Intraspecific Karyotypic Polymorphism and Cytogeography of *Lysimachia mauritiana* (Primulaceae) on Several Islands in the Okinawa and the Daito Groups of the Ryukyu Archipelago, Japan

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**Summary**

A coastal biennial herb, *Lysimachia mauritiana*, exhibited remarkable intraspecific karyotypic diversity, especially in the Ryukyu Archipelago of Japan (the Ryukyus). The species displayed five chromosome numbers (*2n*= 16, 17, 18, 19, 20) and 18 cytotypes in the Ryukyus alone. Our serial investigations elucidated that: (1) 11 cytotypes on Takarajima Island (Is.) showed the highest intra- and inter-populational cytotype polymorphism in the Ryukyus, (2) a total of 15 cytotypes was recognized and several cytotypes coexisted in every locality on Amamioshima, Kakeromajima and Tokunoshima Is., and (3) closely located two Islands of Amamioshima and Tokunoshima had different dominant cytotypes, 16 (*6m*) and 18 (*6m*), respectively. In the present study, to explore whether a similar karyotypic polymorphism exists on their neighbor islands belonging to the Okinawa and the Daito Groups, a total of 610 plants from 51 localities on 10 islands were analyzed karyomorphologically and cytogeographically. As a result, five chromosome numbers (*2n*= 16, 17, 18, 19, 20) and 13 cytotypes were recognized in the areas. Okinawajima Is. was divided into two areas according to cytotype distribution patterns. Northern and western areas facing the East China Sea showed intra- and inter-populational polymorphism in cytotype. By contrast, southern, eastern and northeastern areas facing the Pacific Ocean showed a single cytotype in a locality. In the Daito Group, 20 (*4m*) TS cytotype with a wide distribution in southern Taiwan was found in Japan for the first time. The cytotype probably descended from currents of southern Taiwan and/or the Philippines.

**Key words** Cytogeography, Cytotypic variation, Intraspecific karyotypic polymorphism, *Lysimachia mauritiana*, Ryukyu Archipelago of Japan.

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Our most recent study focusing on the intraspecific karyotypic polymorphism and cytogeography in the Ryukyus revealed that five chromosome numbers (*2n*= 16–20) and 15 cytotypes were recognized on Amamioshima, Kakeromajima and Tokunoshima Is. belonging to the Amami Group of the Ryukyus. Most localities showed intra-populational variations in cytotype (Kono *et al.* 2015). The islands had different dominant cytotypes, namely 16 (*6m*) on Amamioshima Is. and 18 (*6m*) on Tokunoshima Is. On Kakeromajima Is., every locality showed different cytotype structure. Additionally on Amamioshima Is., it was more likely that cytotypes in the northwestern area facing the East China Sea might be converged to 16 (*6m*) in the near future according to dynamic analyses in cytotype.

To explore whether a similar wide-ranging karyotypic polymorphism exists on the neighboring islands belonging to the Okinawa and the Daito Groups, detailed
Table 1. Collection localities and karyotypes of *Lysimachia mauritiana* on Okinawajima Is. and its neighbor islands in the Okinawa Group.

<table>
<thead>
<tr>
<th>Population</th>
<th>Locality [Latitude (N), Longitude (E)]</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okinawajima Island</td>
<td></td>
<td>2n</td>
<td>Formula</td>
<td></td>
</tr>
</tbody>
</table>
| OK-1       | Hedo, Kunigami-son  
(26°52′25″, 128°15′48″′) | 20        | 4m+2sm+14t           | 31                       | 34                       |
| OK-2       | Ginama, Kunigami-son  
(26°51′03″, 128°15′12″′) | 18        | 6m+2sm+10t           | 3                        | 5                        |
| OK-3       | Uka, Kunigami-son  
(26°49′53″, 128°14′46″′) | 18        | 5m+3sm+11t           | 1                        | 13                       |
| OK-4       | Jashiki, Kunigami-son  
(26°46′57″, 128°12′57″′) | 20        | 4m+4sm+12t           | 12                       | 12                       |
| OK-5       | Hentona, Kunigami-son  
(26°44′57″, 128°10′58″′) | 19        | 6m+4sm+1st+8t        | 1                        | 13                       |
| OK-6       | Kijoka, Ogimi-son  
(26°42′38″, 128°08′44″′) | 18        | 5m+3sm+11t           | 1                        | 18                       |
| OK-7       | Inamine, Nago-shi  
(26°38′09″, 128°02′31″′) | 18        | 6m+3sm+1st+10t       | 1                        | 1                        |
| OK-8       | Yonamine, Nukijin-son  
(26°42′27″, 127°57′02″′) | 20        | 4m+2sm+2st+12t       | 10                       | 15                       |
| OK-9       | Imadomari, Nukijin-son  
(26°42′07″, 127°55′32″′) | 19        | 5m+2sm+1st+11t       | 1                        | 1                        |
| OK-10      | Shinzato, Motobu-cho  
(26°42′05″, 127°53′50″′) | 18        | 6m+2sm+1st+9t        | 6                        | 8                        |
| OK-11      | Sakinomoto, Motobu-cho  
(26°37′27″, 127°53′21″′) | 20        | 4m+2sm+2st+12t       | 1                        | 1                        |
| OK-12      | Miyazato, Nago-shi  
(26°35′30″, 127°58′15″′) | 18        | 6m+3sm+9t            | 18                       | 24                       |
| OK-13      | Ahuso, Onna-son  
(26°30′26″, 127°53′50″′) | 17        | 7m+2sm+8t            | 4                        | 6                        |
| OK-14      | Onna, Onna-son  
(26°28′53″, 127°50′45″′) | 16        | 6m+1sm+3st+7t        | 2                        | 12                       |
| OK-15      | Maeda, Onna-son  
(26°26′37″, 127°46′20″′) | 16        | 6m+2sm+2st+6t        | 12                       | 15                       |
| OK-16      | Uza, Yomitani-son  
(26°26′24″, 127°42′48″′) | 17        | 7m+3sm+7t            | 1                        | 13                       |
| OK-17      | Namihira, Yomitani-son  
(26°23′50″, 127°43′10″′) | 19        | 5m+1sm+3sm+10t       | 2                        | 10                       |
| OK-18      | Toguchi, Yomitani-son  
(26°21′58″, 127°44′14″′) | 18        | 6m+2sm+2st+10t       | 7                        | 14                       |
| OK-19      | Mihama, Chatan-cho  
(26°18′29″, 127°45′39″′) | 18        | 4m+2sm+2st+10t       | 6                        | 6                        |
| OK-20      | Yahuso, Urasoe-shi  
(26°15′18″, 127°41′47″′) | 18        | 4m+2sm+2sm+10t       | 20                       | 20                       |
| OK-21      | Kyan, Itoman-shi  
(26°15′18″, 127°41′47″′) | 18        | 4m+2sm+2sm+10t       | 4                        | 4                        |
| OK-22      | Chinenkudeken, Nanjo-shi  
(28°10′28″, 127°49′48″′) | 18        | 4m+2sm+2st+10t       | 8                        | 8                        |
| OK-23      | Sashihikusozaki, Nanjo-shi  
(26°10′28″, 127°47′27″′) | 18        | 4m+2sm+2sm+10t       | 17                       | 17                       |
| OK-24      | Itarashiki, Yonaharu-cho  
(26°11′33″, 127°46′17″′) | 18        | 4m+2sm+2sm+10t       | 12                       | 12                       |
| OK-25      | Awasu, Okinawa-shi  
(26°19′11″, 127°50′36″′) | 18        | 4m+2sm+2sm+10t       | 11                       | 11                       |
| OK-26      | Konbu, Uruma-shi  
(26°23′54″, 127°51′17″′) | 18        | 4m+2sm+2sm+10t       | 12                       | 12                       |
karyomorphological and cytogeographical analyses were conducted (Kono et al. 2013, 2015). This is the third report on an intra- and inter-populational karyotypic polymorphism and the cytogeography of L. mauritiana from the middle Ryukyus. The aims of this study were to investigate (1) chromosome numbers and cytotypes, (2) cytotype composition in each population, (3) cytogeography on Okinawajima Is. and its offshore islands from the Okinawa Group and, on Kita- and Minami-daitojima Is. of the Daito Group.

Materials and methods

For karyotypic analyses, the samplings of Lysimachia

<table>
<thead>
<tr>
<th>Population</th>
<th>Locality</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OK-27</td>
<td>Ginoza, Ginoza-son (26°29'07&quot;, 128°00'06&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>11</td>
<td>11</td>
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<tr>
<td>OK-28</td>
<td>Kayo, Nago-shi (26°32'58&quot;, 128°06'24&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>OK-29</td>
<td>Teniya, Nago-shi (26°33'58&quot;, 128°08'13&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OK-30</td>
<td>Gesashi, Higashi-son (26°35'58&quot;, 128°09'02&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>OK-31</td>
<td>Taira, Higashi-son (26°37'51&quot;, 128°09'20&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>15</td>
<td>15</td>
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<tr>
<td>OK-32</td>
<td>Aha, Kunigami-son (26°42'24&quot;, 128°17'28&quot;)</td>
<td>19 4m+2m+2sm+11t</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>OK-33</td>
<td>Ada, Kunigami-son (26°45'17&quot;, 128°19'24&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>11</td>
<td>11</td>
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<tr>
<td>OK-34</td>
<td>Susu, Kunigami-son (26°47'40&quot;, 128°19'05&quot;)</td>
<td>17 5m+2m+2sm+8t</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>OK-35</td>
<td>Oku 1, Kunigami-son (26°49'17&quot;, 128°18'47&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>10</td>
<td>10</td>
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<tr>
<td>OK-36</td>
<td>Oku 2, Kunigami-son (26°50'26&quot;, 128°17'32&quot;)</td>
<td>20 4m+2sm+14t</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19 5m+2sm+12t</td>
<td>1</td>
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<td>18 5m+1m+2sm+10t</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>17 4m+2m+2sm+10t</td>
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</table>

Kourijima Island

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<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
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</thead>
<tbody>
<tr>
<td>KUR-1</td>
<td>Kouri, Nakijin-son (26°41'46&quot;, 128°00'58&quot;)</td>
<td>19 5m+2sm+2st+10t</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 6m+2sm+2st+8t</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>17 7m+3sm+7t</td>
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Yagajijima Island

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<thead>
<tr>
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<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
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<tbody>
<tr>
<td>YGJ-1</td>
<td>Sumuide, Nago-shi (26°39'40&quot;, 128°01'36&quot;)</td>
<td>20 4m+2sm+2st+12t</td>
<td>3</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>18 6m+2sm+2st+8t</td>
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Iejima Island

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<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
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<tbody>
<tr>
<td>IE-1</td>
<td>Higashiemae, le-son (26°43'01&quot;, 127°49'46&quot;)</td>
<td>20 4m+2sm+2st+12t</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 5m+2sm+2st+12t</td>
<td>7</td>
</tr>
<tr>
<td>IE-2</td>
<td>Higashieue 1, le-son (26°44'12&quot;, 127°48'30&quot;)</td>
<td>12 4m+2sm+2st+12t</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>IE-3</td>
<td>Higashieue 2, le-son (26°43'57&quot;, 127°47'23&quot;)</td>
<td>18 4m+1m+2sm+1st+11t</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 6m+2sm+10t</td>
<td>1</td>
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Sesokojima Island

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<th>Locality</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
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</thead>
<tbody>
<tr>
<td>SSK-1</td>
<td>Sesoko, Motobu-cho (26°38'56&quot;, 127°51'22&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>11</td>
<td>11</td>
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</tbody>
</table>

Hamahigajima Island

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<th>Population</th>
<th>Locality</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHG-1</td>
<td>Katsurenhiga, Uruma-shi (26°19'10&quot;, 127°57'57&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>7</td>
<td>7</td>
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</tbody>
</table>

Miyagijima Island

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<thead>
<tr>
<th>Population</th>
<th>Locality</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYG-1</td>
<td>Yonashirokumi, Uruma-shi (26°22'38&quot;, 127°59'12&quot;)</td>
<td>18 4m+2m+2sm+10t</td>
<td>9</td>
<td>9</td>
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Ikeijima Island

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<th>Locality</th>
<th>Karyotype1</th>
<th>No. of plants in 2012</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IKI-1</td>
<td>Yonashirokumi, Uruma-shi (26°33'29&quot;, 127°59'28&quot;)</td>
<td>18 4m+2m+2st+10t</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

1 Predominant karyotypes of respective localities are listed. m, longer metacentric chromosome used as the marker chromosomes.
mauritiana were broadly conducted in the Okinawa Group: Okinawajima, Kourijima, Yagaijima, Iejima, Sesokojima, Hamahigajima, Miyagijima and Ikeijima Is. in 2012 and in the Daito Group: Kita- and Minami-daitojima Is. in 2015, respectively (Tables 1 and 2, Figs. 1 and 2). Okinawajima Is. is the biggest major island of the group with ca. 476 km around, ca. 503 m height and ca. 1207 km² in area. In this study, a total of 610 individuals from 51 localities on 10 islands were karyomorphologically and cytogeographically analyzed; two to 34 plants per locality were collected (average: 12.0 plants). They mostly grow on sandy and/or gravel coasts. Population numbers and locality information are summarized in detail in Tables 1 and 2.

Detailed cytological procedures of collecting, pretreatment, fixation, staining and chromosome observations were described in Kono et al. (2012). Classifications of chromosome morphology were based on the centromere position following Levan et al. (1964). Voucher specimens were deposited in the herbarium of University of the Ryukyus (RYU).

Similarly, in our serial studies of karyotypic polymorphism in L. mauritiana (Oginuma et al. 2004a, 2004b, Kono et al. 2008, 2010, 2012, 2013, 2015), marker chromosomes (longer metacentric chromosomes, m) and cytotype(s) were adopted for the simplification of its wide-ranging karyotypic polymorphism.

### Results and discussion

Chromosome numbers and predominant karyotypic formulae of L. mauritiana in the respective locality are summarized in Tables 1 and 2. Among 610 plants, 12 cytotypes of five chromosome numbers \(2n=16–20\) in the Okinawa Group (Fig. 1) and two cytotypes of two chromosome numbers \(2n=18, 20\) in the Daito Group (Fig. 2) were identified.

### Table 2. Collection localities and karyotypes of Lysimachia mauritiana on Kita- and Minami-daitojima Is. in the Daito Group.

<table>
<thead>
<tr>
<th>Population</th>
<th>Locality</th>
<th>Karyotype (^1)</th>
<th>No. of plants in 2015</th>
<th>Total number of plants (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitadaitojima Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KDT-1</td>
<td>Minami, Kitadaito-son (25°56’38″, 131°19’42″)</td>
<td>20 (4m+4sm+2st+10t)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>KDT-2</td>
<td>Minato, Kitadaito-son (25°57’28″, 131°17’08″)</td>
<td>20 (4m+4sm+2st+10t)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Minamidaitojima Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDT-1</td>
<td>Ikenosawa 1, Minamidaito-son (25°50’34″, 131°13’05″)</td>
<td>18 (4m+2m+2sm+10t)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>MDT-2</td>
<td>Ikenosawa 2, Minamidaito-son (25°51’02″, 131°13’14″)</td>
<td>18 (4m+2m+2sm+10t)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MDT-3</td>
<td>Kita, Minamidaito-son (25°52’13″, 131°14’13″)</td>
<td>20 (4m+4sm+2st+10t)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>MDT-4</td>
<td>Kyuto, Minamidaito-son (25°49’57″, 131°16’02″)</td>
<td>20 (4m+4sm+2st+10t)</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^1\) Predominant karyotypes of respective locality are listed. m, longer metacentric chromosome used as the marker chromosome.

Cytotypes of respective locality in Okinawajima Island (Table 1, Fig. 1)

The appearance frequencies in each locality are described below in descending order of frequency. The same shall apply hereinafter.

1. OK-1
   - Two cytotypes co-occurred: 91.2% in 20 (4m) HD-type; 8.8% in 18 (6m).
2. OK-2
   - Three cytotypes co-occurred: 60.0% in 18 (6m); 20.0% in 19 (5m) and 20 (4m) MR-type.
3. OK-3
   - Two cytotypes co-occurred: 92.3% in 18 (4m); 7.7% in 18 (5m).
4. OK-4
   - Four cytotypes co-occurred: 50.0% in 18 (6m); 25.0% in 17 (7m); 16.7% in 19 (5m); 8.3% in 20 (4m) MR-type.
5. OK-5
   - Three cytotypes co-occurred: 84.6% in 18 (6m); 7.7% in 19 (5m) and 19 (6m).
6. OK-6
   - Four cytotypes co-occurred: 54.1% in 20 (4m) MR-type; 16.7% in 18 (6m) and 19 (5m); 12.5% in 17 (7m).
7. OK-7
   - Four cytotypes co-occurred: 83.2% in 18 (4m); 5.6% in 18 (6m), 19 (5m) and 19 (6m).
8. OK-8
   - Three cytotypes co-occurred: 66.7% in 20 (4m) MR-type; 26.7% in 18 (6m); 6.6% in 19 (5m).
9. OK-9
   - A single cytotype 20 (4m) MR-type occurred.
10. OK-10
    - A single cytotype 20 (4m) MR-type occurred.
11. OK-11
    - A single cytotype 18 (4m) occurred.
12. OK-12
    - Three cytotypes co-occurred: 75.0% in 18 (6m); 16.7% in 17 (7m); 8.3% in 17 (6m).
Two cytotypes co-occurred: 92.3% in 16 (6m); 7.7% in 17 (5m).

(14) OK-14
Two cytotypes co-occurred: 80.0% in 16 (6m); 20.0% in 17 (6m).

(15) OK-15
Two cytotypes co-occurred: 92.3% in 18 (6m); 7.7% in 17 (7m).

(16) OK-16
Two cytotypes co-occurred: 72.4% in 18 (6m); 27.6% in 18 (4m).

(17) OK-17
Three cytotypes co-occurred: 70.0% in 18 (4m); 20.0% in 19 (5m); 10.0% in 18 (6m).

(18) OK-18 to -31
A single cytotype 18 (4m) occurred.

(19) OK-32
Two cytotypes co-occurred: 91.7% in 18 (4m); 8.3% in 19 (4m).

(20) OK-33
Two cytotypes co-occurred: 95.8% in 18 (4m); 4.2% in 17 (5m).

(21) OK-34 and -35
A single cytotype 18 (4m) occurred.

(22) OK-36
Four cytotypes co-occurred: 63.6% in 18 (4m); 18.2% in 20 (4m) HD-type; 9.1% in 18 (5m) and 19 (5m).
Cytotypes of respective locality on offshore islands, Kourijima, Yagajijima, Iejima, Sesokojima, Hamahigajima, Miyagijima and Iketijima Is. (Table 1, Fig. 1)

- (23) KUR-1
  - Three cytotypes co-occurred: 50.0% in 18 (6m); 40.0% in 19 (5m); 10.0% in 17 (7m).
- (24) YGJ-1
  - Two cytotypes co-occurred: 50.0% in 18 (6m) and 20 (4m) MR-type.
- (25) IE-1 and -2
  - A single cytotype 20 (4m) MR-type occurred.
- (26) IE-3
  - Two cytotypes co-occurred: 50.0% in 18 (6m) and 19 (4m).
- (27) SSK-1, HHG-1, MYG-1 and IKI-1
  - A single cytotype 18 (4m) occurred.

Cytotypes of respective locality in Kita- and Minamidaitojima Is. (Table 2, Fig. 2)

- (28) KDT-1 and -2
  - A single cytotype 20 (4m) TS-type occurred.
- (29) MDT-1 and -2
  - A single cytotype 18 (4m) occurred.
- (30) MDT-3
  - Two cytotypes co-occurred: 50.0% in 18 (4m) and 20 (4m) TS-type.
- (31) MDT-4
  - A single cytotype 20 (4m) TS-type occurred.

Intraspecific karyotypic polymorphism and cytogeography in the Okinawa Group
On Okinawajima Is. and the offshore islands, 45 localities were divided into two areas according to patterns of cytotype distribution (Fig. 1). The northern and western localities facing the East China Sea (OK-1 to -17 and -36 including the neighboring four islands) predominantly showed intra-populational polymorphisms in cytotypes with the exception of six localities, i.e., OK-9, -10, -11, IE-1, -2 and SSK-1 having a single cytotype. In the areas, every locality showed a different dominant cytotype. By contrast, the southern, eastern and northeastern localities facing the Pacific Ocean (OK-18 to -35 including its offshore three islands) showed a single cytotype in a locality, except for two localities, OK-32 and -33 showing coexistence of two cytotypes (Fig. 1).

Cytogeographical distribution patterns of major cytotypes in the species were as follows.

1. 20 (4m) HD-type
   - Previously, 20 (4m) cytotype was considered to be one of the most common and major cytotype in the species (e.g., Tanaka and Hizume 1978, Kono et al. 2011). In our studies, we also presumed that it is more likely to be an origin of its karyotypic polymorphism. Thus far, three distinguishable 20 (4m) cytotypes were found based on the analyses of their detailed karyotypic formulae (Kono et al. 2010). The 20 (4m) cytotype distributed only in OK-1 and -36 was a cytotype specific to the area and had never been found in other places before. Its karyotypic formula \(2n=20=4m+2s+14t\) clearly differed from those of 20 (4m) JM-type \(2n=20=4m+2s+4st+10t\) and 20 (4m) MR-type \(2n=20=4m+2s+2st+12t\) previously reported (Kono et al. 2011, 2015). Thus we designated it as ‘20 (4m) HD-type (Hedo cape-type).’
2. 20 (4m) MR-type
   - The cytotype was distributed in small areas on Tokunoshima Is. in the middle Ryukyus (Kono et al. 2015). The cytotype was distributed in the northeastern areas on Okinawajima Is. from Kunigami-son to Motobu-cho (OK-2, -4, -6, -8, -9, -10 and YGI-1). Especially in three localities on the Motobu Peninsula (OK-8, -9, -10), it consisted mostly of cytotypes covering 66.7% in OK-8 and 100.0% in OK-9 and -10. The cytotype was scattered about the islands in the middle Ryukyus.
3. 18 (6m) cytotype
   - The cytotype was distributed from the northern to the eastern areas on Okinawajima Is. with three neighboring islands (OK-1, -2, -4, -5, -6, -7, -8, -12, -15 to -17, KUR-1, YGI-1 and IE-3) facing the East China Sea. No plant with 18 (6m) cytotype was found in areas facing the Pacific Ocean thus far. The cytotype was previously found on Takarajima, Kakeromajima and Tokunoshima Is. in the middle Ryukyus (Kono et al. 2013, 2015); every islands in the southern Ryukyus, except for Yonagunijima Is. (Kono et al. 2010, Oginuma et al. 2004a). It was also dominant in the islands of the southern Ryukyus (Kono et al. 2010) and in every 10 localities on Tokunoshima Is. (Kono et al. 2015).
4. 18 (4m) cytotype
   - The cytotype was distributed broadly from the south-
ern to the northeastern areas on Okinawajima Is. facing the Pacific Ocean and rarely coexisted with other cytotypes in a population. Especially in localities from OK-18 to -31, all plants were 18 (4m) cytotype, and inter-populational cytotype variations have never been seen. Considering our cytogeographical studies, a co-existence place of the two predominant cytotypes, 18 (4m) and 18 (6m), has never been found in the islands of the Amami Group (Kono et al. 2015), but rarely in five localities on Takarajima Is. (Kono et al. 2013). Similar to the Okinawa Group, a similar situation occurred in limited three localities (OK-7, -16 and -17).

(5) 16 (6m) cytotype

The cytotype was found in small areas on Okinawajima Is. (OK-13 and -14). The karyotypic formula of the cytotype (2n=16=6m+2sm+2st+10t) was similar to the dominant cytotype on Amamioshima Is. (Kono et al. 2015). The cytotype was also grown in Takarajima Is. (Kono et al. 2013) and Kakeromajima Is. (Kono et al. 2015); therefore, the similar karyotypic polymorphism coincidentally occurred in several places of the middle Ryukyus.

Intraspecific karyotypic polymorphism and cytogeography in the Daito Group

Among six localities on two small islands of the Daito Group, intra-populational variation in cytotypes was unidentified with the exception of MDT-3 (Fig. 2). On Kita-daitojima Is., a single cytotype 2n=20 (4m) grew in both localities (Fig. 2). By contrast in Minami-daitojima Is., 18 (4m) cytotype occupied MDT-1 and -2; 20 (4m) cytotype in MDT-4; and 18 (4m) and 20 (4m) cytotypes coexisted in MDT-3 (Fig. 2). The karyotypic formula of the 20 (4m) cytotype (2n=20=4m+4sm+2st+10t) was clearly distinguishable from those of other 20 (4m) cytotypes detected in Japan, i.e. JM-type (2n=20=4m+2sm+4st+10t), MR-type (2n=20=4m+2sm+2st+12t) and HD-type (2n=20=4m+2sm+14t) (Kono et al. 2008, 2010, 2011, 2013, 2015), and was similar to that of 20 (4m) TS cytotype grown widely in southern Taiwan including two offshore islands, the Philippines, in the Hawaiian Islands and Rota of the Northern Mariana Islands (Kono et al. 2012). Thus, the present study is the first report to reveal that 20 (4m) TS cytotype has also grown in Japan. In the Daito Group, 20 (4m) TS cytotype is probably descended from sea dispersals from southern Taiwan and/or the Philippines; and 18 (4m) cytotype, from southern and/or eastern coasts on Okinawajima Is. because of its maritime habitat and buoyant capsules (Kono et al. 2012).

Further additional karyomorphological studies and observations using FISH are underway in order to more fully clarify the cytogeographic structure and relationships/origins of cytotype polymorphisms in the species.

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