New chromosome number reports in some polypetalous species from District Kangra (Himachal Pradesh), India

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ABSTRACT. As a part of our programme to explore and evaluate genetic diversity of flowering plants from District Kangra (Himachal Pradesh) India, at present 150 species of Polypetalae have been meiotically worked out. It brings to light new/ varied chromosome number reports for 23 species. The species being cytologically worked out for the first time on world-wide basis include Chaerophyllum capnoides (n = 11), Heracleum brunonis (n = 11), Berberis ceratophylla (n = 14), Sedum trifidum (n = 18), Corydalis meifolia (n = 8), C. thrysiflora (n = 8), Indigofera hamiltonii (n = 8), Viola betonicifolia (n = 7), V. canescens (n = 6), and V. sempervirens (n = 6) along with new intraspecific cytopotypes in case of six species as Astragalus ladakensis (n=8), Geranium pratense (n = 13), G. wallichianum (n = 28), Pelargonium hortorum (n = 18), Hypericum dyeri (n = 9) and Argemone mexicana (n = 7). The species being cytologically worked out for the first time from India include Barbarea vulgaris (n = 8), Capsella bursapastoris (n = 8), Thlaspi alpestre (n = 7), Astragalus hamosus (n = 8), Caragana pygmaea (n = 8), Desmodium retusum (n = 11), Lespedeza cuneata (n = 10) and Oxalis corymbosa (n = 7).

KEYWORDS. Chromosome number reports, Cytotypes, District Kangra, Polypetalae.

Kangra is the most popular district located on the Southwestern end of the Himachal Pradesh, a hilly state in Western Himalayas. Its area extends from low hill subtropical zone of altitude range (400-650 m) in Shiwaliks to high hill wet sub-temperate zone (above 3000 m) in Dhauladhar and Pir Punjal (Bara Bhangal and Chhota Bhangal) through mid hill sub-humid zone (651-1800 m) in Palampur and Dharamshala. The geographical area of District Kangra is 5,739 sq. km and lies in 31°45’0” to 32°28’05” N latitude and 75°35’34” to 77°04’46” E longitudes supports diverse species of Angiospermic flora. In District Kangra, subclass Polypetalae is represented by 52 families and 140 genera (Chowdhery and Wadhwa 1984). It harbours enormous economically, medicinally and ethnobotanically important Angiosperms (Sood et al. 1982; Kapur 1985; Sharma and Maheshwari 2005; Uniyal et al. 2006). The perusal of literature shows that for district Kangra, no attempts has been made for cytological picture of the vast diverse flora except for a few sporadic reports by Bir and Kumari (1975, 1981) on 17 Legume taxa from Dharamshala. To study the genetic diversity at intra- and interspecific levels and to further enrich the chromosomal database of the lesser known Angiospermic species, the present meiotic studies have been carried out from wide spread area covering up to 3,000 m altitude.

MATERIALS AND METHODS
For meiotic studies, flower buds were collected from various localities of the Kangra district of Himachal Pradesh (Table 1). Smears of appropriate sized flower buds were made after fixing these in the Carnoy’s fixative (6 ethanol: 3 chloroform: 1 acetic acid v/v), using standard acetocarmine technique. Pollen fertility was estimated by mounting mature pollen grains in glycerco-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and un-stained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System. Voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

RESULTS AND DISCUSSION
It is interesting to note that the present study has brought to light in one or other way, newer chromosome counts in 23 species belonging to 18 genera and 11 families from different localities with altitudinal range of 500-3,000 m from District Kangra. The data regarding locality with altitude, accession number, present chromosome number, ploidy level, nature of meiotic course and pollen fertility of these species have been given in Table 1. The morphological field notes are provided only for those species which have been cytologically worked out for the first time on world-wide basis. The previous chromosome reports are based on chromosome number compilations by Darlington and Wylie (1955), Fedorov (1974), Kumar and Subramaniam (1986), Index to Plant Chromosome Numbers from 1968 onwards, various Journals, Proceeding volumes and internet. Further, in case of any plant species having same chromosome number reported by many authors, at the most only four latest references have been mentioned in the text. Meiotic abnormalities have been observed in the form of cytomixis, chromosomal stickiness, unoriented bivalents, inter-bivalent connections or formation of laggards and bridges in the species marked with abnormal meiotic course. Brief observations for each species are discussed below.

Heracleum brunonis Benth. ex C. B. Clarke The species is...
### Table 1. Location, altitude, accession number, present chromosome number, meiotic course, ploidy level and pollen fertility in different species from District Kangra.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Location/Altitude (m)</th>
<th>Accession Number (PUN)</th>
<th>Meiotic Chromosome (n)</th>
<th>Meiotic Course*</th>
<th>Ploidy Level (x)</th>
<th>Pollen Fertility (%age)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family: Apiaceae</strong></td>
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<tr>
<td>1. <em>Heracleum brunonis</em> (DC.) Benth.</td>
<td>Patti, 32°13'N 76°10'E/900</td>
<td>55199</td>
<td>11</td>
<td>N</td>
<td>2x</td>
<td>95.50</td>
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<tr>
<td><strong>Family: Berberidaceae</strong></td>
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<td>2. <em>Berberis ceratophylla</em> G. Don</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>55080</td>
<td>14</td>
<td>ABN</td>
<td>2x</td>
<td>70.56</td>
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<td><strong>Family: Brassicaceae</strong></td>
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<tr>
<td>3. <em>Barbarea vulgaris</em> R.Br.</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52796</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>93.70</td>
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<tr>
<td>4. <em>Capsella bursa-pastoris.</em> (L.) Medik</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52722</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>98.00</td>
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<tr>
<td>5. <em>Thlaspi alpestre</em> L.</td>
<td>Baragran, 32°02’N 76°50’E/3,000</td>
<td>52742</td>
<td>7</td>
<td>N</td>
<td>2x</td>
<td>92.30</td>
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<td><strong>Family: Crassulaceae</strong></td>
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<tr>
<td>6. <em>Sedum trifidum</em> Wall.</td>
<td>Triund, 32°16’N 76°22’E/3,000</td>
<td>55122</td>
<td>18</td>
<td>N</td>
<td>4x</td>
<td>91.45</td>
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<td><strong>Family: Fumariaceae</strong></td>
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<td>7. <em>Corydalis meifolia</em> Wall.</td>
<td>Lohadari, 32°02’N 76°50’E/1,600</td>
<td>55202</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>96.00</td>
</tr>
<tr>
<td>8. <em>Corydalis thyrsiflora</em> Prain</td>
<td>Lohadari, 32°02’N 76°50’E/1,600</td>
<td>55128</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>95.89</td>
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<td><strong>Family: Fabaceae</strong></td>
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<td>9. <em>Astragalus hamosus</em> L.</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52737</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>93.20</td>
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<td>10. <em>Astragalus ladakensis</em> N. P. Balakr.</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52710</td>
<td>8</td>
<td>ABN</td>
<td>2x</td>
<td>73.50</td>
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<tr>
<td>11. <em>Caragana pygmaea</em> (L.) DC</td>
<td>Triund, 32°16’N 76°22’E/3,000</td>
<td>55195</td>
<td>8</td>
<td>N</td>
<td>2x</td>
<td>90.00</td>
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<tr>
<td>12. <em>Desmodium retusum</em> G. Don</td>
<td>Patti, 32°13’N 76°10’E/900</td>
<td>55201</td>
<td>11</td>
<td>N</td>
<td>2x</td>
<td>90.76</td>
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<td>13. <em>Indigofera hamiltonii</em> Graham</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>55078</td>
<td>8</td>
<td>ABN</td>
<td>2x</td>
<td>60.78</td>
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<tr>
<td>14. <em>Lespedeza cuneata</em> G. Don</td>
<td>Patti, 32°13’N 76°10’E/900</td>
<td>55200</td>
<td>10</td>
<td>N</td>
<td>2x</td>
<td>91.00</td>
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<td>15. <em>Geranium pratense</em> L.</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>55094</td>
<td>13</td>
<td>ABN</td>
<td>2x</td>
<td>62.87</td>
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<td>16. <em>Geranium wallachianum</em> D. Don</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52734</td>
<td>28</td>
<td>ABN</td>
<td>4x</td>
<td>63.97</td>
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<tr>
<td>17. <em>Pelargonium hortorum</em> L. H. Bailey</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300</td>
<td>52739</td>
<td>18</td>
<td>N</td>
<td>4x</td>
<td>86.60</td>
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<td>18. <em>Hypericum dyeri</em> Rehder</td>
<td>Biching, 32°02’N 76°50’E/2,200</td>
<td>54300</td>
<td>9</td>
<td>N</td>
<td>2x</td>
<td>89.90</td>
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<td><strong>Family: oxalidaceae</strong></td>
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<td>19. <em>Oxalis corymbosa</em> DC.</td>
<td>Tal-mata, 32°14’N 76°12’E/1,400</td>
<td>52738</td>
<td>7</td>
<td>ABN</td>
<td>2x</td>
<td>61.90</td>
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<td><strong>Family: Papaveraceae</strong></td>
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<td>20. <em>Argemone mexicana</em> L.</td>
<td>Dehra, 31°52’N 76°12’E/530</td>
<td>52743</td>
<td>7</td>
<td>N</td>
<td>2x</td>
<td>90.00</td>
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<td><strong>Family: violaceae</strong></td>
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<tr>
<td>21. <em>Viola betonicifolia</em> Sm.</td>
<td>Buragran, 32°02’N 76°50’E/3,000</td>
<td>54303</td>
<td>7</td>
<td>N</td>
<td>2x</td>
<td>98.00</td>
</tr>
<tr>
<td>22. <em>Viola canescens</em> Wall.</td>
<td>Tal-mata, 32°14’N 76°12’E/1,400</td>
<td>54303</td>
<td>6</td>
<td>N</td>
<td>2x</td>
<td>91.69</td>
</tr>
<tr>
<td>23. <em>Viola sempervirens</em> Greene</td>
<td>Chhota Bhangal, 32°02’N 76°50’E/2,300m</td>
<td>55096</td>
<td>6</td>
<td>N</td>
<td>2x</td>
<td>92.80</td>
</tr>
</tbody>
</table>

Meiotic course *N = Normal; *ABN = Abnormal.
found on moist rocky slopes between the altitudinal range of 500-1,500 m in District Kangra. It is characterized as pubescent herb, flowers greenish - white to purple and fruits glabrous brown. The flowering and fruiting is seen during the months of August-September. The species is cytologically worked out for the first time on world-wide basis showing chromosome count of \( n = 11 \) (Fig. 1A) and which is in conformity with the earlier chromosomal reports of the allied species of the genus.

**Berberis ceratophylla** *G. Don*  The species is found between the altitudinal range of 2,000-3,000 m in District Kangra. It is characterized as an undershrub, stem pale yellowish, flowers yellow and fruit dark red. The flowering and fruiting is seen during the months of May-August. The species is cytologically worked out for the first time on world-wide basis and the present haploid count of \( n = 14 \) (Fig. 1B) conforms to chromosomal reports of the allied species of the genus.

**Barbaraea vulgaris** *W. T. Aiton*  The gametic number \( n = 8 \) (Fig. 1C) for the species is reported for the first time from India and conforms to the earlier report of \( 2n = 16 \) (Harriman 1981; Hill 1989; Albers and Probsting 1998; Koch *et al.* 1999) from outside India.

**Capsella bursa-pastoris** *L.* *Medik*  The gametic number \( n = 8 \) (Fig. 1D) for the species is reported for the first time from India and conforms to the earlier report of \( 2n = 16 \) (Khatoon 1991; Neuffer and Eschner 1995; Aksoy *et al.* 1999; Runemark 2000) from outside India.

**Thlaspi alpestre** *L.*  The present chromosome count of \( n = 7 \) (Fig. 1E) is reported for the first time from India and is in line with the previous chromosomal reports from outside India by Manton (1932), Gadella and Kliphuis (1966), Holmgren (1971) and Laane (1971).

**Sedum trifidum** *Wall.*  The species is found between the altitudinal range of 2,500-3,500 m in District Kangra. It is characterized as a perennial herb with glabrous stem, leaves long, strap-shaped, tapering to a stalkless base and flowers pink. The flowering and fruiting is seen during the months of August-September. The \( n = 18 \) (Fig. 1F) is the first chromosome count for the species on world-wide basis and is in conformity with the earlier chromosomal reports of the allied species of the genus.

**Corydalis meifolia** *Wall.*  The species is found between the altitudinal range of 1,500-2,500 m in District Kangra. It is an erect herb with leafy stems and flowers yellow with purple tips. The flowering and fruiting is seen during the months of July-September. The species is cytologically worked out for the first time on world-wide basis and the present haploid count of \( n = 8 \) (Fig. 1G) conforms to chromosomal reports of the allied species of the genus.

**Corydalis thyrsiflora** *Praun*  The species is found between the altitudinal range of 1,500-2,500 m, distributed on moist and shady places in forest. It is a short stemmed herb with woody rootstocks and yellow flowers with purple tip. The flowering and fruiting is seen during the months of July-September. The species is cytologically worked out for the first time on world-wide basis showing haploid count of \( n = 8 \) (Fig. 1H) conforms to chromosomal reports of the allied species of the genus.

**Astragalus hamosus** *L.*  The chromosome number \( n = 8 \) (Fig. 1I) is reported for the first time from India and is in line with the earlier report of \( 2n = 16 \) (Maassoumi 1986, 1987) from outside India.

**Astragalus ladakensis** *N. P. Balakr.*  The present chromosome count of \( n = 8 \) (Fig. 1J) adds a new diploid cytotype for the species. The species is earlier known to exhibit only \( 2n = 32 \) (Ashraf and Gohil 1986; Wang *et al.* 1994; Gu and Sun 1996).

**Caragana pygmaea** *L.* *DC.*  The species exhibits \( n = 8 \) (Fig. 1K) at various stages of meiosis, makes a new report from India and confirms the earlier report of \( 2n = 16 \) (Frahm-Lelivela 1962; Mesicek and Sajak 1995) from outside India.

**Desmodium retusum** *G. Don*  The chromosome number \( n = 11 \) (Fig. 1L) is reported for the first time from India and is in line with the earlier report of \( 2n = 22 \) (Manandhar and Sakya 2003) from outside India.

**Indigofera hamiltonii** *Graham.*  The species is found between the altitudinal range of 2,000-3,000 m in District Kangra. It is characterized as a tall shrub with dark red flowers and pods glabrous and linear. The flowering and fruiting is seen during the months of April-July. The present chromosome count of \( n = 8 \) (Fig. 1M) is the first chromosome count for the species on world-wide basis and conforms to chromosomal reports of the allied species of the genus.

**Lespedeza cuneata** *G. Don*  The chromosome number \( n = 10 \) (Fig. 1N) is reported for the first time from India and is in line with the earlier report of \( 2n = 20 \) (Lee 1969; Clewell 1971; Liu *et al.* 1985; Hill 1989) from outside India.

**Geranium pratense** *L.*  The present chromosome count of \( n = 13 \) (Fig. 1O) for the species adds an anuploid cytotype on world-wide basis. The species is already known to have variable chromosome numbers as \( 2n = 24 \) (Tjebbes 1928; Tischler 1934) and \( 2n = 28 \) (Hollingsworth *et al.* 1992; Dobes *et al.* 1997; Albers and Probsting 1998; Petrova and Stanimirova 2001).

**G. wallichianum** *D. Don*  The haploid count of \( n = 28 \)
Hypericum dyeri Rehder The present chromosome count of $n = 7$ (Fig. 1R) adds a new anaploid cytotype for the species on world-wide basis. The species is previously known to have diploid cytotypes from India with $2n = 20$ (Mehra and Sareen 1969).

Oxalis corymbosa DC. PMCs at various stages of meiosis depict $n = 7$ (Fig. 1S). This makes a new record from India and confirms the previous report of $2n = 14$ (Naranjo et al. 1982; Xu et al. 1992) from outside India.

Argemone mexicana L. The present chromosome count of $n = 7$ (Fig. 1T) adds a new diploid cytotype for the species on world-wide basis. The species is previously known to have $2n = 28$ (Bir and Sidhu 1980; Koshly and Mathew 1988; Safonova 1991; Trivedi and Trividi 1992) and $2n = 112$ (Diers 1961).

Viola betonicifolia Sm. The species is found between the altitudinal range of 2,500-3,500 m, distributed on moist and shady places of forest in the District Kangra. It is characterized a short stemmed herb with lanceolate leaves and violet flowers. The flowering and fruiting is seen during the months of June-July. The species is cytologically worked out for the first time on world-wide basis showing chromosome count of $n = 7$ (Fig. 1U) and conforms to chromosomal reports of the allied species of the genus.

Viola canescens Wall. ex Roxb. The species is found between the altitudinal range of 1,000-2,000 m in District Kangra. It is an annual herb with tufted, ovate- cordate, crenate leaves and blue flowers. The flowering and fruiting is seen during the months of April-September. The present chromosome count of $n = 6$ (Fig. 1V) is the first chromosome count for the species on world-wide basis and is in conformity with reports of the allied species of the genus.

Viola sempervirens Greene The species is found on rocky slopes between the altitudinal range of 2,000-2,500 m in District Kangra. It is found growing on moist slopes. It is characterised as perennial herb with round leaves and pale yellow flowers. The flowering and fruiting is seen during the months of August-October. The species is cytologically worked out for the first time on world-wide basis showing chromosome count of $n = 6$ (Fig. 1W) and is in conformity with the chromosomal reports of the allied species of the genus.

**ACKNOWLEDGEMENTS** The authors are grateful to the University Grants Commission, New Delhi for providing financial assistance under the DRS SAP III of UGC as well as to Department of Science and Technology, New Delhi for FIST programmes. We are also highly thankful to the Director and other staff of Herbarium of Botanical Survey of India, Dehradun for their help in the identification of the plant species.

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