Variation in Ploidy and Karyological Diversity in Different Herbaceous Peony Cultivar Groups

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ABSTRACT. The karyotypes of 21 herbaceous peony (Paeonia) cultivars were studied using root tip squashes revealing a wide variety of ploidy levels. There were three tetraploid (2n = 4x = 20), 11 triploid (2n = 3x = 15), and one diploid (2n = 2x = 10) cultivars in the hybrid group; five triploid (2n = 3x = 15) cultivars in the Itoh group; and one diploid (2n = 2x = 10) cultivar in the lactiflora group (LG). The asymmetry index (AI) ranged from 59.61% (‘Cytherea’) to 64.03% (‘Little Red Gem’). The karyotypes of all peony cultivars were 2A with 60% metacentric (m), 20% submetacentric (sm), and 20% subtelocentric (st) chromosomes. The karyotypic background of these 21 herbaceous peony cultivars is discussed in the context of the evolution of ploidy and the three cultivar groups. These results provide cytological information that would assist in a peony hybridization program.

Herbaceous peonies are perennial flowers belonging to section Paeonia of the genus Paeonia in the Paeoniaceae. Herbaceous peonies usually blossom from April to June and have a variety of flower patterns and flower colors, making them famous traditional flowers in China and offering them an extremely important position in the global cut-flower market. They have a variety of flower patterns and flower colors, making them one of the most popular flowers in the world. However, they have not been systematically studied. Paeonia lactiflora (section lactiflora) and P. peregrina (section Moutan) are both diploid (2n = 2x = 10). P. lactiflora is usually 2n = 2x = 10, a triploid (2n = 3x = 15), and one diploid (2n = 2x = 10). Cultivars of the hybrid group (HG) include ‘Zhu Sha Pan’ and ‘Cream Delight’. The asymmetry index (AI) ranged from 59.61% (‘Cytherea’) to 64.03% (‘Little Red Gem’). The karyotypes of all peony cultivars were 2A with 60% metacentric (m), 20% submetacentric (sm), and 20% subtelocentric (st) chromosomes. The karyotypic background of these 21 herbaceous peony cultivars is discussed in the context of the evolution of ploidy and the three cultivar groups. These results provide cytological information that would assist in a peony hybridization program.
Karyotype analysis, which is the comparison of chromosome measurements, is a traditional cytogenetic step to compare genomes among related species. In this study, a karyotypic analysis of the chromosomes of 21 non-Chinese cultivars of herbaceous peony is reported. The characteristics and variation in chromosome number and karyotype have also been analyzed. Cytological data of these cultivars will allow better guidance of future breeding work.

Materials and Methods

PLANT MATERIALS. The herbaceous peonies used in this study (Table 1) were planted at the Xiaotangshan cultivation base, Beijing Forestry University, National Engineer Research Center for Floriculture, Beijing, China.

METHODS. Actively growing root tips (1–3 cm) were cut and pretreated with cycloheximide for 6–8 h in the dark, fixed in Carnoy’s solution (100% ethanol:acetic acid = 3:1) for 24 h, and then stored in ethanol at 4°C until use. Root tips were hydrolyzed in 1 M HCl at 60°C for 10 min, washed with distilled water three times and stained by improved carbol fuchsin for 5–10 min. Karyokinetic observations were made and documented by Leica Application Suite version 3 (Leica Microsystems, Wetzlar, Germany). All chemicals (100% ethanol, acetic acid, HCl, and carbol fuchsin) were purchased from Lanyi Co. (Beijing, China). Chromosome measurements were made using five well-spread metaphase plates per population. Karyotype analysis was performed using the criteria described by Chen et al. (2003) and Li and Zhang (1982). Karyotype symmetry was classified according to Stebbins (1971) as shown in Table 2 in this article. The AI percent (long arm length/total genome length × 100%) was determined according to the calculation defined by Arano (1963). The index of relative length [IRL (chromosome length/mean genome length)] and composition of the relative length of the genome [CRL (chromosome length/genome length)] were

Table 1. Information about the group, flower color, and parents of the 21 herbaceous peony cultivars that were used in the study of variation in ploidy and karyological diversity.

| Code | Group | Cultivar | Flower color | Parents |
|------|-------|---------|--------------|---------|
| A    | Lactiflora | Henry Sass | Pure white | Unknown |
| B    | Itoh | Border Charm | Yellow | 'Carr East' × Paeonia lactiflora 'Yaso-okina' × P. lutea |
| C    | Itoh | Going Bananas | Yellow | Unknown |
| D    | Itoh | Old Rose Dandy | Dark yellow with dark red | Unknown |
| E    | Itoh | Prairie Charm | Yellow | P. lactiflora 'Miss America' × Paeonia lactiflora |
| F    | Itoh | Lemon Dream | Yellow | P. lactiflora 'Martha Washington' × Paeonia 'Golden Era' |
| G    | Hybrid | Carina | Brilliant scarlet | P. lactiflora × P. peregrina |
| H    | Hybrid | Chalice | White | P. lactiflora × P. macropylla |
| I    | Hybrid | Command Performance | Cardinal red | P. lactiflora × Paeonia 'Good Cheer' (P. officinalis 'Alba Plena' × P. peregrina) |
| J    | Hybrid | Cytherea | Bright rose pink | P. lactiflora × P. peregrina |
| K    | Hybrid | Etched Salmon | Pink | Unknown |
| L    | Hybrid | Fairy Princess | Red | Unknown |
| M    | Hybrid | Henry Bockstoe | Dark red | P. officinalis × P. lactiflora |
| N    | Hybrid | Joker | Pink | Unknown |
| O    | Hybrid | Lovely Rose | Pink | P. lactiflora × P. peregrina |
| P    | Hybrid | Little red gem | Light red | Unknown |
| Q    | Hybrid | Many Happy Returns | Red | P. lactiflora 'Nippon Splendor' × Paeonia 'Good Cheer' (P. officinalis × P. peregrina) |
| R    | Hybrid | Old Faithful | Red | Unknown |
| S    | Hybrid | Prairie Moon | Pale yellow | P. lactiflora 'Laura Magnuson' × Paeonia 'Archangel' (P. lactiflora × P. macropylla) |
| T    | Hybrid | Roy Pehrson's Best Yellow | Soft yellow | Unknown |
| U    | Hybrid | Scarlet O’Hara | Red | P. officinalis × P. lactiflora |

Table 2. Classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971) and relative chromosome length of 21 herbaceous peony cultivars.

| Lt/Stz | IRLx | IRL typew |
|-------|------|----------|
| <2:1 | 0.0 | 1A |
| 2:1–4:1 | 0.01–0.50 | 2A |
| >4:1 | 0.51–0.99 | 3A |
| >4:1 | 1.00 | 4A |
| L = long chromosomes, M2 = medium chromosomes 2, M1 = medium chromosomes 1, S = short chromosomes. |

Karyotype analysis, which is the comparison of chromosome measurements, is a traditional cytogenetic step to compare genomes among related species. In this study, a karyotypic analysis of the chromosomes of 21 non-Chinese cultivars of herbaceous peony is reported. The characteristics and variation in chromosome number and karyotype have also been analyzed. Cytological data of these cultivars will allow better guidance of future breeding work.

Materials and Methods

PLANT MATERIALS. The herbaceous peonies used in this study (Table 1) were planted at the Xiaotangshan cultivation base, Beijing Forestry University, National Engineer Research Center for Floriculture, Beijing, China.
calculated according to Kuo et al. (1972). The resulting classification is compiled in Table 2. Chromosome morphology was determined using arm ratio (long arm/short arm). Accordingly, chromosomes were classified as either m (1.01–1.70), sm (1.71–3.00), or st (3.01–7.00) (Levan et al., 1964). The ratio of longest to shortest chromosome (Lt/St) and centromere index percent (short arm length/chromosome length \( \times 100\% \)) were also calculated. Another AI to measure karyotype asymmetry proposed by Paszko (2006) was also used in this study. The AI is defined as the product of a component expressing the relative variation in chromosome length (\( CV_{CL} \)) and a component expressing the relative variation in centromeric index (\( CV_{CI} \)). Relationships between these parameters are summarized by the following equation: \( AI = CV_{CL} \times CV_{CI}/100 \).

**Results**

**Chromosome number of 21 cultivars.** The metaphase chromosomes and karyotypes of each studied species are shown in Figs. 1 and 2, respectively. Karyotypes of the studied species are compared in Table 3. No aneuploidy was observed in any species. The basic chromosome number, which is reported for the first time for these 21 cultivars, is \( x = 5 \) although they have different ploidies. The cultivars in the LG were diploid with 10 chromosomes (\( 2n = 2x = 10 \)), the IG cultivars were triploid with 15 chromosomes (\( 2n = 3x = 15 \)), and the HG had diploid, triploid, and tetraploid cultivars. ‘Little Red Gem’ was diploid with 10 chromosomes (\( 2n = 2x = 10 \)), ‘Old Faithful’, ‘Roy Pehrson’s Best Yellow’ and ‘Scarlet O’Hara’ were tetraploids with 20 chromosomes (\( 2n = 4x = 20 \)), whereas the remaining HG cultivars were triploid with 15 chromosomes (\( 2n = 3x = 15 \)).

**Karyotype parameters of 21 cultivars.** The karyotypes of the studied taxa are reported here for the first time. The chromosomes of all cultivars were composed of three
Fig. 2. Chromosome idiograms of 21 peony cultivars that were used in the study of variation in ploidy and karyological diversity. The relative length of the short and long arm of the chromosome and classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971) are clearly shown. The order of letters corresponds to the letters in Table 1 (A–U); scale = 10 μm. x-axis: Five pairs of chromosome idiograms of 21 peony cultivars. The first, second, and third pairs of chromosome idiograms of each peony cultivar are metacentric (m), and the arm ratio of each chromosome is from 1.01 to 1.70; the fourth pair of chromosome idiograms of each peony cultivar is submetacentric (sm), and the arm ratio of each chromosome is from 1.71 to 3.00; the fifth pair of chromosome idiograms of each peony cultivar is subtelocentric (st), and the arm ratio of each chromosome is from 3.01 to 7.00. y-axis: chromosome relative length (%) of 21 peony cultivars. Zero on the y-axis represents the location of the centromere, with zero as the boundary. Greater than zero on the y-axis is the relative length (%) of the short arm of the chromosome. Less than zero on the y-axis is the relative length (%) of the long arm of the chromosome.
| Code | Cultivar               | Ploidy  | Relative length (%) | Mean arm ratio | Lt/St | AI (%) | IRL               | Karyotype | Avg centromere index (%) | CVCL | CVCI | AI |
|------|------------------------|---------|---------------------|----------------|-------|--------|-------------------|-----------|--------------------------|------|------|----|
| A    | Henry Sass             | 2n = 2x = 10 | 16.98–23.67         | 2.03           | 1.39  | 62.26  | 4M2 + 6M1         | 2A        | 35.61                    | 12.20 | 25.11 | 3.06 |
| B    | Border Charm           | 2n = 3x = 15 | 15.79–24.28         | 1.83           | 1.54  | 61.02  | 9M2 + 6M1         | 2A        | 37.76                    | 16.60 | 24.16 | 4.01 |
| C    | Going Bananas          | 2n = 3x = 15 | 15.19–24.71         | 1.87           | 1.63  | 61.93  | 9M2 + 6M1         | 2A        | 36.86                    | 17.17 | 21.69 | 3.72 |
| D    | Old Rose Dandy         | 2n = 3x = 15 | 15.75–24.75         | 1.71           | 1.57  | 61.98  | 6M2 + 9M1         | 2A        | 38.99                    | 16.02 | 20.90 | 3.35 |
| E    | Prairie Charm          | 2n = 3x = 15 | 16.04–25.11         | 1.88           | 1.57  | 61.71  | 3L + 3M2 + 9M1    | 2A        | 37.35                    | 15.57 | 22.87 | 3.56 |
| F    | Lemon Dream            | 2n = 3x = 15 | 14.84–25.19         | 2.17           | 1.70  | 62.41  | 9M2 + 6M1         | 2A        | 35.89                    | 18.03 | 28.94 | 5.22 |
| G    | Carina                 | 2n = 3x = 15 | 15.83–25.27         | 1.90           | 1.60  | 61.60  | 3L + 3M2 + 9M1    | 2A        | 37.03                    | 17.83 | 25.66 | 4.58 |
| H    | Chalice                | 2n = 3x = 15 | 16.30–24.25         | 1.95           | 1.49  | 62.81  | 6M2 + 9M1         | 2A        | 36.27                    | 14.16 | 23.30 | 3.30 |
| I    | Command Performance    | 2n = 3x = 15 | 15.21–25.55         | 1.96           | 1.68  | 60.80  | 3L + 6M2 + 6M1    | 2A        | 37.55                    | 19.60 | 27.39 | 5.37 |
| J    | Cytherea               | 2n = 3x = 15 | 14.13–26.19         | 1.92           | 1.85  | 59.61  | 3L + 6M2 + 3M1 + 3S | 2A   | 38.31                    | 20.84 | 29.48 | 6.14 |
| K    | Etched Salmon          | 2n = 3x = 15 | 15.97–23.69         | 1.90           | 1.48  | 61.80  | 6M2 + 9M1         | 2A        | 37.22                    | 13.47 | 23.21 | 3.13 |
| L    | Fairy Princess         | 2n = 3x = 15 | 16.22–24.72         | 1.88           | 1.52  | 61.57  | 6M2 + 9M1         | 2A        | 37.39                    | 15.14 | 24.94 | 3.78 |
| M    | Henry Bockstoce        | 2n = 3x = 15 | 15.81–24.87         | 1.94           | 1.57  | 60.75  | 6M2 + 9M1         | 2A        | 37.89                    | 16.03 | 27.92 | 4.48 |
| N    | Joker                  | 2n = 3x = 15 | 16.32–24.94         | 1.86           | 1.53  | 61.05  | 6M2 + 9M1         | 2A        | 37.74                    | 15.39 | 25.31 | 3.90 |
| O    | Lovely Rose            | 2n = 3x = 15 | 15.39–23.00         | 1.78           | 1.49  | 60.05  | 3L + 3M2 + 6M1 + 3S | 2A   | 38.81                    | 13.21 | 23.37 | 3.09 |
| P    | Little Red Gem         | 2n = 2x = 10 | 14.78–23.59         | 2.00           | 1.60  | 64.03  | 6M2 + 2M1 + 2S    | 2A        | 34.87                    | 16.69 | 19.34 | 3.23 |
| Q    | Many Happy Returns     | 2n = 3x = 15 | 15.07–24.62         | 1.82           | 1.63  | 60.59  | 6M2 + 6M1 + 3S    | 2A        | 38.11                    | 17.16 | 24.38 | 4.18 |
| R    | Old Faithful           | 2n = 4x = 20 | 14.44–25.40         | 1.82           | 1.76  | 60.90  | 4L + 4M2 + 8M1 + 4S | 2A   | 38.00                    | 19.18 | 22.77 | 4.37 |
| S    | Prairie Moon           | 2n = 3x = 15 | 14.96–23.68         | 1.92           | 1.58  | 61.16  | 9M2 + 3M1 + 3S    | 2A        | 37.47                    | 16.95 | 24.73 | 4.19 |
| T    | Roy Pehrson's Best     | 2n = 4x = 20 | 15.46–24.63         | 1.86           | 1.59  | 59.96  | 12M2 + 8M1        | 2A        | 38.47                    | 17.80 | 26.31 | 4.68 |
| U    | Scarlet O'Hara         | 2n = 4x = 20 | 15.74–24.08         | 1.98           | 1.53  | 62.88  | 12M2 + 8M1        | 2A        | 36.01                    | 14.75 | 23.42 | 3.45 |

Chromosome length/total chromosome length.
Long arm/short arm.
Ratio of longest chromosome to shortest chromosome.
Classification of karyotype in relation to their degree of asymmetry according to Stebbins (1971).
Long arm length/total genome length × 100.
Short arm length/chromosome length × 100.
Relative variation in chromosome length.
Relative variation in centromeric index.
Index to measure karyotype asymmetry proposed by Paszko (2006), where $AI = \frac{CV_{CL} \times CV_{CI}}{100}$. 
sections of m chromosomes, one section of sm chromosomes, and one section of st chromosomes. As Figs. 1 and 2 and Table 2 show, the IRL of the studied cultivars varied considerably. The first pair of chromosomes from ‘Cytherea’ (HG) had the longest relative total length of 26.29%, while that of ‘Lovely Rose’ (HG) had the shortest relative total length of 23.00%. With a relative total length of 16.98%, the last pair of chromosomes from ‘Henry Sass’ (HG) was the longest and that from ‘Cytherea’ (HG) was the shortest with a relative total length of 14.13% among all the studied species. The genomes of ‘Henry Sass’ (HG), ‘Border Charm’ (IG), ‘Going Bananas’ (IG), ‘Old Rose Dandy’ (IG), ‘Lemon Dream’ (IG), ‘Chalice’ (HG), ‘Etched Salmon’ (HG), ‘Fairy Princess’ (HG), ‘Henry Bockstoce’ (HG), ‘Joker’ (HG), ‘Roy Pehrson’s Best Yellow’ (HG), and ‘Scarlet O’Hara’ (HG) were one of two chromosome types, a medium long chromosome (M1) or a medium short chromosome (M2). The genomes of ‘Prairie Charm’ (IG), ‘Carina’ (HG), ‘Command Performance’ (HG), ‘Little Red Gem’ (HG), ‘Many Happy Returns’ (HG), and ‘Prairie Moon’ (HG) were composed of three chromosome types: long chromosomes (L), M1, and M2. The genomes of ‘Cytherea’ (HG), ‘Lovely Rose’ (HG), and ‘Old Faithful’ (HG) were composed of four types of chromosomes: L, M1, M2, and short (S).

Only 2A symmetry was found, and the karyotypes of the studied species were somewhat symmetric. The AI varied from 59.61% ['Cytherea' (HG)] to 64.03% ['Little Red Gem' (HG)]. The mean arm ratio ranged from 1.71 ['Old Rose Dandy’ (IG)] to 2.17 ['Lemon Dream’ (IG)]. The AI values varied from 3.06 ['Henry Sass’ (HG)] to 6.14 ['Cytherea’ (HG)].

Discussion

Since the 1960s, studies related to chromosome number, karyotype, and genome composition have been widely used in biosystematics research and studies on the phylogenetic and evolutionary relationships among biological taxa (Yang et al., 2014). The basic chromosome number of *Paeonia* is *x* = 5, all of them being euploid (Wang, 2010). Polyploidy is an important aspect of evolutionary diversification and is one of the most important cytogenetic mechanisms in plant evolution and rapid speciation (Grant, 1981; Jian et al., 2013; Levin, 2002; Stebbins, 1971). The parent of the LG is *P. lactiflora* (2n = 2x = 10), so the cultivars of this group are usually diploid; e.g., ‘Henry Sass’. Because there is only one triplidium tree peony, ‘Shou’ An Hong’ (Li and Zhang, 1982), due to its triplidium status in the HG, the female parent may be tetraploid herbaceous peony, whereas the male parent may be diploid tree peony, but this should be confirmed in a future study. The ploidy range of the HG is very wide, with diploid, triplidium, and tetraploid species having been found in this study. For example, ‘Little Red Gem’ is the self-cross of ‘Gwenda F2’, which itself is the cross of *P. tenuifolia* (2n = 2x = 10) and *P. mlokosewitschii* (2n = 2x = 10). In this study, ‘Little Red Gem’ was verified to be a diploid. The female parent of ‘Chalice’ is *P. lactiflora* (2n = 2x = 10), whereas its male parent is *P. macrophylla* (2n = 4x = 20), and ‘Henry Bockstoce’ is the cross of *P. officinalis* (2n = 4x = 20) and *P. lactiflora* (2n = 2x = 10), which were confirmed as triplidium in this study.

Except for five cultivars of the IG, the remaining 16 cultivars are the hybrid of herbaceous peony. The parents of the IG usually belong to *P. lactiflora* and *P. lactea* (Sun, 2007). The karyotype of herbaceous peony is 2n = 2x = 10 = 6m + 2sm + 2st, and the karyotype of *P. lactea* is usually 2n = 2x = 10 = 6m + 2sm + 2st, but Gong found that a Ludian population is 2n = 10 = 5m + 2sm + 3st (Gong et al., 1991, 1999; Hou et al., 2006). Most herbaceous and tree peonies belong to 2A (Sang et al., 2004), whereas Hong et al. (1988) and Liu (2016) found cases of 2B among a population of *P. obovata* and the hybrid of ‘Zhu Sha Pan’ and ‘Cream Delight’, respectively. In this study, however, the karyotypes of all 21 herbaceous peony cultivars were 2n = 2x = 10 = 6m + 2sm + 2st, 2n = 3x = 15 = 9m + 3sm + 3st, 2n = 4x = 20 = 12m + 4sm + 4st, and 2A.

According to Stebbins (1971), the evolutionary trend of karyotypes in the plant kingdom is from symmetry to asymmetry. Thus, relatively primitive plants in systematic evolution have relatively symmetric karyotypes, whereas derivative or more advanced plants may have an asymmetric karyotype (Deng et al., 2009, 2011; Stebbins, 1971). In our study, we also used the AI index, a new AI that was developed by Paszko (2006) to give a single value that assesses karyotype asymmetry. The AI index has the advantage of allowing a high degree of precision and sensitivity to assess karyotype asymmetry, and higher values of the AI index are considered to indicate higher levels of karyotypic heterogeneity (Paszko, 2006, Zhang et al., 2013). Generally, the mean of cultivars of the HG (4.12) had higher asymmetry karyotypes than cultivars of the IG (3.97) and the LG (3.06). A cultivar of the LG, ‘Henry Sass’, had the lowest AI index (3.06). Unfortunately, only a single cultivar was used in the LG in this study, so additional studies on a larger pool of cultivars are needed to validate our findings. Only *P. lactiflora* is involved in the hybridization of cultivars of the LG. However, in the HG and IG, especially the former, different species or cultivars of different groups have been used in the hybridization of these cultivars, such as *P. lactea*, *P. tenuifolia*, *P. mlokosewitschii*, *P. macrophylla*, *P. officinalis*, and others. The AI index can indicate an evolutionary trend, and primitive species or cultivars usually have a lower AI index, a plant having a higher AI value would indicate that it is more advanced (Deng et al., 2011; Gao et al., 2012; Zhang et al., 2013).

Differences among karyotypes and chromosomes can reflect genetic, morphological, and cytological diversity, as shown for studies on *Vernonia* (Angulo and Massimiliano, 2009), *Rosa odorata* (Jian et al., 2010), wild *Rosa* (Jian et al., 2013), and *Forsythia* (Shen et al., 2015), which have used karyotypes and chromosomes to reflect the genetic diversity of these species or cultivars. The karyotypes of all 21 herbaceous peony cultivars in this study are similar, but their ploidies differ. To establish a future study on evolutionary trends and genetic diversity, chromosome banding, in situ hybridization, and molecular markers should be combined to provide more useful information for a breeding program.

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