Phenomenon of Cytomixis and Intraspecific Polyploidy (2x, 4x) in *Spergularia diandra* (Guss.) Heldr. & Sart. in the Cold Desert Regions of Kinnaur District (Himachal Pradesh)

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**Summary** *Spergularia diandra* (Guss.) Heldr. & Sart. (Family: Caryophyllaceae), worked out presently from the valleys of Ganjul and Hangrang in the Kinnaur district of Himachal Pradesh, exhibits intraspecific diploid (2x=n=9) and tetraploid (4x=n=18) cytotypes. The species has been worked out chromosomally for the first time from India and adds a new 4x cytotype to the already existing 2x cytotype known from outside of India. The individuals of 2x cytotype are restricted to the Ganjul Valley while those of 4x cytotype are present in Hangrang valley. The individuals of the 2x and 4x cytotypes could be distinguished on the basis of habit, plant size, number of branches, bushy nature, and fleshy and size of leaves. The diploid plants showed spindle abnormalities during meiosis resulting into heterogeneous sized fertile/stained pollen grains and some pollen sterility (10.00–12.00%). On the other hand, some plants of 4x cytotype showed the phenomenon of cytomixis involving chromatin transfer among proximate meiocytes, and chromatin stickiness resulting into 5.00–8.00% unstained/sterile pollen grains. The cytomixis in the 4x cytotype is the first ever record for the species and seems to be a natural phenomenon under direct genetic control as the plants with and without cytomixis grow under the same environmental conditions.

**Key words** Ganjul and Hangrang valleys, Intraspecific cytotypes, Diploid, Tetraploid, Cytomixis, Heterogeneous sized fertile pollen grains.

*Spergularia diandra* (Guss.) Heldr. & Sart. (Family: Caryophyllaceae), also described under the name *Arenaria diandra* Guss., is referred to as the Lesser sand spurrey, Small sand spurrey or Alkali sand spurrey. The species, which is commonly found in damp clay or sandy and saline or calcareous soils along sandy beaches, river shores and roadsides, is distributed in Mediterranean Europe, North Africa, South Western and East Asia. It grows as an annual herb with ascending, slender, glandular pubescent stems which are erect to diffusely spreading, much-branched proximally and distally (Fig. 1). Leaves are linear, somewhat fleshy, apex obtuse and stipules inconspicuous, silvery to dull tan, triangular or rarely lanceolate. Flowers with white and oblong-elliptic petals are present in 4–8 compound cymes. Capsule ovoid and greenish tan become purple-black at maturity.


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Islands (Spain) of Europe (Dalgaard 1987) and Mediterranean regions (Runemark 1996). While analyzing the cytomorphological diversity in the flowering plants from the cold desert regions of the Kinnaur district in Himachal Pradesh, we have been able to detect the existence of intraspecific dip-
Phenomenon of Cytomixis and Intraspecific Polyploidy (2x, 4x) in *S. diandra* in the Cold Desert Regions

Some of the diploid (2x) and tetraploid (4x) plants showed the phenomena of cytomixis, associated meiotic abnormalities and pollen malformation.

In this paper the aim was to study in detail the course of meiosis including microsporogenesis and pollen fertility in both the cytotypes. The aim here was also to study the distribution pattern and to differentiate the 2 cytotypes on morphological characters.

**Materials and methods**

Materials for male meiotic studies were collected during the months of September in 2008 and 2009 from 10 wild plants growing in Ganjul Valley (Pooh, 2840 m, 31°45’47″N and 78°35’20″E) and Hangrang Valley (Chango, 3050 m, 31°58’34″N and 78°35’33″E) of the Kinnaur district of Himachal Pradesh (India). Voucher specimens of the cytologically worked out individuals were deposited in the Herbarium, Department of Botany, Punjabi University Patiala (PUN* 53741, 53742).

For male meiotic studies, young and unopened floral buds were fixed in freshly prepared Carnoy’s fixative (6 ethanol: 3 chloroform: 1 glacial acetic acid, v/v/v) for 24 h and preserved in 70% alcohol at 4°C. Anthers were squashed in 1% acetocarmine and preparations were studied for meiotic chromosome counts and detailed meiotic behavior in pollen mother cells (PMCs) at different stages. Pollen viability was estimated through stainability tests for which anthers from mature flowers were squashed in glycerol–acetocarmine (1:1) mixture and 1% aniline blue dye. Well-filled pollen grains with fully stained cytoplasm were scored as fertile while those that were shriveled up and had partially or unstained cytoplasm were counted as sterile. Pollen grain size was measured using an occlulomicrometer. Images of chromosome counts, meiotic abnormalities, sporads and pollen grains were photomicrographed from the freshly prepared slides with a Nikon 80i eclipse microscope and a Leica Qwin Digital Imaging System.

**Results**

While surveying dicotyledonous plants for cytological study from the Ganjul Valley and Hangrang Valley in the Kinnaur district of Himachal Pradesh (India), we have detected the existence of 2x and 4x cytotypes in *S. diandra*. Out of the total of 10 individuals scored presently, 2 plants collected from Ganjul Valley showed a meiotic chromosome count of *n*=9 while the rest of the plants studied from the Hangrang Valley showed a tetraploid chromosome number of *n*=18.

The 2 cytotypes could be differentiated in the field on the basis of habit, plant height, number of branches, and fleshiness and size of leaf (Table 1). Tetraploid plants which are confined to Hangrang Valley are relatively more common than the diploids growing in Ganjul Valley. Further, the 2 cytotypes could also be differentiated on the basis of their bushy nature as 4x plants were observed to have more branching and are bushy compared to the 2x plants. The leaves of both the cytotypes are linear but differentiated in being more fleshy in 2x plants compared to 4x plants. Pollen grains in tetraploid plants are of uniform size whereas in the diploids, pollen grains are of heterogeneous sizes.

**The diploid (n=9)**

The individuals collected for chromosomal studies from Ganjul Valley growing on sandy slopes and among stones along roadsides existed at diploid level with a meiotic chromosome count of *n*=9 as confirmed by the presence of 9 bivalents at M-I (Fig. 2) and 9:9 chromosomes distribu-
tion at A-I (Fig. 3). However, a few PMCs showed the presence of laggards (1.23–2.65%) and chromatin bridges (0.55–1.22%) during A-I (Fig. 4). The lagging chromosomes in such PMCs failed to get included at poles and organized into micronuclei during sporad formation (Fig. 5). Consequently, the individuals showed some pollen malformation in the form of pollen sterility (10.00–12.00%) and apparently fertile pollen grains of 3 different sizes (Large: 33.93–35.82 \( \mu m \), 6.90%; Typical: 28.27–30.16 \( \mu m \), 82.76%; Small: 20.73–22.62 \( \mu m \), 10.34%) (Fig. 6).

The tetraploid (n=18)

The male meiotic course studied on the basis of 8 individuals collected from the Hangrang Valley showed a tetraploid chromosome count of \( n=18 \) as confirmed from the presence of 18 bivalents at M-I (Fig. 7) and 18 : 18 chromosomes distribution at A-I (Fig. 8). Out of 8 tetraploid individuals, 2 showed perfectly regular meiotic course, normal sporad formation and 100.00% fertile pollen grains. On the other hand, the remaining 6 individuals showing normal 18 bivalents depict the phenomenon of chromatin transfer among neighbouring meiocytes (Figs. 9–11) and chromatin stickiness (Fig. 12). The chromatin transfer occurred through long narrow cytomictic channels by forming 1–2 strands. Consequent to meiotic irregularities in meiocytes, these individuals showed 5.00–8.00% unstained/sterile pollen grains (Fig. 13).

The data on chromosome number, meiotic course, microsporogenesis and pollen grain size and pollen fertility in both the cytotypes are given in Table 2.

**Discussion**

*S. diandra* has been counted chromosomally for the first time from India. Further, the present study also records the existence of intraspecific 2x and 4x cytotypes in the Kinnaur district of Himachal Pradesh in India (Asia). Outside of India, only diploid cytotype with \( n=9 \) is known from Morocco and Algeria in North Africa (Humphries *et al.* 1978, Galland 1988), the Canary Islands

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**Table 1.** Comparison of morphological characters in the 2x and 4x cytotypes of *S. diandra*

<table>
<thead>
<tr>
<th>Characters</th>
<th>The diploid (n=9)</th>
<th>The tetraploid (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>Sandy slopes, among stones along roadsides</td>
<td>Sandy slopes, among roadsides</td>
</tr>
<tr>
<td>Habit</td>
<td>Less bushy</td>
<td>More bushy</td>
</tr>
<tr>
<td>Plant size (cm)</td>
<td>28.82–34.23 (31.58±2.79)</td>
<td>42.80–56.00 (51.26±4.32)</td>
</tr>
<tr>
<td>No. of branches</td>
<td>3–5 (4±1)</td>
<td>7–9 (8±1)</td>
</tr>
<tr>
<td>Leaves (i)</td>
<td>Linear</td>
<td>Linear</td>
</tr>
<tr>
<td>(ii) Size (mm)</td>
<td>10.0–15.8 (10.80±1.1)</td>
<td>15.0–22.0 (17.2±1.65)</td>
</tr>
<tr>
<td></td>
<td>6.0–7.0 (6.2±0.46)</td>
<td>4.0–6.0 (5.0±0.92)</td>
</tr>
<tr>
<td>(iii) Fleshiness</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Flowers</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Pollen grains (i)</td>
<td>Heterogeneous sized</td>
<td>Uniform sized</td>
</tr>
<tr>
<td></td>
<td>Large: 33.93–35.82 (34.81±0.95)×33.93–35.82 (34.81±0.95); 6.90%</td>
<td>28.27–29.40 (28.77±0.57)×28.27–29.40 (28.77±0.57); 6.90%</td>
</tr>
<tr>
<td></td>
<td>Medium: 28.27–30.16 (29.12±0.96)×28.27–30.16 (29.12±0.96); 82.76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small: (20.73–22.62 (21.67±0.68)×20.73–22.62 (21.67±0.68); 10.34%</td>
<td></td>
</tr>
<tr>
<td>(ii) Fertility (% age)</td>
<td>88.00–90.00 (89.00±1)</td>
<td>92.00–100.00 (95.12±3.22)</td>
</tr>
</tbody>
</table>

Values in parentheses represent the mean±standard deviation.
Table 2. Meiotic course, microsporogenesis, pollen grain size, frequency and pollen fertility in different individuals of the 2x and 4x cytotypes of S. diandra

<table>
<thead>
<tr>
<th>Cytotype</th>
<th>Cytomixis</th>
<th>Meiotic course</th>
<th>Microsporogenesis</th>
<th>Pollen grains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% age of PMCs involved</td>
<td>Meiotic stages</td>
<td>% age of PMCs with chromatin stickiness</td>
<td>% age of PMCs with laggard at A-I</td>
</tr>
<tr>
<td>2x1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.65</td>
</tr>
<tr>
<td>2x2</td>
<td>3.47</td>
<td>M-I</td>
<td>2.50</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5.88</td>
<td>M-I, T-II</td>
<td>10.31</td>
<td>—</td>
</tr>
<tr>
<td>4x1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4x2</td>
<td>3.66</td>
<td>T-II</td>
<td>19.49</td>
<td>—</td>
</tr>
<tr>
<td>4x3</td>
<td>1.52</td>
<td>T-II</td>
<td>6.38</td>
<td>—</td>
</tr>
<tr>
<td>4x4</td>
<td>1.88</td>
<td>T-II</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4x5</td>
<td>2.12</td>
<td>M-I, T-II</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4x6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4x7</td>
<td>—</td>
<td>—</td>
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<td>—</td>
</tr>
</tbody>
</table>

M-I=Metaphase-I; A-I=Anaphases-I; T-I=Telophases-I; values in parentheses represent the mean±standard deviation.
The intraspecific 2x and 4x cytotypes recorded presently in *S. diandra* from cold desert regions of the Indian Himalayas could be differentiated in the field on the basis of some morphological characters (habit, plant size, number of branches and fleshy and size of leaf). The tetraploid plants were observed to be much taller and possessed large fleshy leaves in a smaller quantity. It is thus apparent that morphological characters in both the cytotypes of the species are directly correlated with the increase in ploidy level which has also been reported in *Andropogon gerardii* Vitman (Keeler and Davis 1999), *Centaurea jacea* L. (Hardy et al. 2000), *Parasenecio auriculata* (DC.) J.R. Grant (Nakagawa 2006), *Dactylis* L. (Amirouche and Misset 2007), *Centaurea phrygia* L. (Koutecký 2007), *Rorippa amphibia* L. (Luttikhuizen et al. 2007), *Centaurea stoebbe* L. (Spaniel et al. 2008, Mráz et al. 2011) and *Ranunculus hirtellus* (Kumar and Singhal 2011). Pollen grains are of uniform size in the tetraploid cytotype whereas those of 2x cytotype are of 3 different sizes, large, medium (typical) and small sized. The heterogeneous sizes of pollen grains in the 2x cytotype could be the consequence of spindle irregularities. Further, the typical sized pollen grains in 2x and 4x cytotypes were observed to be of the same size indicating that the increase in the ploidy level has not affected the pollen grain size in the 4x cytotype. Kumar and Singhal (2011) have also reported in *Ranunculus hirtellus* that increase in ploidy level does not affect the size of pollen grains.

Both the cytotypes are found to grow under the same climatic conditions but the individuals of 4x cytotype are restricted to the Hangrang Valley at the altitude of 3050 m whereas the 2x cytotype is restricted to the Ganjul Valley at 2840 m.

In spite of normal bivalent formation and their regular segregation during anaphases/telophases, a few PMCs in the individuals of the 2x cytotype showed some spindle abnormalities re-
resulting in the presence of laggards and chromatim bridges. The lagging chromosomes failed to get included at poles and organized into the formation of micronuclei or micropollen as reported by Bhat et al. (2006) and Singhal and Kumar (2008a). Out of 8 tetraploid individuals, 6 plants showed the phenomenon of cytomixis involving chromatin transfer among proximate PMCs leading into some sterile pollen grains (5.00–8.00%). The phenomenon of cytomixis, coined by Gates (1911), is reported here for the first time in the species. Cytomixis only occurs in the tetraploid and not in the diploid individuals in S. diandra, confirming the views of earlier workers that the phenomenon of chromatin transfer is more prevalent in polyploids than their diploid counterparts (Kamra 1960, Semyarkhina and Kuptsou 1974, Basavaiah and Murthy 1987, Sheidai and Attaei 2005, Singhal et al. 2007). Chromatin transfer occurred through long sized cytoplasmic channels forming 1–2 chromatin strands involving 2 PMCs in 1.52–5.88% cases from late stages of meiosis-I and continued up to T-II. The same results are found in some cases where the transfer of chromatin or chromosomes may take place until the later stages of meiosis and the size of cytomictic channels may increase to form conspicuous transfer of chromatin material among inter-PMCs (Falistocco et al. 1995, Haroun 1995, Singhal and Kumar 2008a, b, 2010, Shabrangi et al. 2010, Mursalimov and Deineko 2011). The inter-PMCs transfer of chromatin material results in chromosome stickiness and consequently pollen sterility. Similar findings on the impact of chromatin transfer in causing chromatin stickiness and consequently pollen sterility have been recorded earlier in Vicia faba (Haroun et al. 2004), Caltha palustris (Kumar and Singhal 2008), Meconopsis aculeata (Singhal and Kumar 2008a), Hippophae rhamnoides (Singhal et al. 2008), Anemone rivularis (Singhal et al. 2009a) and Clematis orientalis (Kumar et al. 2010).

The phenomenon of cytomixis has been reported in a large number of angiospermic plants, and many researchers consider cytomixis to be of considerable evolutionary significance (Falistocco et al. 1995, Morikawa and Leggett 1996, Malallah and Attia 2003, Singhal and Kumar 2010). Some of the possible causes and explanations put forth by earlier researchers include the effect of fixation (Haroun 1995), pathological changes (Morisset 1978), physiological control (Bahl and Tyagi 1988), chemicals and herbicides (Haroun 1995), environmental stress and pollution (Haroun et al. 2004), temperature (Kumar and Tripathi 2008), stress factors and genetic control (Malallah and Attia 2003), pressure difference (Morisset 1978) and clumped chromatin bridges during premeiotic anaphase (Mendes and Rijo 1951). In the present case, cytomixis seems to be a natural phenomenon under direct genetic control as the plants with and without cytomixis grow under the same environmental conditions as reported by several researchers (Singhal and Gill 1985, Bellucci et al. 2003, Haroun et al. 2004, Lattoo et al. 2006, Singhal et al. 2007, 2008, 2009a, b, 2010, 2011, Kumar and Singhal, 2008, Singhal and Kumar, 2008a, b, 2010, Kumar et al. 2008a, b 2010, 2011, 2012, Himshikha et al. 2010).

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