Ploidy Level, Karyotype, and DNA Content in the Genus Lonicera

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Abstract. Ploidy levels and genome sizes have significant implications in plant evolution and crop improvement. Species of Lonicera L. have long been cultivated as medicinal, ornamental crops, or both. However, chromosome numbers, karyotypes, and DNA contents have only been documented in a few species, of which some controversies regarding basic chromosome numbers and karyotypes remain. This study analyzed the chromosome numbers and karyomorphology of 11 cultivars across four species and also the DNA content of 10 cultivars representing six species of Lonicera. Among them, the chromosome numbers of nine cultivars are reported for the first time. Results showed that the basic chromosome number of \( x = 9 \) was constant, and chromosome numbers of \( 2n = 18, 27, 36, \) or \( 54 \) were observed, suggesting that polyploidy exists in the genus. Five cultivars are diploid with \( 2n = 18 \); one cultivar is triploid, four are tetraploid, and one is hexaploid. The karyotypes of all studied cultivars are \( 3B \) or \( 3A \), except Lonicera sempervirens 'Crimson Cascade' that is \( 2B \) based on the Stebbins' asymmetry classification of karyotypes. The asymmetry index (A1) values vary from 0.47 to 0.60. The chromosome lengths range from 0.77 to 4.09 \( \mu \)m. Total karyotype lengths differ from 33.55 to 78.71 \( \mu \)m. The 1C-value of 10 cultivars varies 3-fold, ranging from 1.158 to 3.664 pg. Information gathered from this study could be valuable for improving breeding efficiency in the development of new cultivars of Lonicera with enhanced medicinal, ornamental value, or both.

The genus Lonicera L., commonly known as honeysuckle, is a member of the family Caprifoliaceae and comprises more than 200 species (Naujokszys et al., 2007). This genus is mainly distributed in temperate and subtropical areas, with some species extending their range into tropical areas of India, Malaysia, and the Philippines (Rehder, 1903; van Steenis, 1946). The region of greatest species diversity is the Himalayas and China (Ammal and Saunders, 1952). Some species, including Lonicera confusa (Sweet) DC., Lonicera fulvotomentosa Hsu et S.C. Cheng, Lonicera japonica Thunb., Lonicera hypoglauca Miq., and Lonicera macranthoides Hands.-Mazz., are medicinal plants (Zeng et al., 2017). Dried flowers and buds of honeysuckles are known as flos loniceras and have been a recognized herb of traditional Chinese medicine for more than 1500 years (Li et al., 2015). Floss loniceras have been used to treat arthritis, diabetes mellitus, fever, and viral infections (Li et al., 2015; Shang et al., 2011). Some species have been used as ornamentals worldwide including L. japonica, Lonicera sempervirens L., Lonicera periclymenum L., and Lonicera flava Sims (Georges et al., 1992). Because of their different growth habitats and great adaptability, a few species, such as L. japonica (Schirenbach, 2004) and Lonicera maackii (Rupr.) Herder. (Gorchov and Trisel, 2003), are considered as invasive plants in some regions. One species, Lonicera caerulea L., also known as blue honeysuckle, is a shrub producing edible fruits (Miyashita et al., 2011).

Despite their economic importance, chromosome numbers have only been documented in less than 30% of the described species based on the index to plant chromosome number databases (IPCN, 2017). The basic chromosome number of \( x = 9 \) was reported by Ammal and Saunders (1952). However, Benko-Iseppon and Morawetz (2000) suggested that the basic chromosome number of the family Caprifoliaceae was \( x = 8 \) or 9. In addition, karyotypes have only been described in 36 entities (Table 1), of which some controversies remain in L. japonica. As listed in Table 1 (Deneau et al., 2007; Guo et al., 1993; Li et al., 2009; Wang et al., 2005; Yan et al., 2014; Yang et al., 2011; Zhang et al., 2017), there are 17 different karyotypes for L. japonica based on the Stebbins’ karyotypes (Stebbins, 1971).

Polyploidy occurs in the genus Lonicera (Ammal and Saunders, 1952; Solov’eva and Plekhanova, 2003). Ploidy levels and genome sizes play important roles in plant evolution (Solits et al., 2003). The 2C nuclear DNA amount of Lonicera varies 6-fold based on the Plant DNA C-values Database (2012) and reports of Bennett et al. (2000) and Bennett and Leitch (2012). The DNA amount of the unreplicated haploid genome (1C-value) is highly characteristic for different taxa. Species’ DNA amounts (1C-value) are key diversity characters of fundamental significance with many important uses (Bennett and Leitch, 2005). However, there are only six 1C-values for Lonicera so far (Bennett and Leitch, 2012; Olszewska and Osiecka, 1984; Zonneveld et al., 2005). Moreover, ploidy levels and genome sizes are important in crop improvement (Bennett, 1998). Because of the lack of reliable chromosome and genomic information, current breeding on Lonicera has been primarily focused on the selection of naturally occurring mutations (Chen et al., 2013; Wang et al., 2013; Zeng et al., 2017).

The objectives of this study were to determine the chromosomal numbers, ploidy levels, and karyotypes of some important cultivars of selected Lonicera species as well as their DNA contents. It is anticipated that information gathered from this study could help improve medicinal and ornamental value of Lonicera species and cultivars through plant breeding.

Materials and Methods

Lonicera cultivars listed in Tables 2 and 3 were grown in a container substrate comprising 60% peat, 20% perlite, and 20% vermiculite in a greenhouse. Root tips were collected and pretreated with 0.1% colchicine (Phytotechnology laboratories, Shawnee Mission, KS) for about 5 h before being fixed in Carnoy I (glacial acetic acid:absolute ethanol = 1:3). The samples were then macerated in a 1:1 mixture of 45% acetic acid (Fisher Scientific Inc., Pittsburgh, PA) and 1 m HCl (Fisher Scientific Inc.) at 60 °C for 4 min and stained and squashed in carbol fuchsin (Sigma-Aldrich Inc., St. Louis, MO). The root tips were squashed with forceps in...
Table 1. A summary of somatic chromosome numbers of *Lonicera* species that have been reported in the literature.

| Taxa                              | Karyotype formula | Stebbins’ type | No. (2n) | Origin     | Reference     |
|-----------------------------------|-------------------|----------------|----------|------------|---------------|
| *Lonicera affinis* Hook. & Arn.   | 34m + 2sm         | —              | 18       | Japan      | Lin and Sasaki (2011) |
| *Lonicera caerulea* L.            | 36m + 2sm         | 2A             | 36       | Romania    | Truta et al. (2013) |
| *L. caerulea*                     | —                 | 2A             | 18       | Russia     | Solov’eva and Plekhanova (2003) |
| *Lonicera gracilipes* Miq. var.   | 6m + 10sm + 2st   | 2A             | 18       | China      | Wang and Wang (2005) |
| *Lonicera hypoglauca* Miq.         | 2m + 14sm (3SAT) + 2st (2SAT) | 3A | 18       | China      | Guo et al. (1993) |
| *Lonicera japonica* Thunb.         | 8m + 8sm + 2st    | 2B             | 18       | China      | Li et al. (2009) |
| *Lonicera japonica* var. glabra   | 4m + 14sm         | 2B             | 18       | China      | Yan et al. (2014) |
| *Lonicera japonica* var. miyagusukiana | 12m (2SAT) + 4sm (2SAT) + 2st (2SAT) | 2B | 18       | China      | Yang et al. (2011) |
| *Lonicera macranthoides*          | 2m + 14sm + 2st   | 2B             | 36       | China      | Zhang et al. (2002) |
| *Lonicera macranthoides* var.     | 8m + 8sm + 2st    | 2B             | 18       | China      | Yan et al. (2014) |
| *Lonicera periclymenum* var.      | 2m + 12sm + 4st   | 3B             | 18       | China      | Wu et al. (2011b) |
| *Lonicera periclymenum* var. Wisleyensis | 2m + 12sm + 4st | 3B | 18       | China      | Zheng et al. (2007) |
| *Lonicera japonica* var. chinensis | 2m + 14sm + 2st   | 3A             | 18       | Japan      | Denda et al. (2007) |
| *L. japonica* ‘Damaohua’           | 4m + 28sm (2SAT) + 4st | 3A  | 36       | Japan      | Denda et al. (2007) |
| *L. japonica* ‘Jinfeng 1’          | 2m + 14sm + 2st   | 3A             | 18       | China      | Zhang et al. (2017) |
| *L. japonica* ‘Jizhuahua’          | 8m + 8sm + 4st (4SAT) | 3B  | 18       | China      | Zhang et al. (2017) |
| *L. japonica* ‘Sijjinjinhua’       | 4m + 8sm + 6st    | 3A             | 18       | China      | Zhang et al. (2017) |
| *Lonicera macranthoides* (Rupr.) Maxim. | 8m + 10sm        | 2A             | 18       | China      | Wang and Wang (2005) |
| *Lonicera macranthoides* var.     | 2m + 8sm + 8st    | 3B             | 18       | China      | Mei et al. (2006) |
| *Lonicera periclymenum* L.         | 10m + 8sm        | 2A             | 18       | Europe     | Ammal and Saunders (1952) |
| *L. periclymenum* var. Belgica     | —                 | —              | 36       | Cultivated | Ammal and Saunders (1952) |
| *L. periclymenum* var. Hjotyseniss | —                 | —              | 36       | —          | Suerrey Ammal and Saunders (1952) |
| *Lonicera similis* Hemsl.          | 2m + 14sm + 2st   | 3A             | 18       | China      | Yan et al. (2014) |
| *Lonicera sempervirens* L.         | 2m + 12sm + 4st   | 3B             | 18       | China      | Wang (2014) |
| *Lonicera syringantha* Maxim.      | 2m + 14sm + 2st   | 3B             | 18       | China      | Wang (2016) |
| Four diploid cultivars             | 4m + 12sm + 2st   | 3B             | 18       | China      | Wang (2016) |
| Eight tetraploid cultivars         | 8m + 22sm + 6st   | —              | 36       | Russia     | Solov’eva and Plekhanova (2003) |

* m = median centromeric chromosome; sm = submedian centromeric chromosome; st = subterminal centromeric chromosome; SAT = satellite chromosome.

* Dashes indicate no data available.

Table 2. Ploidy level, chromosome numbers, and karyotypic characteristics of the 11 studied *Lonicera* cultivars.

| Taxon                              | Ploidy level (2n) | Karyotype formula | Chromosome length range (μm) | TKL* (μm) | A1* | A2* | No.** | Stebbins’s type | Figure* |
|-----------------------------------|-------------------|-------------------|-------------------------------|------------|-----|-----|-------|----------------|---------|
| *Lonicera japonica* ‘Jinfeng 1’   | 18                | 2                 | 2m + 10sm + 6st               | 1.40–2.46  | 33.55 | 0.57 | 0.22 | 3B             | 1A, 2A  |
| *L. japonica* ‘Jinfeng 1’         | 36                | 4                 | 4m + 28sm + 4st               | 0.77–1.83  | 52.11 | 0.50 | 0.26 | 3B             | 1B, 2B  |
| *Lonicera macranthoides*          | 18                | 2                 | 2m + 8sm + 8st                | 1.99–4.09  | 44.86 | 0.60 | 0.23 | 3B             | 4IC, 2C |
| *L. macranthoides* ‘Baiyun’       | 18                | 2                 | 2m + 16sm                    | 1.27–2.35  | 35.05 | 0.52 | 0.20 | 3A             | 2ID, 2D |
| *L. macranthoides* ‘Longhua’      | 18                | 2                 | 2m + 14sm + 2st               | 1.31–3.03  | 38.86 | 0.50 | 0.24 | 3B             | 3IE, 2E |
| *L. macranthoides* ‘Jincuilé’     | 18                | 2                 | 2m + 10sm + 6st (2SAT)        | 1.55–3.27  | 38.70 | 0.54 | 0.21 | 3B             | 4IF, 2F |
| *Lonicera periclymenum* ‘Graham Thomas’ | 54             | 6                 | —                            | —          | —    | —   | —     | —              | 1G      |
| *Lonicera sempervirens* ‘Major Wheeler’ | 36         | 4                 | 4m + 30sm + 2st               | 0.79–2.82  | 73.72 | 0.52 | 0.26 | 3B             | 4IH, 3A |
| *L. sempervirens* ‘Crimson Cascade’ | 36         | 4                 | 6m + 28sm + 2st               | 1.02–2.39  | 70.31 | 0.47 | 0.24 | 2B             | 3II, 3B |
| *L. sempervirens* ‘John Clayton’  | 36                | 4                 | 4m + 22sm + 10st              | 1.65–3.33  | 78.71 | 0.57 | 0.22 | 3B             | 3IJ, 3C |
| *L. sempervirens* ‘Magnifera’      | 27                | 3                 | 3m + 24sm                    | 0.93–2.30  | 45.98 | 0.49 | 0.25 | 3B             | 3IK, 3D |

* TKL = total karyotype length.
* A1 = intrachromosomal asymmetry index.
* A2 = interchromosomal asymmetry index.
* No. = the number of metaphase cells measured.
* Chromosome numbers and karyotypes presented in the figures of this article.
* Dashes indicate no data.

... a drop of 45% acetic acid on a glass slide, then covered with a cover glass, and squashed again. Cover glasses were removed by freezing glass slides in a freezer. The slides were dried at room temperature and passed through three solutions: 50%, 75%, and 100% ethanol (Fisher Scientific Inc.). Slides were sealed with a euparal mounting medium (Asco Laboratories Ltd., Manchester, UK). Metaphase chromosomes were observed, and photographs were taken under the Olympus Motorized Inverted Research Microscope Model IX81 (Olympus Corporation, Tokyo, Japan). Chromosome numbers of a minimum of 20 cells of each collection were counted at a well-spread metaphase stage. Karyotype formulae were established by measuring mitotic–metaphase chromosomes taken from photographs. In most cases, measurements of two to four cells were made for karyotypic analysis. The nomenclature used for the description of the chromosome morphology followed the description of Levan et al. (1964) where m = median centromeric chromosome, sm = submedian centromeric chromosome, st = subterminal centromeric chromosome, and t = terminal centromeric chromosome. The karyotype asymmetry was determined using the karyotype asymmetry indices A1 (intrachromosomal asymmetry...
index), A2 (interchromosomal asymmetry index) (Zarco, 1986), and the categories of Stebbins (1971).

Ploidy levels were analyzed by flow cytometry (Partec PA; Partec GmbH, Münster, Germany). According to the concept of Suda et al. (2006), we used the term “DNA ploidy level.” The prefix “DNA” indicates that ploidy levels are mostly inferred from nuclear DNA content without knowledge of the exact chromosome numbers. Sample treatments followed the method of Cui et al. (2009). A fresh young leaf was chopped with a 0.2 mL drop of nuclei extraction buffer (CyStain ultraviolet precise P; Partec GmbH). After incubation for 2 min at room temperature and filtration through a Partec 50 μm CellTrics disposable filter, DNA ploidy levels were analyzed using flow cytometry. DNA contents were also calculated using leaves of *Pisum sativum* ‘Ctirad’ (2C = 9.09 pg) (Dolezel and Bartos, 2005) as the internal standard. Ten cultivars from six species were examined. The analysis was considered to be acceptable when the CV was within 5%. For each sample, four replicates of the same plants were performed within 1 d. Relative DNA content was estimated by the ratio between the fluorescence intensity of the sample and internal standard: sample relative DNA content = sample peak mean/standard peak mean. The relative DNA content was then calculated for each sample as the average of the four replications.

**Results**

**Chromosome number.** Chromosome numbers of 11 cultivars across four species are presented in Table 2 and their chromosome morphology and sizes are illustrated in Fig. 1. Five diploid cultivars had the chromosome number 2n = 18: *L. japonica* ‘Jinfeng 1’, *L. macranthoides*, *L. macranthoides* ‘Jincuilei’, *L. macranthoides* ‘Baiyun’, and *L. macranthoides* ‘Jincuilei’. One triploid cultivar, Table 3. DNA ploidy level, relative DNA content, z and nuclear DNA content in *Lonicera* cultivars as estimated by flow cytometry.

| Taxon                     | n | DNA ploid level (x) | Chromosome numbers (2n) | Relative DNA content | Difference within population (foldy) | 2C value (pgx) (Mean ± sd) | Mean 1C value (pgx) | 1Cx-value (Mbp)w | CVvMean ± SD | Minimum | Maximum |
|---------------------------|---|---------------------|-------------------------|----------------------|--------------------------------------|-----------------------------|---------------------|------------------|-------------|---------|---------|
| *Lonicera caerulea*       | 4 | 4                   | 36                      | 0.520 ± 0.005        | 0.516                                | 1.023                       | 4.730 ± 0.042       | 2.365            | 1.159     | 0.96    |
| ‘Kamchatka’               |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |
| *Lonicera flava*          | 4 | 4                   | 36                      | 0.525 ± 0.006        | 0.520                                | 1.027                       | 4.772 ± 0.050       | 2.386            | 1.169     | 1.14    |
| *Lonicera japonica*       | 4 | 2                   | 18                      | 0.255 ± 0.004        | 0.249                                | 1.044                       | 2.316 ± 0.038       | 1.158            | 1.135     | 1.57    |
| *L. japonica* ‘Jinfeng 1’ | 4 | 2                   | 18                      | 0.258 ± 0.006        | 0.248                                | 1.056                       | 2.345 ± 0.053       | 1.173            | 1.149     | 2.33    |
| *L. japonica* ‘Jinfeng 1’ | 4 | 2                   | 18                      | 0.511 ± 0.005        | 0.504                                | 1.028                       | 4.645 ± 0.047       | 2.323            | 1.138     | 0.78    |
| *Lonicera macranthoides*  | 8 | 2                   | 18                      | 0.277 ± 0.004        | 0.270                                | 1.048                       | 2.516 ± 0.036       | 1.258            | 1.233     | 1.44    |
| ‘Jincuilei’               |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |
| *Lonicera periclymenum*   | 4 | 6                   | 54                      | 0.796 ± 0.009        | 0.784                                | 1.032                       | 7.238 ± 0.084       | 3.619            | 1.182     | 1.13    |
| ‘Belgia’                  |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |
| *L. periclymenum*         | 4 | 6                   | 54                      | 0.806 ± 0.007        | 0.800                                | 1.021                       | 7.328 ± 0.060       | 3.664            | 1.197     | 0.87    |
| ‘Graham Thomas’           |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |
| *L. sempervirens*         | 8 | 4                   | 36                      | 0.530 ± 0.003        | 0.524                                | 1.025                       | 4.816 ± 0.042       | 2.408            | 1.180     | 0.57    |
| ‘Major Wheeler’           |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |
| *L. sempervirens*         | 8 | 3                   | 27                      | 0.415 ± 0.005        | 0.407                                | 1.032                       | 3.770 ± 0.043       | 1.885            | 1.232     | 1.20    |
| ‘Magnifica’               |   |                     |                         |                      |                                      |                             |                     |                  |             |         |         |

*Relative DNA content was estimated by the ratio between a sample and internal standard: sample relative DNA content = sample peak mean/standard peak mean.

yDifferences of relative DNA content (fold) within population was calculated as maximum relative DNA content/minimum relative DNA content.

x1 pg = 980 Mbp (Bennett et al., 2000).

w1Cx = DNA content of one nonreplicated monoploid genome with chromosome number x.

vCV = coefficient of variation of relative DNA content was calculated as (SD/mean) · 100.

![Fig. 1. Somatic chromosomes at mitotic metaphase of *Lonicera* cultivars. (A) *Lonicera japonica* ‘Jinfeng 1’; (B) *L. japonica* ‘Jinfeng 1’; (C) *Lonicera macranthoides*; (D) *L. macranthoides* ‘Baiyun’; (E) *L. macranthoides* ‘Longhua’; (F) *Lonicera periclymenum* ‘Jincuilei’; (G) *Lonicera sempervirens* ‘Major Wheeler’; (H) *L. sempervirens* ‘Crimson Cascade’; (J) *L. sempervirens* ‘John Clayton’; (K) *L. sempervirens* ‘Magnifica’. Scale bar = 10 μm.](image)
L. sempervirens ‘Magnifera’, had $2n = 27$. Four tetraploid cultivars had $2n = 36$: L. japonica ‘Jinfeng 1’, L. sempervirens ‘Major Wheeler’, L. sempervirens ‘Crimson cascade’, and L. sempervirens ‘John Clayton’. Lonicera periclymenum ‘Graham Thomas’ was hexaploid with $2n = 54$.

**Karotype.** Karyotype formulae and related parameters for the 11 cultivars are summarized in Table 2 and their karyotypes are illustrated in Figs. 2 and 3. The karyotypes consisted of median centromeric (m), submedian centromeric (sm) chromosomes, a few subterminal centromeric (st), but no terminal centromeric chromosome (t). Thus, the karyotypes were moderately symmetrical and fell in the Stebbins’ 3B, 3A, or 2B category of asymmetry (Table 2). The A1 values ranged from 0.47 to 0.6. The A2 values differed from 0.2 to 0.26. Most species had small-sized chromosomes (Table 2) according to the classification of Lima de Faria (1980). The chromosome length ranged from 0.77 to 4.09 μm. Total karyotype length (TKL) varied from 33.55 to 78.71 μm.

**DNA ploidy level.** The DNA ploidy levels of 10 cultivars representing six species are shown in Table 3. Lonicera japonica, L. japonica ‘Jinfeng 1’, and L. macranthoides ‘Jincuilei’ showed 2Cx DNA content. Lonicera sempervirens ‘Magnifica’ had 3Cx DNA content. Lonicera caerulea ‘Kamchatka’, L. flavida, L. japonica ‘Jiufeng 1’, and L. sempervirens had 4Cx DNA content. Lonicera periclymenum ‘Belgia’ and L. periclymenum ‘Graham Thomas’ had 6Cx DNA content. The Cx-value was designated as DNA content of a monoploid genome with chromosome number $x$ according to Greilhuber et al. (2005). The results of the flow cytometric analysis provided additional evidence that diploid (2n = 2x = 18), triploid (2n = 4x = 36), tetraploid (2n = 4x = 36), and hexaploid (2n = 6x = 54) populations exist in the genus Lonicera (Table 2).

**Discussion**

The genus Lonicera encompasses a number of medicinally important species (Li et al., 2015; Shang et al., 2011; Zeng et al., 2017) and also some popular ornamental plants that can grow vigorously in a wide range of environments (Wu et al., 2011a). Because of the lack of fundamental genetic information, such as chromosome numbers, karyotypes, and DNA contents, there has been no documented breeding scheme for genetic improvement of Lonicera species. The current effort has been primarily limited to the selection of sports or induced mutations (Chen et al., 2013; Wang et al., 2013; Zeng et al., 2017). The present study investigated some important cultivars of Lonicera species and documented their ploidy levels, karyotypes, and DNA contents, which could be potentially valuable for improving breeding efficiency of Lonicera species.

This study showed that the basic chromosome number of $x = 9$ is constant as the mitotic number (2n) counts are 18, 27, 36, and 54 (Tables 2 and 3) depending on the investigated cultivars. Our observations agree with the conclusion of Ammal and Saunders (1952) and also the reports of others.
listed in Table 1 that the basic chromosome number is \( x = 9 \), not 8 as suggested by Bedi et al. (1982). Furthermore, these are the first reports of chromosome numbers for *L. japonica* ‘Jinfeng 1’; *L. macranthoides* ‘Jincuilei’, ‘Longhua’, and ‘Baiyun’; *L. sempervirens* ‘Crimson Cascade’, ‘John Clayton’, ‘Magnifera’, and ‘Major Wheeler’; and *L. periclymenum* ‘Graham Thomas’.

Karyotype formulae and quantitative analysis indicate that there are interspecific and intraspecific instabilities in the studied *Lonicera* cultivars (Tables 2 and 3), which are different from the observations of Solov’eva and Plekhanova (2003) who reported that karyotypes of 23 natural populations from *L. aerulea* had considerable generic resemblance because of their similar chromosome morphology and variation range of chromosome length. Both quantitative (Tables 2 and 3) and qualitative (Figs. 2 and 3) data showed interspecific differences, and the most variable characters include the ploidy levels, TKL, and the number of sm chromosomes. At the intraspecific level, different cultivars have different karyotype formulae. The differences in karyotype morphology may represent differences in genetic recombination or gene arrangement (Stebbins, 1971), resulting in morphological, physiological, and biochemical differences in plants. The karyotype of *L. macranthoides* was in agreement with the observations of Mei et al. (2006). However, ‘Baiyun’, ‘Longhua’, and ‘Jincuilei’ have different karyotypes. The three cultivars were mutants of *L. macranthoides*; they produce large flowers, but the flowers never open and contain high chlorogenic acid (CGA) content (Chen et al., 2013; Wang et al., 2009, 2013). The karyotype of *L. japonica* ‘Jinfeng 1’ is closely similar to that of *L. japonica* ‘Sijijinyinhua’ listed in Table 1, but the latter has four satellite chromosomes. There are 17 karyotype formulae among the reported *L. japonica* cultivars (Table 1); such a high karyotype variation suggests great genetic diversity of this species, thus possessing a wide range of adaptability. This may partially explain why *L. japonica* is considered an invasive species in some parts of the world. The B karyotype of *L. japonica* ‘Jinfeng 1’ in this study differs from the results of Zhang et al. (2017) who reported that it was a 3A type. In addition, the index A1, developed by Zarco (1986), is considered to have a high degree of precision and sensitivity in assessing karyotype asymmetry. In general, higher values of the A1 index are considered to be higher levels of karyotypic heterogeneity. In the present study, all cultivars fell in the Stebbins’ 3B or 3A category of asymmetry, except *L. sempervirens* ‘John Clayton’ with 2B (Table 2), and the A1 values ranged from 0.47 to 0.6. Therefore, *Lonicera* is generally characterized by possessing asymmetrical karyotypes, with a predominance of sm chromosomes.

Polyploidy occurred in *Lonicera* species ranging from triploid to hexaploid. Polyploidy may change breeding systems and cause shifts to asexual reproductions (Negrón-Ortiz, 2007). Polyploid plants generally have larger plant organs (Sasnauskas et al., 2007; Zhang et al., 2010) and greater adaptability than diploid plants (Lewis, 1980). Polyploidization is characterized by variations in the amount of nuclear DNA (C-value), with simultaneous changes in chromosome structures and numbers (Negrón-Ortiz, 2007). In the present study, *L. caerulea* ‘Kamchatka’ and *L. flava* are tetraploids. Miyashita et al. (2011) reported that both diploid and tetraploid *L. caerulea* plants exist in Japan, whereas tetraploid plants were much more widely distributed than the diploids.
suspecting the increased adaptability of the polyploid plants. *Lonicera flava* was reported to be a diploid (Rüdenberg and Green, 1966); however, the species in the present study was a tetraploid. *Lonicera sempervirens*, a native to the eastern United States, is a tetraploid species. It is considered to be an allopolyploid (Ammal and Saunders, 1952), and Schierenbeck et al. (1995) speculated that its likely diploid congeners could be *L. flava* and *Lonicera dioca*. *L. sempervirens* was considered to be a diploid (Rüdenberg and Green, 1966). Our study, however, did not include this species, it is unknown if tetraploid *L. dioca* exists. Among the three tetraploid cultivars of *L. sempervirens*, karyotype formulas and chromosome morphologies varied (Table 2; Fig. 3), thus each has different pheno- types. ‘Major Wheeler’ is a nonstop bloomer from later spring to fall with showy clusters of large orange-red flowers. ‘Crimson Cascade’ has multiple pharmacological actions including antioxidation, antibacteria, antivirus, anti-inflammatory, and anti-oxidative activities (Yang et al., 2004). The CGA can strongly suppress adeno-associated 3 and 7 viruses, coxsackie B3 and B5 virus, and respiratory syncytia virus (Hu et al., 2001). Luteolin shows antiviral actions and has been used as an influenza virus neuraminidase inhibitor (Liu et al., 2008). Thus, further research on the development of interspecific hybrids is warranted.

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