comprises 8 genera (10%) whereas it is 7 genera (8.75%) under dibasic category. The doubtful polybasic is with least number of genera i.e. 6 (7.5%).

Graph 6. Categorization of 80 genera on the basis of basic chromosome numbers and their respective frequencies under each category.

5.3. Polyploidy: Polyploidy is the process of genome doubling that gives rise to organisms with multiple sets of chromosomes. Polyploidy can arise either by multiplication of a basic set of chromosomes (autopolyploidy) or as a result of hybridization of genomes of two related species (allopolyploidy). It may occur due to abnormal somatic doubling in mitosis, or by fusion of unreduced gametes during meiosis.
This phenomenon is a major factor in evolution and speciation of plants. Research on polyploidization and speciation has been studied for a century. Polyploidy occurs in diverse eukaryotes including, animals, fungi and protozoa. However, it reaches its peak in plants, where 50–100 per cent of flowering plants are believed to have a polyploid ancestry (Cui et al. 2006; Soltis et al. 2009) and 20–40 per cent of existing flowering plant species thought to be neopolyploids (Stebbins 1971). The exact number of polyploid Angiosperm species differs from author to author e.g. 50 per cent (Darlington 1937), 30-35 per cent (Stebbins 1950) and 40-70 per cent (Masterson 1994). Whereas Lewis (1980) assigned 70-80 per cent of dicots as polyploids, many lineages show evidence of ancient polyploidy (paleopolyploidy) in their genomes (Meyers & Levin 2006). As stated earlier, there are differences in assigning the frequency of the polyploid species in Angiosperms which are created due to different criteria taken for considering the base number for a genus.

In the present study, 39 species (29%) came out to be polyploids with a majority of 28 species of tetraploids, 9 species of hexaploids and 2 species with octaploidy. The remaining 95 species are diploids. Many cytologists from India have estimated the frequency of polyploidy in various woody Angiosperms such as 24.06 per cent (Mehra 1972), 23 per cent (Bedi 1982) and 22.5 per cent (Singhal 1982). Similarly, Kumar (2010) reported 36.4 per cent polyploidy in Polypetalous members from Lahaul & Spiti cold deserts and Kaur (2012) reported 25 per cent in dicots from Kinnaur, H.P. Similar studies in India on family basis show that polyploidy exhibited by Compositae is 32.4 per cent (Gupta 1981), in Acanthaceae and Lamiaceae 13.53 per cent and 29.21 per cent, respectively (Saggoo 1983) and in Papilionaceae 23.24 per cent (Kumari 1984). Overall data of the presently worked out species shows that 50 species (37.3%) show intraspecific eupolyploidy. Presently, intraspecific polyploidy is reported in 3 species such as Calamintha vulgaris (2n=20, 40), Elsholtzia ciliata (2n=16, 32) and Mentha longifolia (2n=24, 48). While Elsholtzia ciliata is proposed to be a segmental allopolyploid on the basis of frequency of quadrivalents, the remaining two show allopolyploid behaviour. In these intraspecific polyploids morphological differences are significant and presented in results (see: Results & discussion). Tetraploid cytotype of Elsholtzia ciliata shows qualitative as well as quantitative morphological differences from its diploid partner, besides broader ecological habitats. There is a gigas effect in tetraploids of Calamintha vulgaris,
though both cytotypes occur sympatrically and inhabit same habitat. Although polyploids differ from their diploid ancestors in terms of distribution and habitat because of combined genome from two parents (Malik et al. 2012), this is not always true (c.f. Arrigo & Barker 2012). In Mentha longifolia the diploid and tetraploid cytotypes show morphological variation besides distributional differences with tetraploids being more common at higher altitudes. Polyploids often differ markedly from their progenitors in morphological, physiological, or life history characteristics (Levin 1983; Ramsey & Schemske 2002), including heterosis and these differences may contribute to the establishment and success of a polyploid species in novel ecological settings. It is thus hypothesized that polyploidy may serve as an important mechanism for ecological diversification, especially in harsh environments (see: Otto 2007).

As per te Beest et al. (2012) polyploids have greater role in facilitating plant invasions because of pre-adaptation and subsequent adaptation due to larger genetic diversity. It is proved in some species experimentally that polyploidy increases leaf size mainly by increasing the cell elongation rate, but not the duration of the period of elongation, and thus increases final cell size (Sugiyama 2005). Also the gigas effect which is generally seen in polyploids (Stebbins 1971; Levin 2002) need not be always directly related (Otto & Whitton 2000) and organ or plant size may remain unchanged or even decreases; different traits in different species experience phenotypic selection (Balao et al. 2011). According to Lavana et al. (2012), polyploidy is associated with increased concentration of secondary metabolites and the expected increase in body size is decided by the type of accumulation of secondary metabolites.

At present, out of 39 polyploid species reported, incidence of polyploidy in Asteraceae is 34.5 per cent (20 species) with 14 species being tetraploids and 5 species hexaploids (Graph 7) and 1 species octaploid. The remaining 38 species (65.5%) came out as diploid. In Lamiaceae, percentage of polyploidy is presently calculated to be 23 per cent (6 species). Family Scrophulariaceae reveals 42.1 per cent polyploidy, with 7 polyploid species being tetraploids and 1 being hexaploid.

Among the 4 species of Solanaceae, 2 are hexaploids and remaining 2 diploids. In Campanulaceae the only tetraploid race of Codonopsis rotundifolia is
presently reported for the first time. The newly found polyploid races in some species or first time from previously explored areas hints towards the active state of evolution in such cases.

Graph 7. Incidence of polyploidy in 3 major families

The different ploidy levels in the presently observed species are 2x, 4x, 6x and 8x. The number of species representing each ploidy level is shown Graphically (Graph 8) and the same is shown family wise in 3 major families at present viz. Asteraceae, Lamiaceae and Scrophulariaceae (Graph 9).

Graph 8. Ploidy levels in 134 species presently worked out
5.3.1. Meiosis in polyploids

Normal meiosis depends upon the interrelated events of homologous chromosomes recognition, intimate association, synapsis and recombination (de Muyt et al. 2009). For correct segregation of chromosomes at anaphase-I, the formation of stable bivalents at metaphase-I is required which in turn needs meiotic crossover of homologous chromosomes. In polyploids (particularly neopolyploids) quadrivalents or other multivalent are formed at diakinesis and metaphase-I in high frequency which hinders normal meiosis. However many of the nowadays polyploids do not show quadrivalent or multivalent formation suggesting their cytological diploidization (Cifuentes et al. 2010), the gradual process in which bivalent formation overcomes multivalents leading to chromosomally and genetically balanced gametes. The reason for gradual loss of homology over generations is thought to be due to cryptic chromosomal aberrations. Autopolyploids have more than two copies of each chromosome hence crossovers can be formed between more than two homologues, resulting in multivalents at diakinesis and metaphase-I and chromosome missegregation at anaphase I. The stability of multivalents is far less than that of bivalents leading to an increased ratio of mistakes and hence reduced pollen fertility. Presently, multivalent formation is seen in 6 polyploid species one being octaploid and remaining 5 tetraploids with majority from Asteraceae except *Elsholtzia ciliata* (2n=4x=32) which is a member of Lamiaceae. Among these, *Anaphalis nepalensis, Elsholtzia ciliata, Scrophularia decomposita* and *Senecio chrysanthemoides* reveal only quadrivalents, besides bivalents which may arise due to homologies between the chromosomes. However, *Chrysanthemum leucanthemum* and
Senecio graciliflorus also depict hexavalents, besides quadrivalents and bivalents suggesting chromosome homologies and/or structural heterozygosity in these species. The effect of multivalent formation in these polyploids appears to be insignificant in terms of causing pollen sterility in Scrophularia decomposita (79-97%) and Senecio graciliflorus (95-97%). However, it is reduced to below 80 per cent in others. Darlington (1965) attributed the sterility in the polyploids to the multivalent formation. Frequency of the multivalents is directly correlated with the pollen sterility (Gottschalk 1978). Keeping in view the low range of multivalent (1-2/ cell) and high pollen fertility these can be proposed here as allopolyploids (Stebbins 1950). In some allopolyploids, the homeologous chromosomes from parents may be almost similar to one another as are the homologous chromosomes and hence leading to quadrivalent.

Theoretical models predicted that quadrivalent formation is expected to reduce if there is, no pairing preference, synapsis initiation at both ends of chromosomes and pronounced distal chiasmata location (see: Gillies 1989).

Presently, in Chrysanthemum leucantherum, Elsholtzia ciliata and Senecio chrysanthemoides pollen fertility is significantly reduced to 66 per cent, 40 per cent and 75 per cent, respectively. In this way and also keeping in view the range and frequency of multivalents, they are considered as segmental allopolyploids. Rest of the polyploid species (33 species) which contribute the majority of the polyploidy in the present study (84%) show no multivalent formation and behave like diploids. This normal diploid-like meiotic behaviour of polyploids is thought to result from the divergence between homeologous chromosomes (in allopolyploids), which may already exist and/or be accentuated at the onset of polyploid formation (Le Comber et al., 2010) and involve the rearrangement of large chromosome fragments, or from the activity of Pairing homeologous (Ph) genes (see: Jenczewski & Alix, 2004).

Since in neopolyploid populations fertility selection usually results in increased bivalent formation (Ramsey & Schemske 2002), the genesis or selection of Ph-like genes is a logical process to consider (Cifuentes et al. 2010).

Among the 6 polyploid species of Lamiaceae under present investigation, multivalents have been observed in only one species Elsholtzia ciliata. Thoppil (1993) also could not find multiple associations in most of the Labiates in his study on South
Indian Lamiaceae. Hence small chromosome size is one of the reasons for absence of multivalents.

An important factor in polyploids is gene redundancy. The polyploids have more than twice as many copies of any particular gene which shields the individual from the deleterious effects of recessive mutations. It also helps diversify gene function over time. In other words, extra copies of genes not required for normal function may get used in new and entirely different ways, leading to evolutionary selection (Adams & Wendel 2005).

There are selective advantages in sexuality through polyploidy e.g. by breaking certain self-incompatibility systems, thereby allowing self-fertilization. This might be the result of the interactions between parental genomes in allopolyploids (Comai 2005). Onset of asexual reproduction, which is associated with polyploidy in both plants and animals is another switch. This switch in reproductive strategies may improve fitness in static environments.

Although almost all Angiosperms have experienced at least one round of polyploidy during their evolution, much of the original genetic redundancy was erased by a massive removal of some but not all duplicated gene copies (Doyle et al., 2008). This process of ‘genetic diploidization’ is not random, and duplicated gene copies of some functional categories have been shown to be preferentially retained or lost (Doyle et al., 2008).

5.3.2. Polyploidy and habitat correlation: generally the ecological amplitude of polyploids is thought to be broader than that of their ancestors, as they combine features of the parental genomes (Brochmann et al. 2004; McIntyre 2012). The intraspecific polyploids of Elsholtzia ciliata seem to show such variation as tetraploids are found growing on both sloped and plane surfaces, unlike diploids which flourished only on slopes where moisture cannot accumulate. Polyploids usually have different geographical ranges than their diploid progenitors (Lewis, 1980). Various ecological factors also have a bearing on the distribution of polyploids e.g. polyploids were found to be more frequently distributed in wet soils and meadows as opposed to more stable habitats with drier soils or forest communities, respectively (Grant 1981). This helps explain the habitat niche difference between the two cytomorphotypes.
5.3.3. Polyploidy and habit correlation: It is a known fact that the conditions for establishment of polyploidy are more favoured in perennials than annuals/biennials, because the time available for union of unreduced gametes is longer in perennials. Further, the unbalanced polyploids have more chances of establishment and stabilization in perennials than in annuals or biennials. Otto and Whitton (2000) observed a positive relation between polyploidy and herbaceous dicots. However, Vamosi and Dickinson (2006) in their study concluded that there is no evidence of correlation between polyploidy and herbaceous growth habit. Polyploids are more in perennials as compared to annuals (Stebbins 1971). There is frequently observed a shift in polyploids from annual to perennial habit (Sano, 1980), which might be necessary due to the slower metabolism caused by an increased cell size, with the advantage of greater longevity (Garbutt & Bazzaz 1983).

Out of 134 presently studied species, 76 species (56.7%) are perennial herbs, 42 species (31.3%) are annual herbs, 11 species (8.2%) are biennials and 5 species (3.73%) are shrubs/undershrubs. Total number of polyploid species as stated earlier is 38 (28.35%).

It is estimated presently that incidence of frequency of polyploidy is maximum (31.6%) in perennial herbs with 24 polyploid species, followed by annual herbs (28.6%) with 12 polyploid species and biennials (27.3%) with 3 polyploid species (Graph 10). All the 5 shrubs/under-shrubs are diploids.

![Graph 10. Overall incidence of polyploidy frequency in different life forms](image)

In family Asteraceae the incidence of polyploidy in perennial herbs is 39.3 per cent (11 species), followed by 33 per cent in annual herbs and 28.6 per cent (2
species) in biennials (Graph 11). In Lamiaceae among a total of 5 presently studied annual species 2 (40%) are polyploid. Here the incidence of polyploidy within perennials is 21 per cent with 4 polyploid species out of total 19 perennials (Graph 12). In case of Scrophulariaceae same results are also obeyed (Graph 13) with annuals showing highest incidence of polyploidy (75%) with 3 polyploid species out of 4, followed by biennials (50%) with 1 species out of 2 and is least in perennials (30.8%) with 4 out of 13. Similarly, positive correlation of polyploidy with annual habit has also been found by Otto and Whitton (2000). However less number of polyploids presently reported and not much significant difference in overall perennial polyploids from annual polyploids prevent to make a solid generalization.

Graph 11. Relation between incidence of polyploidy and life forms in Asteraceae

Graph 12. Relation between incidence of polyploidy and life forms in Lamiaceae
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Graph 13. Relation between incidence of polyploidy and life forms in Scrophulariaceae

5.3.4. Correlation between habit and altitudinal range: It is generally seen that there exists a relation between altitude and habit of plants, with perennials dominating the higher altitudinal ranges and annuals being more frequent at lower altitudes. In temperate range (1650-2500 m) frequency of perennial herbs and annuals is 41.6 per cent for each. In subalpine range (2600-3200 m) these values are 66 per cent and 21 per cent, respectively. In alpine range (above 3200 m) the frequency of perennial herbs is highest (74%) and that of annuals is lowest (26%) when compared to temperate and subalpine ranges (Graph 14).

Graph 14. Distribution of different life forms with respect to altitudinal range
5.3.5. Correlation between polyploidy and altitudinal range: The polyploidy is generally thought to increase with increase in altitude and latitude (de Wet 1980; Brochmann et al. 2004) and the phenomenon of polyploidization arises more often in species inhabiting high altitude cold climates (Brochmann et al. 2004). This is mainly because most of the perennials inhabit high altitudes and also meiotic abnormalities due to cold harsh climate create unreduced gametes. The ratio of polyploids:diploids has been generally found to increase with latitude and altitude although not always true with respect to later (Hieter & Griffiths 1999). However, this is not always necessary and some studies show even inverse relation (Husband & Schemske 1998; Schonswetter et al. 2007).

In the present study percentage of polyploidy is higher (31%) in subalpine/alpine plants with 23 species being polyploid out of total 74 species collected from this zone (Graph 15, Table 22). On the other hand the polyploidy percentage is lower (26.6%) with 16 species being polyploid out of total 60 species collected from temperate zone. Proportionally more polyploids at high altitude cold regions are due to their higher ecological tolerances, higher growth and adaptation rates than their diploids (Vamosi & Dickinson 2006).

However, in northern latitudes the increased cold tolerance is not necessarily responsible for high incidence of polyploidy (Soltis et al. 2004), but rather a confounding effect with the dominance of perennial life forms in these regions.

Presently, Mentha longifolia collected from temperate altitude (1900 m) came out to be diploid (2n=2x=24), whereas its accession from higher altitude (3000 m) of subalpine range is tetraploid (2n=2x=48). However the intraspecific cytotypes found in Calamintha vulgaris and Elsholtzia ciliata show no clear cut demarcation in altitudinal distribution pattern.

Table 22: Relationship between polyploidy and altitude in the presently studied species.

<table>
<thead>
<tr>
<th>Altitudinal zone</th>
<th>Total species collected</th>
<th>Different Ploidy levels (number of species)</th>
<th>Total number of polyploids</th>
<th>percentage of polyploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperate</td>
<td>60</td>
<td>2x (44), 4x (10), 6x (6)</td>
<td>16</td>
<td>26.6</td>
</tr>
<tr>
<td>Subalpine/alpine</td>
<td>74</td>
<td>2x (51), 4x (18), 6x (3), 8x (2)</td>
<td>23</td>
<td>31</td>
</tr>
</tbody>
</table>
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Graph 15: Distribution of polyploids with respect to altitudinal range

5.4. Aneuploidy: The numerical change not involving the whole set of chromosomes (as in eupolyploidy) results into individuals with loss or gain of one or a few chromosomes or even chromosome parts. Irregularities in chromosome segregation due to gene mutation or other factors may lead to aneuploidy (Cohen 2002). Laggard formation, asynapsis or desynapsis, spindle abnormalities, chromatin stickiness and bridge formations can lead to loss or gain of chromosomes in the daughter cells and hence aneuploids. As per Sharma (1990) chemicals like that used in agriculture are responsible for causing aneuploidy in many ways such as affecting spindle mechanisms or chiasma frequency. His study shows 45 plant species exhibiting aneuploidy without any apparent cause or source of stress. In such cases aneuploidy could arise due to nutritional status of the soil, irrigation by polluted water, the ageing of seeds, parthogenesis of plants, cryptotoxins in plants, pesticide applications and pesticide residues. Environmental factors such as pH and temperature may further aggravate the problems.

Trisomics are the best documented aneuploids (Khush 1973) and different types of trisomics may show different phenotypes as in case of Datura (Blakeslee 1921). Aneuploid generations come into origin when diploids form polyploids through triploid bridges (Henry et al. 2005).

Presently, aneuploid report for Swertia ciliata (2n=26) is documented for the first time. Although Swertia is a polybasic genus, the aneuploidy here is speculated on the basis of its previous chromosome report of 2n= 24 from India. Meiotic course in
Swertia ciliata is variously abnormal with unoriented bivalents at metaphase-I, early disjunction of bivalents, chromatin bridges and laggards leading to reduced pollen fertility (77.4%). Aneuploidy sternly affects on phenotype and viability probably because of unbalanced dosage of many genes (Birchler et al. 2001) which may cause disruption in vital cellular processes (Talukdar 2011). However in some cases the effects may be alleviated by the epigenetic silencing of the unpaired chromosomes (Bean et al. 2004). Although poor growth has been generally considered as an obvious outcome of trisomy due to meiotic instability, reverse phenomenon like vigorous growth is not uncommon in trisomic population (Khush et al. 1984; Talukdar & Biswas 2007).

5.5. **Meiotic abnormalities:** Normal meiosis shows balanced behavior of chromosomes at various stages and leads to 4 healthy haploid (n) gametes. Further, recombination during the early stages of meiosis allows the exchange of genetic information, serving as an important source of genetic diversity. The success of meiosis depends mainly on a complex and prolonged prophase I that involves homologous chromosome pairing, synopsis and recombination (Page & Hawley 2003; Schwarzacher 2003) which in turn is controlled by different genes in different organisms (Li et al. 2005).

During the meiotic course different types of meiotic irregularities have been presently observed which include multivalent formation (mainly quadrivalents), univalents, interbivalent matrical connections/threads, bivalent associations, cytomixis, chromatin stickiness, nonsynchronous disjunction of bivalents at metaphase-I, synocyte formation, chromatin bridges, chromosomal laggards, unoriented bivalents, chromosome fragments and multipolar cells. Besides, in Centaurea iberica synaptic mutation results into univalents at diakinesis. In Hieracium vulgatum (2n=54) non-synchronous meiosis is reported in which some chromosomes show leading stage whereas the others lag behind at other pole in the form of diffuse chromatin mass of early prophase. To my knowledge this type of irregularity in meiosis is here reported for the first time. A total of 68 species (50.7%) show meiotic irregularities of one or more types (Graph 16). Among these are included highly abnormal species and also those with just chromatin stickiness or little frequency of cytomixis. Such anomalies usually lead to abnormal microsporogenesis like monads, dyads, triads and polyads which are seen with and/or
without micronuclei thereby reducing the pollen fertility in many cases. The most common meiotic irregularity observed in these Kashmir Himalayan species appears to be chromatin transfer among proximate PMCs i.e. cytomixis (25 species), followed by chromatin stickiness (21 species) which is thought to have interrelation with cytomixis (Graph 16). All these meiotic abnormalities are taken one by one as under.

**5.5.1. Structural heterozygosity and multivalents:** The occurrence of quadrivalents or hexavalents in diploids and hexavalents in tetraploids is an indication of structural heterozygosity (translocation). In case of polyploids multivalent formation can be explained by complete or partial homologies. Reciprocal translocations are the most frequent types of mutations in different crop plants (Sadanaga & Newhouse 1982; Singh 2003) and these are considered as an important source of intraspecific chromosomal structural polymorphism (Candela *et al.* 1979; Talukdar 2009) e.g. by forming new linkage groups. Previously such chromosomal behavior has been seen to occur naturally in a number of Himalayan species viz. *Chrysanthemum* spp. (Mehra & Remanandan 1974; Gill & Gupta 1981), *Artemisia parviflora* (Gupta *et al.* 2010), *Euphorbia pilosa* (Saggoo & Farooq 2011) and many others.

*Oenothera* is a classical example for hybrid variegation (Kirk & Tilney-Bassett 1978) and partial or complete permanent translocation heterozygosity in plants (Levin 2002; Golczyk *et al.* 2005). Belling (1927) suggested reciprocal translocation between non-homologous chromosomes conducive for ring formation in
Datura and in this way helped the geneticists to interpret the same for Oenothera. Therefore, it was said that for each chromosome, the two ends are homologous with the ends of different chromosomes. At least every alternate chromosome in the ring and in some cases other chromosomes also must have resulted from interchanges between non-homologous chromosomes (Burnham 1962). When the chromosomes in the complex translocation heterozygote undergo synapsis, they lead to ring chromosomes. Translocation heterozygosity can be ascertained by presence of semi-sterility and low seed set and can then be confirmed at meiosis by quadrivalent formation.

Multivalents/quadrivalents of open chain or ring type in the present study are observed in 10 species (7 from Asteraceae alone) out of which 5 species are tetraploids and the remaining 5 are diploids.

Among diploids Dipsacus mitis and Mertensia echioides show presence of 1 quadrivalent whereas Artemisia absinthium and A. gmelinii depict 1-2 quadrivalents during the meiosis. In all these cases pollen fertility is above 70 per cent and therefore the reasons for the quadrivalent formation appears to be structural heterozygosity with alternate disjunction.

In tetraploids, quadrivalents are presently reported in Anaphalis contorta (1 IV), Chrysanthemum leucanthemum (1-3 IV), Senecio chrysanthemoides (1 IV), Senecio graciliflorus and Scrophularia decomposita (1-2 IV each). In octaploid species Anaphalis nepalensis (1 IV) is reported. Heterozygosity is common in Chrysanthemum spp. and is supposed to be a cause of homologies or reciprocal translocation (Gill & Gupta 1981) and applies to other tetraploids too. Among tetraploids, Chrysanthemum leucanthemum and Senecio graciliflorus also depict hexavalent formation pointing to structural heterozygosity in these species. Presently highest frequency of chromosomes involved in multivalent formation is recorded in Chrysanthemum leucanthemum (19.3%). However, on the basis of their observations on a large number of artificially produced autotetraploids covering many genera and families Morrison and Rajathy (1960) estimated that about 66 per cent chromosomes are involved in multivalent formation. Preferential pairing, chromosome size, selection of meiotic fertility, limitation of chiasma formation, etc. have been usually held responsible for the predominance of bivalent formation (see: Venkateshwarlu &
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Rao 1976). It is also believed that heterozygosity for interchanges may interfere with multivalent formation.

5.5.2. Secondary bivalent associations/ interbivalent connections: The secondary associations where the individuality of bivalents is not affected as the chiasma formation between the associated bivalents doesn’t take place, is presently reported in *Centaurea iberica, Elsholtzia ciliata, Pedicularis pectinata* and *Senecio chrysanthemoides* showing the association of 2-5 bivalents, 2-4 and 2 bivalents, respectively. As per Darlington (1928) these are residual attractions between non-homologous chromosomes. Secondary associations may reflect partial or complete homology between the chromosomes and suggest polyploid origin of the species. The presence of high range of quadrivalents due to associations in 4x *Elsholtzia ciliata* with abnormal meiotic course suggests it to be a neopolyploid. Previously, the phenomenon has been reported in a number of Angiosperms viz. *Cosmos bipinnatus, Lactuca macrorhiza* (Remandan & Mehra 1974), *Physalis peruviana* (Bala & Gupta 2011), to mention a few. Gupta et al. (2010) reported 5 associated groups in *Senecio nudicaulis* as a result of secondary bivalent associations hinting towards x=5 as the original base number. Such associations are more common in polyploids as compared to diploids (Mehra 1982) and may help in presuming the base numbers in plants (Mathew & Philip 1979; Mukherjee & Datta 2006).

In other cases, like various species of Cichorieae (particularly *Lactuca* and allied genera) chromatin thread between the bivalents at metaphase-I and/or diakinesis appears as a usual phenomenon. This has been presently observed in *Cicerbita lessertiana, Lactuca decipiens* and *Prenanthes brunoniana*. Such type of chromatin/matrical strands between the bivalents has been commonly observed both in plant and animal meiosis (Klasterska & Natarajan 1974; Klasterska et al. 1977; Bressa et al. 2002). Presently these chromatin/matrical threads are between the bivalents far apart and hence term like ‘secondary association’ seems not to be applicable. Repetitive DNA has been attributed to such presence of connections between the bivalents (Yunus & Yasmineh 1971).

5.5.3. Synaptic mutation: It includes asynapsis that is failure of pairing of homologous chromosomes which may be due to genetic or environmental factors (Cai & Xu 2007) and desynapsis wherein the pairing is normal but it is not retained until
anaphase-I, leading to premature separation. Asynaptic and desynaptic mutants have been documented in a number of plant species (see: Koduru & Rao 1981) including maize, rice, wheat, soybean and Arabidopsis thaliana. This process is presently met in Centaurea iberica where 8-20 univalents/PMC are observed at diakinesis/metaphase-I with a frequency of 33.3 per cent PMCs. The further microsporogenesis is abnormal with dyads and triads, besides normal tetrads. Asynapsis or desynapsis results into univalent formation. In this abnormal behavior most of the bivalents appear as univalents at diakinesis and/or metaphase-I which are either lost or get randomly transmitted to daughter cells resulting in gametes with more or less than normal chromosomes and hence eventually leading to aneuploids. Univalents may also undergo misdivision resulting in to acrocentric, telocentric or accentric chromosomes or isochromosomes (Friebe et al. 2005). The synapsis is under genetic control and such genes in Arabidopsis, Brassica and Rice have already been isolated through analysis of mutants that display decreased fertility (see: Chang et al. 2009). Maity and Datta (2009) suggested monogenic inheritance of desynapsis in synaptic mutant Corchorus fascicularis with no significant reduction in pollen fertility from normal plants.

5.5.4. B-chromosomes: These are dispensable or accessory chromosomes and were first discovered in plants by Longley (1927). About 23 per cent of Dicots are known to have B-chromosomes (Jones & Rees 1982). Alone in Compositae there are 170 species with B-chromosomes (Gupta 1981).

Presently, 3 species Campanula cashmeriana (2n=28+0-1B), Dipsacus mitis (2n=18+ 0-1B) and Verbascum thapsus (2n=34 +0-2B) are reported for the first time with B-chromosomes. The presence of the B-chromosomes is confirmed with their number varying from PMC to PMC, besides showing typical characteristics enough to consider them as Bs (Camacho et al. 2000). While the meiotic course in Campanula cashmeriana, and Dipsacus mitis is abnormal, it is normal in Verbascum thapsus.

The main characteristics of the B-chromosomes include, they are present besides normal diploid content of the plant, are darkly stained, short in length, morphologically indistinguishable from As, usually moving apart from As and drifting in nucleus as univalents and follow non Mendelian pattern of inheritance (Cheng et al. 2000; Kaniki 2009). Their origin is believed to be from autosomes
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(Jones & Rees 1982) and various mechanisms of their formation have been already proposed (Cabrero et al. 2003; Medeiros et al. 2006). B-chromosomes have been considered to be genetically inert but some studies reveal their role in pollen sterility and increased chiasma frequency (Sheidai et al. 2002; Kumar & Naseem 2012). Mani and Thoppil (2005) concluded the effect of B-chromosomes on the essential oil biosynthesis and chemical characterization in a cytotype of *Salvia coccinea* from Western Ghats, India. Presently the population of *Verbascum thapsus* with B-chromosomes shows decreased pollen fertility than the population without Bs.

5.5.5. Cytomixis: Chromatin transfer from one PMC to neighbouring PMC/s through cytoplasmic channels or by direct fusion has been previously found in a number of Angiosperms and results into hypoploid or hyperploids cells or even syncytes and hence unreduced gametes.

This phenomenon was first recorded by Kornicke (1901) in *Crocus sativus*. Such a phenomenon has a profound impact on the meiotic process, meiotic end-products, and the overall reproductive potential of the species. The literature suggests many factors responsible for cytomixis, including temperature (Narain 1976), stress factors coupled with genetic control (Ghanima & Talaat 2003) and direct genetic control (Bellucci et al. 2003; Haroun et al. 2004). It has also been attributed to fortuitous causes such as sublethal artifacts produced by fixation, mechanical injury or pathological anomalies (Takats 1959; Gottschalk 1970). The most plausible explanation for cytomixis is the incomplete wall formation during pre-meiotic mitosis and/or the retaining of the wide plasmodesmata between the pollen mother cells.

Presently, cytomixis is observed in 25 species (18.65%) of the total species studied constituting 36.7 per cent of the meiotically abnormal species. The phenomenon takes place at early prophase-I in 20 (80%) species and at metaphase-I, anaphase-I/II or telophase-I/II in 4 (16%) species. However, *Mertensia echioides* shows it from P-I to metaphase-I of meiosis.

Majority of these species are diploids (76%) which include *Artemisia absinthium, Campanula cashmeriana, Dracocephalum nutans, Erigeron alpinus, Gentiana carinata, Mertensia echioides, Origanum vulgare, Plantago major, Solidago canadensis, S. virga-aurea, Stachys floccosa, S. sericea and Myriactis nepalensis* all showing other meiotic abnormality/s too. The remaining diploid species
undergoing cytomixis are Calamintha umbrosa, Crepis sancta, Erigeron alpinus, Lycopus europaeus, Plantago depressa and Salvia moorcroftiana all showing no other meiotic irregularity. Tetraploid species showing cytomixis constitute 24 per cent and include Bidens biternata, Calamintha vulgaris and Perilla frutescens with otherwise normal meiosis and Codonopsis rotundifolia, Elsholtzia ciliata and Senecio chrysanthemoides showing other meiotic abnormality/s in addition to cytomixis. Hence, there are 16 (64%) species mainly diploids showing other meiotic abnormalities in addition to cytomixis. The maximum frequency of PMCs undergoing cytomixis is found in Dracocephalum nutans (40%) followed by Erigeron alpinus (38.4%) and is least in Bidens biternata (4.7%) ignoring the differences at population level (e.g. Artemisia absinthium P-2 shows only 2.9%). Interestingly in 14 of these species (58.3%) pollen fertility is reduced to less than 80 per cent though only 8 species (33.3%) reveal abnormal microsporogenesis. In most of these species, pollen size shows heterogeneity that can be explained to be due to extra or less chromosomes in gametes. According to Levan (1941), Zheng et al. (1987), Ghanima and Talaat (2003), and Kim et al. (2009), cytomixis plays a major role in chromosomal diversity and speciation of taxa through creation of hypoploid and/or hyperploids cells and hence gametes. Hypoploid and hyperploids PMCs arising due to cytomixis have been presently observed in Mertensia echiioides, Plantago major and Stachys sericea.

5.5.6. Syncyte formation: As defined by Levan (1941), syncyte formation involves the fusion of two or more pollen mother cells (PMCs, or nuclei), usually in early prophase of the first meiotic division, therefore, giving rise to 2n gametes after meiosis. As per Golubovskaya (1989), syncyte formation in maize is caused by mutations in pam genes. According to some previous studies, cell fusion may be caused by environmental and genetic factors (Nirmala & Rao 1996). Syncyte formation occurs through mechanisms like disorders in cytokinesis during the premeiotic mitosis, abnormal spindle, failure of first or second meiotic division, or direct cell fusion. Recently, Singhal et al. (2011) reported syncyte formation in Lindelofia longiflora from H.P, India. Presently it is reported in Mertensia echiioides (2n=24) in which the PMCs with double chromosome number (2n=48) are observed leading to fertile 2n pollen grains of almost double the size of normal n pollen grains. Besides, multivalent formation, chromatin stickiness and cytomixis are the other irregularities observed in this species. Syncyte formation in the presently investigated
species might act as a hint for probable presence of polyploid populations/races and thus demands comprehensive cytological studies in the genus or family in general and species in particular on population basis in order to explore the expected intraspecific cytogenetic variability and to understand comprehensively the causes and consequences of meiotic/chromosomal abnormalities.

5.5.7. Chromatin stickiness: It is characterized by intense clustering of chromosomes during any phase of the cell cycle (Rao et al. 1990). Beadle (1932) reported chromosome stickiness in maize for the first time and attributed the irregularity to a recessive mutant gene called sticky (st). As per Evans (1962) it occurs due to partial dissociation of nucleoproteins and changes in the chromosomal organization. The phenomenon has been reported in different Brachiaria species (Mendes-Bonato et al. 2001; Pagliarini et al. 2008; Risso-Pascotto et al. 2009) with suggestions that chromosome stickiness may be under genetic control or it might also be caused by environmental factors such as X-rays, temperature and soil elements. The changes in the surface properties of chromosomes, due to mutations affecting histones that under normal conditions are important in chromatid separation and segregation, causes chromosomes to adhere to each other on coming in contact (Ritambhara & Kumar 2010).

Clumping of bivalents or chromosomes results in the loss of individuality of these structures and is presently seen in 20 species (14.9%) of the total species studied, constituting 29.4 per cent of meiotically abnormal species. In almost all species, it is seen at metaphase-I with the exception of Lamium album where it is observed at diakinesis and Swertia cordata where it is seen at metaphase-II, besides metaphase-I. The other species showing this phenomenon exclusively at metaphase-I include Anaphalis nepalensis, Cirsium wallichii, Codonopsis ovata, Digitalis lanata, Dipsacus inermis, Dracocephalum nutans, Elsholtzia ciliata, Euphrasia paucifolia, Gentiana carinata, Mertensia echioides, Myriactis wallichii, Nepeta cataria, N. nervosa, Origanum vulgare, Phlomis bracteosa, Plantago depressa, Stachys sericea and S. floccosa. These species mostly depict pyknosis in the chromatin material due to adhesion of chromosomes. The maximum frequency of PMCs showing chromatin stickiness is presently observed in Swertia cordata (42-52%) followed by Elsholtzia ciliata (42%), Dracocephalum nutans (40%) and is least in Codonopsis ovata (3-
4.2%). In many of these species pollen viability is reduced (see: Table 1) as has been seen previously in some species (de Souza & Pagliarini 1996; Kumar & Singhal 2011).

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5.5. Non-synchronous disjunction/separation of bivalents: Some bivalents can either separate earlier (early disjunction) or later (late disjunction) before entering anaphase-I stage. This non-synchronous separation may lead to gametes with extra or deficient number of chromosomes. In the present study such behavior of bivalents is depicted by 17 species (12.6%) constituting 25 per cent of meiotically abnormal ones. These include *Anaphalis nepalensis*, *Euphrasia officinalis*, *Leonurus cardiaca*, *Nepeta erecta*, *N. nervosa*, *Onopordum acanthium*, *Plantago lanceolata*, *P. major*, *Polemonium caeruleum*, *Salvia moorcroftiana*, *Senecio chrysanthemoides* and *Veronica serpyllifolia* all showing early disjunction of 1 or more bivalents and *Lactuca scariola*, *Picris hieracioides*, *Solidago virga-aurea* and *Veronica laxa* showing late disjunction of bivalents. However, *Swertia petiolata* shows early as well as late disjunction in different PMCs. While *Veronica laxa* and *Leonurus cardiaca* show the highest and almost same frequency of such PMCs i.e. 14.2 and 14 per cent respectively, it is seen with minimum frequency in *Plantago major* (4.2%).

Among these, species 4 are polyploids and remaining 13 diploids. Delayed separation of chromosomes is often seen in multivalent and large size bivalents probably due to late terminalization. Presently in tetraploids, the multivalents are seen in *Anaphalis nepalensis* and *Senecio chrysanthemoides*. Different sized bivalents are observed in *Euphrasia officinalis*, *Lactuca scariola* and *Salvia moorcroftiana*. Also chromatin stickiness which is thought to be also responsible for non-synchronous disjunction is seen in *Anaphalis nepalensis*, *Euphrasia paucifolia*, *Lamium album*, *Nepeta nervosa* and *Swertia petiolata*. As per Koul (1971), non-synchronization in separation is sometimes caused by the change in homology of the chromosome partners. Except in *Anaphalis nepalensis*, non-synchronous disjunction appears to be of no cytogenetical significance as it does not affect the distribution of chromosomes at anaphase-I or further meiosis.

5.5.9. Lagging chromosomes/laggards: Chromosomes/chromatin that fail to reach respective poles at anaphase or telophase stages form laggards. Failure of one or more chromosomes to move towards respective poles at anaphase or telophase is observed
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in 7 species (5.2%) of the 134 species studied constituting a frequency of 10.3 per cent of the meiotically anomalous species. These include *Artemisia absinthium*, *Anaphalis nepalensis*, *Campanula cashmeriana*, *Codonopsis rotundifolia*, *Myriactis nepalensis*, *Nepeta govaniana* and *Swertia ciliata*. The maximum percentage of such PMCs depicting this irregular behavior of chromosomes is presently found in *Codonopsis rotundifolia* (21.8-25%) followed by *Campanula cashmeriana* (12%) with least frequency in *Swertia ciliata* (4%). Unoriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers and may thus result into laggards (Nicklas & Ward 1994).

Fifty seven per cent of these species are diploids and remaining polyploids. To make a comparison that whether polyploids or diploids show more laggard formation is not possible here due to lack of intraspecific polyploids in the presently studied species with laggards. However, polyploidy increases the occurrence of spindle irregularities, which can lead to the disordered segregation and hence laggard formation. Laggards arise by spindle abnormalities and are also thought to result from late chiasma terminalization (Pagliarini 2000) which may happen in some situations due to large size of bivalents.

Laggards usually fail to reach the poles and form micronuclei (Utsunomiya et al. 2002), leading to the formation of micro-pollen or gametes with unbalanced chromosome numbers (Mansuelli et al. 1995; Ranjbar et al. 2011).

5.5.10. Chromatin bridges: Presently chromatin bridge formation is seen at anaphase-I/II or telophase-I stages in 6 species (4.4%) of the total analyzed species constituting 8.8 per cent of the meiotically abnormal species. These include *Centaurea iberica*, *Elsholtzia ciliata*, *Mazus japonicus*, *Pedicularis rhinanthesoides*, *Phlomis bracteosa* and *Taraxacum officinale*. Highest percentage of such PMCs with chromatin bridges is met within *Centaurea iberica* (3-17%) and lowest in *Mazus japonicus* (3.4%). Chromatin bridge formation mainly arises as a result of paracentric inversions and a large number of bridges indicate structural hybridity. Chromatin stickiness is also responsible for the bridge formation particularly at anaphase and telophase stages as has been seen earlier (Ranjbar et al. 2011). In animals role of protein complexes called condensing I and condensing II has already been documented (Green et al. 2012).
5.5.11. Unoriented/off plate bivalents: Proper orientation of bivalents at metaphase-I is decided by interactions between centromeres of bivalents and also centromere spindle attachments. Presence of unoriented bivalents is presently seen in 7 species (5.2%), constituting 10.3 per cent of the meiotically abnormal ones. These unoriented bivalents range from 1-4 per cell and are observed presently in *Myriactis nepalensis, Solidago canadensis, Anaphalis nepalensis, Senecio graciliflorus, Mertensia echoides, Gentiana carinata* and *Swertia ciliata*. Proper disjunction of bivalents after metaphase-I may be disrupted unless there is proper orientation at this stage. This may lead to lagging chromosomes that may ultimately get eliminated during further meiotic course and hence arise aneuploid gametes. Interestingly among these species, laggard formation at anaphase and/or telophase stages has been observed in *Anaphalis nepalensis, Myriactis nepalensis* and *Swertia ciliata*. The frequency of PMCs depicting this irregularity is highest in *Swertia cordata* (19.3-34.2%), followed by *Gentiana carinata* (20%), *Myriactis nepalensis* (11.7-20%) and is least in *Mertensia echoides* (5%).

5.5.12. Extra-chromatin mass and chromatin fragments: Extra chromatin is observed in *Campanula cashmeriana*, besides some aneuploid PMCs, thus pointing to cytomixis as a probable reason (Ranjbar *et al*. 2011). Presently in *Solidago virgaurea*, 2 chromosome fragments in 6.25 per cent PMCs at metaphase-I are seen. Fragments may be generated by chromosome breaks due to environmental conditions like irradiation or inversions.

5.5.13. Abnormal microsporogenesis: Abnormal meiosis in many species leads to further defects in microsporogenesis like monads, dyads, triads, polyads and tetrads with or without micronuclei. This is presently found in 18 species (13.43%) of the total investigated species constituting 26.5 per cent of the meiotically abnormal taxa. These include *Anaphalis nepalensis, Calamintha umbrosa, C. vulgaris, Campanula cashmeriana, Centaurea iberica, Erigeron alpinus, Euphrasia officinalis, E. paucifolia, Gentiana carinata, Lycopus europaeus, Origanum vulgare, Onopordum acanthium, Plantago depressa, P. major, Senecio chrysanthenoides, Stachys sericea, Swertia ciliata, Taraxacum officinale* and *Veronica deltigera*. All the species with abnormal microsporogenesis except *Veronica deltigera* show one or more meiotic abnormalities. Abnormal microsporogenesis in most of the cases particularly in those with high frequency reduces the pollen fertility. Monads, dyads, triads and polyads
are produced as a result of abnormal cytokinesis during meiosis, which generates sterile pollen grains (Caetano-Pereira & Pagliarini 2001). For example failure of first division may lead to monads and failure of second division may lead to dyads. Triad formation arises as a result of failure of second meiosis at one pole only, whereas polyads are generally produced due to spindle abnormalities. The micronuclei are the fate of laggards and further lead to micropollen or chromosomally unbalanced gametes.

5.6. Interpopulation variabilities in meiotic course: There are usually interpopulation differences in the frequencies of meiotic abnormalities within a species with one or more accessions showing no or low frequency of a particular meiotic anomaly than other. In *Artemisia absinthium* high altitude populations show more number of abnormalities than that of low altitudes but it is not a general rule. Similarly, in *Crepis sancta* low altitude population (P-1) from Pulwama shows no cytomixis but its high altitude accession (P-2) from Kellar shows cytomixis. This indicates the presence of genetic diversity in such species. In the present results, however, generally the high altitude accessions appear to show more frequency of abnormal PMCs than in low altitude ones thus indicating genetic-environmental interactions.

5.7. Heterogeneous pollen size: Formation of heterogeneous sized pollen grains is found within same population in 7 species (5.2%) of the total species studied meiotically at present. Of these *Artemisia absinthium, Stachys floccosa, Mertensia echioides, Campanula cashmeriana* and *Codonopsis rotundifolia* show cytomixis whereas *Nepeta cataria* shows stickiness and *Veronica beccabunga* is altogether normal. Therefore, generally cytomixis is directly related to origin of heterogeneous sized pollen grains but its genetic origin in some species cannot be overruled.

5.8. Pollen sterility: It has been usually found that pollen fertility has a direct bearing on nature of meiotic course with irregular meiosis reducing the pollen fertility. Presently most of the meiotically abnormal species show reduced pollen fertility (see: Table 1). This occurs because various meiotic abnormalities ultimately lead to formation of such pollens grains that are deficient in one or more chromosomes or chromosome segments. However sterility may not be entirely due to the production of aneuploid gametes/chromosomal abnormalities, but also due to genetic reasons as has
been found in *Avena sativa* and *Cereale secale* (Baptista-Giacomelli *et al.* 2000). Presently, some species such as *Campanula colorata, Dubyaea hispida, Jaeschkea gentianoides, Mentha longifolia* (4x), *Saussurea taraxacifolia, Scrophularia scopolii,* etc. show pollen fertility less than 80 per cent but show normal meiosis (see Table 1), hence gene mutations and/or cryptic structural changes might be a reason. On the other hand there is high pollen fertility but abnormal meiosis in some species like *Anaphalis nepalensis, Anthemis cotula, Picris hieraciodes, Mertensia echioides, Solidago virga-aurea,* etc. (see: Table 1). Since glycerol-acetocarmine actually gives apparent pollen fertility, therefore in these cases using other staining techniques may give different results. As has been seen in *Crocus sativus* glyceracetocarmine reveals high pollen fertility although there is high sterility.