Research Article

Karyomorphological Studies in Some Species of Parnassia L. (Saxifragaceae s.l.) in East Asia and Intraspecific Polyploidy of P. palustris L.

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Received 24 September 2011; Revised 29 November 2011; Accepted 26 January 2012

Academic Editor: Teresa Garnatje

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Karyomorphological information is one of the most important characters for cytotaxonomy. We described karyomorphology of 14 species of Parnassia in East Asia. They had commonly the resting chromosomes of the simple chromocenter type and the mitotic prophase chromosomes of the proximal type. The somatic chromosome number of \(2n = 14\) was shown in three species, that of \(2n = 18\) was shown in six species, that of \(2n = 18\) or \(36\) was shown in two species, that of \(2n = 32\) was shown in one species, that of \(2n = 36\) or \(36 + 1 ∼ 8\) was shown in one species, and that of \(2n = 18, 27, 36\) or \(45\) was shown in one species. They were commonly monomodal (gradual) decrease in length from the largest to the smallest chromosomes. However, their absolute chromosome sizes were different from each other. Average chromosome sizes were recognized as three types small (1.21 µm), medium (1.7–2.36 µm), and large (3.1–3.75 µm). Parnassia palustris showed four cytotypes that could be likely cytogeographically correlated with habitats, polyploidy, and sizes of pollen grains.

1. Introduction

The genus Parnassia L., traditionally classified in the monotypic subfamily Parnassiioideae, the family Saxifragaceae sensu lato [1], is currently placed in the family Parnassiaceae, closely related to Saxifragaceae in the order Celastrales [2, 3]. The genus Parnassia consists of approximately 70 species [4], that are perennial herbs and grow in wet grassy plains and forests in the temperate to arctic regions of the Northern Hemisphere. Among the species of this genus, 63 species are found in China [4], five species are distributed in Russia [5] of which four species are common to China and Russia, and three species grow in Japan [6].

The chromosome numbers of 33 species of Parnassia have been documented as \(2n = 18, 27, 32, 33, 34, 35, 36, 37, 36 + 1 ∼ 8\)s, 43, 44, 45, 48, and 54 including intraspecific polyploid and aneuploid (e.g., [24, 28–35]; Table 1).

The present study contributes cytological data for the species of Parnassia, mainly in its East Asian distribution range, and based on karyomorphology with respect to resting nuclei, mitotic prophase chromosomes and the mitotic metaphase chromosomes, the distribution of intraspecific polyploidy and aneuploidy, and cytogeographic patterns.

2. Materials and Methods

Total 400 samples of 14 species of Parnassia were collected in 75 localities in Russia, Mongolia, China, and Japan (Figure 1; Table 2) and were brought and cultivated in pots in shade place in the experimental garden of Showa Pharmaceutical University. Somatic chromosome observations were made in meristematic cells of fresh root tips. They were pretreated in 2 mM 8-hydroxyquinoline at ca 20°C for 4 hours and then fixed in 45% acetic acid at ca 2°C for 10 minutes. They were macerated in a mixture of 45% acetic acid and 1 N hydrochloric acid (1:1) at ca 60°C for 20–23 seconds and were then stained with 2% aceto-orcein at room temperature.
Table 1: Chromosome numbers of *Parnassia* previously reported.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome number</th>
<th>Selected bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. tenella</em> Hook.f. et Thoms.</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. esquirii</em> Lévl.</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. faberi</em> Oliv.</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. chinensis</em> Franch.</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. epunctulata</em> J.T. Pan</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. venusta</em> Jien</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. myosorensis</em> Heyne ex Wigjt et Arn.</td>
<td>2n = 18</td>
<td>Funamoto et al. 1996 [9]</td>
</tr>
<tr>
<td><em>P. scapa</em> Mattf. var. <em>yushuensis</em> J.T. Pan</td>
<td>2n = 18</td>
<td>Funamoto et al. 1996 [9]</td>
</tr>
<tr>
<td><em>P. aflat. cacuminum</em> Hand.-Mazz. f. <em>yushuensis</em> Ku</td>
<td>2n = 18</td>
<td>Funamoto et al. 2001 [10]</td>
</tr>
<tr>
<td><em>P. pusilla</em> Wall.</td>
<td>n = 9</td>
<td>Malla et al. 1979 [11]</td>
</tr>
<tr>
<td><em>P. oreophylla</em> Hance</td>
<td>2n = 18, 36</td>
<td>Funamoto et al. 1994, 1996 [10, 12]</td>
</tr>
<tr>
<td><em>P. subsapa</em> C.Y. Wu ex Ku</td>
<td>2n = 18</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. trinervis</em> Drude</td>
<td>2n = 18</td>
<td>Funamoto et al. 1996 [9]; Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. viridiflora</em> Batalin</td>
<td>2n = 18, 36</td>
<td>Funamoto et al. 1998 [13], 2001 [10, 13]</td>
</tr>
<tr>
<td><em>P. laxmannii</em> Pall.</td>
<td>2n = 18, 36</td>
<td>Funamoto et al. 2002 [14]; Murín et al. 1984 [15]</td>
</tr>
<tr>
<td><em>P. alpicola</em> Makino</td>
<td>2n = 18</td>
<td>Funamoto 1986 [16]</td>
</tr>
<tr>
<td><em>P. bifolia</em> Nekras.</td>
<td>2n = 36, 36 + 1 ~ 8s</td>
<td>Funamoto et al. 2002 [14]; Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. wightiana</em> Wall.</td>
<td>2n = 14, 36</td>
<td>Funamoto et al. 1998 [13]; Malla et al. 1981 [17]</td>
</tr>
<tr>
<td><em>P. monochorifolia</em> Franch.</td>
<td>2n = 48</td>
<td>Ding et al. 2005 [7]</td>
</tr>
<tr>
<td><em>P. foliosa</em> Hook.f. et Thoms.</td>
<td>2n = 18</td>
<td>Funamotó 1986 [16]</td>
</tr>
<tr>
<td><em>P. grandifolia</em> D.C.</td>
<td>2n = 32</td>
<td>Gastony and Soltis 1977 [18]</td>
</tr>
<tr>
<td><em>P. caroliniana</em> Michaux</td>
<td>2n = 32</td>
<td>Gastony and Soltis 1977 [18]</td>
</tr>
<tr>
<td><em>P. asarifolia</em> Vent.</td>
<td>2n = 32</td>
<td>Gastony and Soltis 1977 [18]</td>
</tr>
<tr>
<td><em>P. glauca</em> Raf.</td>
<td>2n = 32, 36</td>
<td>Gastony and Soltis 1977 [18]; A. Löve and D. Löve 1980 [19]</td>
</tr>
<tr>
<td><em>P. palustris</em> L.</td>
<td>2n = 18, 27, 32, 33, 34, 35, 36, 37, 43, 44, 45, 54</td>
<td>Hultgård 1987 [20]; Lövkvist and Hultgård 1999 [21]; Funamoto et al. 2006, 2008 [22, 23]</td>
</tr>
<tr>
<td><em>P. obtusiflora</em> Rupr.</td>
<td>2n = 36</td>
<td>A. Löve and D. Löve 1982 [24]</td>
</tr>
<tr>
<td><em>P. kotzebuei</em> Cham.</td>
<td>2n = 18, 36</td>
<td>Packer 1964 [25]; Löve and Ritchie 1966 [26]</td>
</tr>
<tr>
<td><em>P. fimbriata</em> Koenig.</td>
<td>2n = 36</td>
<td>Packer 1964 [25]</td>
</tr>
<tr>
<td><em>P. townsendii</em> Robinson</td>
<td>2n = 36</td>
<td>Bye and Soltis 1979 [27]</td>
</tr>
</tbody>
</table>

in moist chamber with 45% acetic acid for ca 30 minutes and were prepared by the conventional squash method. Measurements of chromosomes in 14 species were made in each average length in several good metaphase cells.

Morphological types of the resting and the mitotic prophase chromosomes were classified for Tanaka [36, 37], and the classification of mitotic metaphase chromosomes followed Levan et al. [38]. Taxonomical treatment followed Czerepanov [5], Ku and Hultgård [4], and Akiyama [6]. The voucher specimens of the plants used in this study were deposited in Funamoto’s Herbarium in Showa Pharmaceutical University.

3. Results and Discussion

3.1. Resting Nuclei and Mitotic Prophase Chromosomes. Fourteen species of *Parnassia* in East Asian (Table 2) had common karyomorphological characters in the resting and the mitotic prophase chromosomes: the resting chromosomes were classified as the simple chromocenter type which had several small heteropycnotic bodies per nucleus according to Tanaka [36, 37], and the mitotic prophase chromosomes were classified as the proximal type in which the early condensed segments were confined to the proximal regions of both short and long arms or short arm, showing clear condensation
Table 2: Chromosome numbers in 14 species of *Parnassia* studied here.

<table>
<thead>
<tr>
<th>Section</th>
<th>Species</th>
<th>Number of locality and samples</th>
<th>Chromosome number (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saxifragastrum</td>
<td><em>P. yunnanensis</em> var. Franch. <em>longistipitata</em> Jien</td>
<td>2 localities 17 samples</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td><em>P. scaposa</em> Mattf. var. <em>yushuensis</em> J.T. Pan</td>
<td>5 localities 17 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. aff. cacuminum</em> Hand.-Mazz. <em>f. yushuensis</em> Ku</td>
<td>1 locality 5 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. oreophila</em> Hance</td>
<td>1 locality 2 samples</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>P. trinervis</em> Drude</td>
<td>7 localities 28 samples</td>
<td>18</td>
</tr>
<tr>
<td>Nectarotrilobos</td>
<td><em>P. viridiflora</em> Batalin</td>
<td>1 locality 3 samples</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>P. laxmannii</em> Pall.</td>
<td>2 localities 11 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. brevistyla</em> (Brieg) Hand.-Mazz</td>
<td>2 localities 7 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. delavayi</em> Franch.</td>
<td>2 localities 5 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. alpicala</em> Makino</td>
<td>1 locality 1 sample</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. virens</em> Wall.</td>
<td>1 locality 4 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. longiflora</em> Hook.et Thoms.</td>
<td>4 localities 25 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. foliosa</em> Mattf. var. <em>yushuensis</em> 18</td>
<td>2 localities 11 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. wallii</em> Jien</td>
<td>1 locality 2 samples</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>P. yunnanensis</em> var. <em>longistipitata</em> Jien</td>
<td>2 localities 17 samples</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td><em>P. scaposa</em> var. <em>yushuensis</em></td>
<td>5 localities 17 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. aff. cacuminum</em> <em>Lyushuensis</em></td>
<td>1 locality 5 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. oreophila</em></td>
<td>1 locality 2 samples</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>P. trinervis</em></td>
<td>7 localities 28 samples</td>
<td>18</td>
</tr>
<tr>
<td>Nectarobilobos</td>
<td><em>P. viridiflora</em> Batalin</td>
<td>1 locality 3 samples</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><em>P. laxmannii</em> Pall.</td>
<td>2 localities 11 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. brevistyla</em> (Brieg) Hand.-Mazz</td>
<td>2 localities 7 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. delavayi</em> Franch.</td>
<td>2 localities 5 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. alpicala</em> Makino</td>
<td>1 locality 1 sample</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. virens</em> Wall.</td>
<td>1 locality 4 samples</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>P. longiflora</em> Hook.et Thoms.</td>
<td>4 localities 25 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. foliosa</em> Mattf. var. <em>yushuensis</em> 18</td>
<td>2 localities 11 samples</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td><em>P. wallii</em> Jien</td>
<td>1 locality 2 samples</td>
<td>36</td>
</tr>
</tbody>
</table>

* s: supernumerary chromosome.

Table 3: Karyotype characters in 14 species of *Parnassia* observed here.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome number (2n)</th>
<th>Longest ± SD</th>
<th>Shortest ± SD</th>
<th>Total ± SD</th>
<th>Average ± SD</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. yunnanensis</em> var. <em>longistipitata</em></td>
<td>32</td>
<td>1.6 ± 0.1</td>
<td>1.0 ± 0</td>
<td>38.6 ± 1.1</td>
<td>1.21 ± 0.08</td>
<td>m</td>
</tr>
<tr>
<td><em>P. scaposa</em> var. <em>yushuensis</em></td>
<td>18</td>
<td>2.8 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>41.2 ± 3.8</td>
<td>2.29 ± 0.21</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. aff. cacuminum</em> <em>Lyushuensis</em></td>
<td>18</td>
<td>2.3 ± 0.1</td>
<td>1.5 ± 0</td>
<td>33.0 ± 0.9</td>
<td>1.83 ± 0.07</td>
<td>m</td>
</tr>
<tr>
<td><em>P. oreophila</em></td>
<td>18</td>
<td>2.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>40.6 ± 3.2</td>
<td>2.26 ± 0.27</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. trinervis</em></td>
<td>36</td>
<td>2.8 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>78.5 ± 4.1</td>
<td>2.13 ± 0.16</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. viridiflora</em></td>
<td>18</td>
<td>2.7 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>42.5 ± 2.9</td>
<td>2.36 ± 0.19</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. laxmannii</em></td>
<td>18</td>
<td>2.0 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>30.7 ± 1.2</td>
<td>1.71 ± 0.11</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. brevistyla</em></td>
<td>14</td>
<td>2.1 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>61.1 ± 1.8</td>
<td>1.70 ± 0.09</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. delavayi</em></td>
<td>14</td>
<td>2.3 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>26.7 ± 1.0</td>
<td>1.91 ± 0.11</td>
<td>m + sm + st</td>
</tr>
<tr>
<td><em>P. foliosa</em></td>
<td>18</td>
<td>2.8 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>39.5 ± 2.8</td>
<td>2.19 ± 0.13</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. alpicala</em></td>
<td>18</td>
<td>2.7 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>41.8 ± 3.4</td>
<td>2.32 ± 0.18</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. bifolia</em></td>
<td>36</td>
<td>2.8 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>61.2 ± 3.9</td>
<td>2.27 ± 0.15</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. wightiana</em></td>
<td>14</td>
<td>2.8 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>81.4 ± 5.3</td>
<td>2.27 ± 0.13</td>
<td>m + sm</td>
</tr>
<tr>
<td><em>P. palustris</em></td>
<td>45</td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0</td>
<td>99.4 ± 2.5</td>
<td>2.21 ± 0.07</td>
<td>m + sm</td>
</tr>
</tbody>
</table>

SD: standard deviation; m: median-centromeric chromosome; sm: submedian-centromeric chromosome; st: subterminal-centromeric chromosome.
transition from the proximal to the distal regions (Tanaka [36, 37]; Figure 2). There was no difference in the resting and the mitotic prophase chromosome types among these 14 species, thus it was concluded that the genus *Parnassia* had the simple chromocenter types at the resting nucleus and the proximal types at the mitotic prophase chromosomes.

3.2. The Mitotic Metaphase Chromosomes. Chromosome numbers in 33 species of *Parnassia* were from 2n = 14 to 2n = 54 (Table 1). Three species had the somatic chromosome number of 2n = 14, five species had that of 2n = 32, one species had that of 2n = 48, and the other 24 species had that of 2n = 18. Intraspecific polyploids were found in *P. oreophylla*, *P. viridiflora*, *P. laxmannii*, *P. palustris*, and *P. obtusiflora*, while intraspecific aneuploids were found in *P. bifolia*, *P. wightiana*, *P. glauca*, and *P. palustris* (Table 1). Particularly, *Parnassia palustris* had various chromosome numbers from 2n = 18 to 54 (e.g., [34, 35]; Table 1). *Parnassia brevistyla* (Figure 3(j)), *P. delavayi* (Figure 3(k)), and *P. wightiana* (Figure 3(n)) had 2n = 14, *P. scaposa* var. *yushuensis* (Figure 3(b)), *P. aff. caucatum* f. *yushuensis* (Figure 3(c)), *P. trinervis* (Figure 3(f)), *P. laxmannii* (Figure 3(i)), *P. alpicola* (Figure 3(l)), and *P. foliosa* (Figure 3(o)) had 2n = 18, *P. oreophylla* (Figures 3(d) and 3(e)) and *P. viridiflora* (Figures 3(g) and 3(h)) had 2n = 18 and 36, *P. palustris* had 2n = 18, 27, 36, and 45 (Figures 3(p)–3(s)), *P. yunnanensis* var. *longistipitata* had 2n = 32 (Figure 3(a)), and *P. bifolia* had 2n = 36 and 36 + 1–8s (Figures 3(m) and 4). *Parnassia palustris* in a plant collected in Scandinavia by Hultgård [20] and Lövkvist and Hultgård [21] was very rarely documented to have the chromosome numbers of 2n = 27 and 45. The chromosome number of 2n = 14 for *P. wightiana* [13] was different from the previous counts of 2n = 36 [17] and 2n = 32 [18], while that of *P. glauca* was also different from the previous count of 2n = 36 [19]. Thus, these two species are necessary to have more cytological studies. A peculiar chromosome number of 2n = 14 for Chinese species of *Parnassia* was reported by Funamoto et al. [10, 13] and Ding et al. [7]. The chromosome number of 2n = 32 was previously reported in four North American species of *Parnassia* such as *P. gandifolia*, *P. caroliniana*, *P. asarifolia*, and *P. glauca* by Gastony and Soltis [18] and that was also found in Chinese species [8].

Aneuploid chromosome numbers of 2n = 36 + 1 ~ 8s for *P. bifolia* could include supernumerary chromosomes since these aneuploid plants had high pollen stainabilities (87.5–98.6%).

3.3. Chromosome Sizes and Basic Chromosome Numbers. Morphological characters of chromosomes in chromosome numbers, chromosome sizes, chromosome complements in centromeric positions, and chromosome alignment from the longest to the shortest one are important information for cytotaxonomy. The chromosome number of 2n = 14 was considered diploid x = 7, that of 2n = 32 was considered tetraploid x = 8, and that of 2n = 18 and 36 was considered diploid and tetraploid x = 9, respectively, if these basic chromosome numbers of x = 7, 8, and 9 were accepted. Plants of the basic chromosome number of x = 7 are found in China, those of x = 8 are found in China and North America, and those of x = 9 are commonly found in the world. Their karyotypes had commonly monomodal gradation in chromosome length from the longest to the shortest chromosomes and had median and submedian centromeric chromosomes, rarely median, submedian, and subterminal centromeric chromosomes (Table 3; Figure 5). Thus, *Parnassia* could be divided by four groups at least from chromosome characters mentioned above. (1) Small-sized and median-centromeric chromosomes ranged from 1.6 to 1.0 μm long with average 1.21 μm long, and had the base number of x = 8. (2) Medium-sized and median- and submedian-centromeric chromosomes ranged from 2.8 to 1.9 μm long with average 2.29 μm long and had the base number of x = 9. (3) Large-sized and median- and submedian-centromeric chromosomes ranged from 3.6 to 2.7 μm long with average 3.10 μm long and had the base number of x = 9. (4) Medium-sized and median-, submedian-, and subterminal-centromeric chromosomes ranged from 2.3 to 1.4 μm long with average 1.91 μm long, and had the base number of x = 7. These cytotaxonomical groupings were not agreed with the taxonomical treatment by Ku [39]. More cytological information and another new technique are necessary to clarify and justify the cytosystematic relationships of *Parnassia*.

3.4. Intraspecific Polyploid and Aneuploid. Intraspecific polyploid of 2n = 18 and 36 for *P. oreophylla*, *P. viridiflora*, and *P. laxmannii*, and that of 2n = 18, 27, 36, 45, and 54 for *P. palustris* were reported, especially this polyploidy for *P. palustris* was found in Scandinavia [20, 21] and Great Britain [34, 35], and 2n = 27 and 45 chromosomes were also found in Russia [22, 23]. For example, 156 samples of this species were collected in 23 localities in Altai Mountains, Tuva and Krasnoyarsk region in Russia, of which 113 samples (72.4%) in 18 sites had 2n = 18 chromosomes, 12 samples (7.7%) in three sites had 2n = 27 chromosomes, 29 samples (18.6%) in five sites had 2n = 36 chromosomes, and two samples (1.3%) in one site had 2n = 45 chromosomes (Figure 6; [22, 23]; Funamoto and Smirnov unpublished).
Figure 2: Karyomorphological comparisons of resting and mitotic prophase chromosomes in 14 species of Parnassia. (a)–(n) The resting chromosomes; (a′)–(n′) the mitotic prophase chromosomes. (a) and (a′) P. yunnanensis var. longistipitata; (b) and (b′) P. scaposa var. yushuensis; (c) and (c′) P. aff. cacuminum f. yushuensis; (d) and (d′) P. oreophyla; (e) and (e′) P. trinervis; (f) and (f′) P. viridiflora; (g) and (g′) P. laxmannii; (h) and (h′) P. brevistyila; (i) and (i′) P. delavayi; (j) and (j′) P. alpicola; (k) and (k′) P. bifolia; (l) and (l′) P. wrightiana; (m) and (m′) P. foliosa; (n) and (n′) P. palustris. Scale bar = 5 µm.
According to pollen stainability and sizes of pollen grains of *Parnassia palustris*, plants of $2n = 18$ and 36 had high pollen stainabilities (81.4–98.2%), while those of $2n = 27$ and 45 had very low pollen stainabilities (0.74–0.85%). Sizes of pollen grains of the species were 27.3–30.3 µm × 25.8–28.1 µm in plants of $2n = 18$, 30.1–32.5 µm × 27.6–30.2 µm in plants of $2n = 27$, and 31.8–33.6 µm × 30.1–31.3 µm in plants of $2n = 36$, respectively. Significant $t$-test in sizes.
Figure 4: Variations of supernumerary chromosomes of *Parnassia bifolia*. Arrow indicates satellites and arrows head indicates supernumerary chromosomes. (a) $2n = 36$. (b) $2n = 36 + 1s$; (c) $2n = 36 + 2s$; (d) $2n = 36 + 3s$; (e) $2n = 36 + 5s$; (f) $2n = 36 + 7s$; (g) $2n = 36 + 8s$. (s) supernumerary chromosomes. Scale bar = 5 $\mu$m.

of pollen grains showed significant difference between the plants of $2n = 18$ and $2n = 36$ with $P < 0.05$ and $P < 0.01$ levels, respectively. Sizes of pollen grains could be correlated with polyploid levels [22, 23]. Similar results were reported with Japanese *Deutzia crenata* [40–42] and Japanese *Hydrangea paniculata* [43].

The plants with the chromosome number of $2n = 18$ were commonly found along the vegetation edges such as in the disturbed riverine, forest meadows and tundra in Russian and Mongolian Altai, and Tuva in Russia, those with the chromosome number of $2n = 27$ were found in placid lake side and together with the plants of the chromosome number
Figure 5: Karyotype comparisons in 14 species of Parnassia. (a) *P. yunnanensis* var. *longistipitata*; (b) *P. scaposa* var. *yushuensis*; (c) *P. aff. cacuminum* f. *yushuensis*; (d) *P. oreophylla*; (e) *P. trinervis*; (f) *P. viridiflora*; (g) *P. laxmannii*; (h) *P. brevistyli*a; (i) *P. delavayi*; (j) *P. alpicola*; (k) *P. bifolia*; (l) *P. wightiana*; (m) *P. foliosa*; (n) *P. palustris*. Arrows indicate satellites. Scale bar = 5 μm.
2n = 18 in Russian Altai, while those with the chromosome number of 2n = 36 were commonly found in narrow stream in mountain slope and mountain meadows in Russian Altai and Krasnoyarsk Region in Russia, and those with the chromosome number of 2n = 45 were rarely found together with the plants with the chromosome number of 2n = 36 in Russian Altai. Thus, *Parnassia palustris* in Altai Mountains and adjacent regions in Russia seemed one of the well-diversified species with respect to chromosome characters. These cytological phenomena speculated that the two plant strains with the chromosome numbers of 2n = 27 and 2n = 45 might be isolated by pollination between nondisjunction diploid gametes producing diploid and tetraploid ovules (2x and 4x) and normal male gamete for diploid pollen (x), since any plant with the chromosome number of 2n = 27 has never been found within the populations of the plants with the chromosome number of 2n = 36. This factor might be caused by various geographical conditions and extremes of climate temperature [22, 23].

Acknowledgments

The authors wish to express sincere appreciation to Professor Dr. Joan Vallés, Barceona University for a long-term leadership of the field of chromosome botany and to commemorate his celebration of retirement. They thank many Chinese, Russian, and Mongolian botanists for guiding their field trips and identifying plant materials. This study was supported by the Grant-in-Aid for the Monbusho International Research Program-Joint Research no. 04044128 (the representative: Ryuso Tanaka) and no. 06044160 (the representative: Katsuhiro Kondo) to the Ministry of Education, Science, Sports and Culture of Japan, and the Grant-in-Aid for Scientific Research Program (A) (1) no. 10044209, no. 14255014, and no. 19255004 (the representative: Katsuhiro Kondo) of the Japan Society for the Promotion of Science.

References


Figure 6: Map of collection sites and polyploidy of *Parnassia palustris* in Altai Mountains and the adjacent regions. Chromosome numbers of 2n = 18 (●), 2n = 27 (▲), 2n = 36 (★), and 2n = 45 (■).


