Karyotype Analysis of Wild *Rosa* Species in Xinjiang, Northwestern China

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ADDITIONAL INDEX WORDS. chromosome, ploidy, rose, taxonomy

ABSTRACT. Wild *Rosa* species, which are highly variable and locally adapted, are widely distributed in the Xinjiang Uygur autonomous region of China. These species possess many important horticultural traits that are not found in the gene pool of modern cultivated roses. However, little is known about their cytological characteristics, because few of them have been karyologically analyzed. Karyological data of 13 samples of seven wild *Rosa* taxa, including *R. berberifolia*, two botanical varieties of *R. spinosissima*, *R. platyacantha*, *R. beggeriana*, *R. acicularis*, and *R. laxa*, were investigated by means of squashes of shoot tips. The results showed that seven samples were diploid ($2n = 2x = 14$), whereas the other six samples were tetraploid ($2n = 4x = 28$). The karyotypes of all the test samples were composed of m and sm chromosomes, which include 1A, 2A, 1B, and 2B. There were differences in asymmetry index, centromere index, and distribution of relative lengths. The karyotype of the tetraploid *R. laxa* var. *laxa* sample from Aksu easily distinguished from the other *R. laxa* var. *laxa* samples tested in having the highest asymmetry index and the most evolved karyotype. This Aksu population merits recognition as a new botanical variety of *R. laxa*. The karyological data, most of which are first reports for their taxa, provide a comprehensive cytogenetic resource that can be used to better understand the taxonomy, evolution, and speciation in the genus *Rosa* and to identify candidate species for breeding programs.

The genus *Rosa* comprises ≈ 150 species that are widely distributed in diverse climates and habitats throughout the Northern Hemisphere (Quest-Ritson and Quest-Ritson, 2003; Rehder, 1940) from subtropical to cold-temperate regions. China's 95 species (65 endemic) account for nearly half of the world's *Rosa* species (Ku and Robertson, 2003). Therefore, China is a center of distribution of *Rosa* as it is with numerous other cultivated plants (Brichet, 2003), and China's roses are a great genetic resource for rose improvement.

The Xinjiang Uygur autonomous region (hereafter referred to as Xinjiang) lies at the northwestern border of China (Fig. 1). The Tianshan Mountains transverse it centrally, dividing Xinjiang into southern and northern regions, which differ greatly from each other in climate. In regard to phytogeography, Xinjiang spans the junction of the Eurasian Forest subregion, the Eurasian Steppe subregion, and the Central Asiatic Desert subregion. Many of the wild *Rosa* species in Xinjiang are

distributed widely (Fig. 2). Although 11 *Rosa* species and three botanical varieties were recorded from Xinjiang in *Flora of China* [Ku and Robertson, 2003 (Table 1)], further research has indicated that more than 20 species occur in Xinjiang with botanical varieties that exhibit all sorts of morphological characteristics (Bao, 1993; Liu, 1993; Liu and Cong, 2000; Ma and Chen, 1990, 1991; Yu et al., 1985, 2011). These botanical varieties have been described by different investigators and are notoriously difficult to distinguish, owing to the presence of intermediate forms (Han, 1995). In addition, nomenclatural synonyms and the selection and breeding of cultivars have added to the confusion (Macphail and Kevan, 2009).

There is much interest worldwide in the wild *Rosa* species from China, because they contributed much of the foundation of modern cultivated roses. Wild *Rosa* species with important traits, such as powdery mildew (*Podosphaera pannosa*) resistance, large flowers and hips, and cold and drought resistance are regarded as valuable breeding materials. Modern roses are primarily composed of eight to 11 *Rosa* species and only a few of the species have been involved. Those other species currently are not reported (Ma and Chen, 1992; Zlesak, 2006). Introgressing additional valuable genes from these species into modern rose germplasm is inevitably a long and difficult process (Zlesak et al., 2007), because of the long generation time and the frequent cross-incompatibility and hybrid sterility between parents of different ploidy levels. Therefore, there is interest in the variation of ploidy within and among wild *Rosa* species. Despite the

Received for publication 7 Aug. 2013. Accepted for publication 25 Nov. 2013. This study was supported by the Ministry of Science and Technology for the Twelfth Five-Year Science and Technology Project, People's Republic of China (Grant No. 2012BAD01B07).

We thank Jian-guo Liang, Hui Xue, Jian-xin Liu, and Yun-hong Wang from Beijing Forestry University for the investigation in Xinjiang; Jaenicke Hannah from the International Society for Horticultural Science, Charles Crane from Purdue University, and Ying-nan Wang and Xue-lian Guo from Beijing Forestry University for their critical review of this manuscript.

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Fig. 1. Location of study sites in Xinjiang, northwestern China (2009–11); ● wild *Rosa* species distributed in Xinjiang, including the 13 studied samples.

Table 1. *Rosa* taxa in Xinjiang, northwestern China, recorded in *Flora of China* (Ku and Robertson, 2003) and related cytological data.

Subgenus	Section	Species	Ploidy	Karyotype
<i>Hulthemia</i>		<i>R. berberifolia</i>	Diploid	No report
<i>Rosa</i>	<i>Pimpinellifoliae</i>	<i>R. spinosissima</i> var. <i>spinosissima</i>	Tetraploid	No report
<i>Rosa</i>	<i>Pimpinellifoliae</i>	<i>R. spinosissima</i> var. <i>altaica</i>	Tetraploid	No report
<i>Rosa</i>	<i>Pimpinellifoliae</i>	<i>R. kokanica</i>	No report	No report
<i>Rosa</i>	<i>Pimpinellifoliae</i>	<i>R. platyacantha</i>	Diploid	No report
<i>Rosa</i>	<i>Pimpinellifoliae</i>	<i>R. foetida</i>	No report	No report
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. beggeriana</i> var. <i>beggeriana</i>	Diploid	$2n = 2x = 6m + 8sm$ (Yang et al., 2008)
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. beggeriana</i> var. <i>lioui</i>	Diploid	No report
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. albertii</i>	No report	No report
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. acicularis</i>	Tetraploid	No report
			Octoploid	
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. oxyacantha</i>	No report	No report
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. laxa</i> var. <i>laxa</i>	Diploid, tetraploid	$2n = 4x = 12m + 16sm$ (Yang et al., 2008)
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. laxa</i> var. <i>mollis</i>	Diploid	No report
<i>Rosa</i>	<i>Cinnamomeae</i>	<i>R. fedtschenkoana</i>	No report	No report

development of DNA sequence-based technology in recent years, cytological markers remain useful for plant identification, evaluation of biodiversity, and the study of plant evolution. It has long been known that some wild species, mainly diploids, represent potentially valuable genetic resources, especially for disease resistance. The wild *Rosa* species that have been reported are euploids with chromosome numbers ranging from $2n = 2x = 14$ to $2n = 10x = 70$ (Crane and Byrne, 2003; Darlington and Wylie, 1955; Jian et al., 2010a, 2012; Ma et al., 1997; Malecka et al., 1990; Malecka and Popek, 1984), and an accurate measurement of ploidy appears to be of enormous significance for cross-breeding. The various ploidy groups are not completely cut off from one another; however, the rose breeder who confines his or her hybridization to one ploidy group of species or botanical varieties will find his or her path smoothed and his or her aims more quickly achieved (Percy, 1964).

Karyotype analysis is a traditional cytogenetic first step in the comparison of genomes among related species (Crane and Byrne, 2003) under the usually correct presumption that karyotypic differences can affect meiotic chromosome pairing and hybrid fertility. Although the karyotypic variation within *Rosa* implies a rich genetic resource for rose breeding, it also frequently impedes breeding progress because of cross-incompatibility and hybrid sterility. Hence, elucidation of the karyotype and the physical structure of chromosomes can rationalize and accelerate the exploitation of genetic variation from wild relatives of crops (Baenziger et al., 2006). What is more, the results might be helpful in elucidation of some taxonomical problems and relations between particular species within this highly differentiated genus.

Therefore, a 3-year investigation on wild *Rosa* species in Xinjiang was conducted from 2009 to 2011 with two objectives:

Table 2. Locations of the tested wild *Rosa* samples in Xinjiang, northwestern China.

Code	Species ²	Location	Latitude and longitude	Altitude (m)	Habitat	Remark
1	<i>R. berberifolia</i>	Bagang Prefecture of Urumqi	43°53.196' N, 87°22.368' E	711	Roadside, waste fields, barren land	
2	<i>R. spinosissima</i> var. <i>spinosissima</i>	Chonghuier village of Buerjin Prefecture in Altai	48°27.042' N, 86°38.962' E	1688	Grassy slopes	Flowers white or yellowish, 2–5 cm in diameter
3	<i>R. spinosissima</i> var. <i>altaica</i>	Wood farm of Haba river in Altai	47°59.988' N, 88°16.509' E	1311	Scrub in forests	Flowers white, 5–7 cm in diameter
4	<i>R. platyacantha</i>	Bayingou in Dushanzi district of Karamay	44°06.340' N, 84°49.170' E	1678	Forest margins	
5	<i>R. beggeriana</i> var. <i>beggeriana</i>	Daxigou in Huoicheng of Ili Kazak	44°23.287' N, 80°45.676' E	1015	Slopes, river sides	Hip red
6	<i>R. beggeriana</i> var. <i>beggeriana</i>	Guozigou of Ili Kazak	44°22.149' N, 80°59.355' E	1241	Side of miscellaneous and deciduous woods	Hip red
7	<i>R. beggeriana</i> var. <i>beggeriana</i>	Secondary forest in Nileke of Ili Kazak	43°47.789' N, 82°27.001' E	1056	Secondary forests	Hip purple to dark brown
8	<i>R. acicularis</i>	Xiaodonggou in Altai	47°58.598' N, 87°07.573' E	982	Roadside, bases of mountains	
9	<i>R. laxa</i> var. <i>laxa</i>	Kuitun	44°06.786' N, 80°49.239' E	782	Barren land	Papery leaflets, 3–6 flowers in a corymb
10	<i>R. laxa</i> var. <i>mollis</i>	Crane River of Altai	47°54.404' N, 88°07.174' E	969	Bush woods on sunny slopes	Papery leaflets, 3–6 flowers in a corymb
11	<i>R. laxa</i> var. <i>laxa</i>	Guozigou of Ili Kazak	44°22.672' N, 80°59.765' E	1254	Side of miscellaneous and deciduous woods	Papery leaflets, 3–6 flowers in a corymb
12	<i>R. laxa</i> var. <i>laxa</i>	Leat side in the area of fifth regiment of Agriculture First Division in Aksu	41°28.756' N, 80°46.795' E	1339	Gobi desert and barren land	Leathery leaflets, 3–9 flowers in a corymb
13	<i>R. laxa</i> var. <i>laxa</i>	Tuomuerfeng natural preservation areas of Wensu in Aksu	41°47.003' N, 80°41.309' E	2112	Rock patch in valleys and by the side of brooks	Paper leaflets, solitary flowers

²Taxonomic names of the species are recorded in *Flora of China* (Ku and Robertson, 2003).

1) to learn more details of the wild *Rosa* species and to compare species and botanical varieties on the basis of morphological and cytological characters; and 2) to exploit useful traits of wild *Rosa* species and identify candidate species for breeding strategies. In this article, the chromosome number and karyotype analysis are reported for 13 samples of seven wild *Rosa* taxa from different subregions of Xinjiang.

Materials and Methods

MATERIALS. All the test samples were collected from the original sources in Xinjiang (Table 2), and the wild *Rosa* species were identified carefully according to the *Flora of China* (Ku and Robertson, 2003). Additional variants, recorded in the primary literature (Bao, 1993; Han, 1995; Liu, 1993; Liu and Cong, 2000; Ma and Chen, 1990; Yu et al., 1985, 2011), were surveyed and analyzed together. Table 2 lists the authorities for taxonomic names used in this article.

METHODS. Shoot tips ≈ 1 cm long were collected from the vigorously growing plants ≈ 0830 HR. The materials were

treated first with $0.002 \text{ mol}\cdot\text{L}^{-1}$ 8-hydroxyquinoline for 50 min at 25°C and then fixed for 4 h in fresh Farmer's fixative (1:3 glacial acetic acid/absolute alcohol) at 4°C . Finally the shoot tips were softened with 1 M hydrochloric acid at 55°C for 10 min and stained for 10 min with carbol-fuchsin before being squashed between a glass slide and a coverslip. The images were captured by a charge-coupled device (CCD) camera (ProgRes C5; Analytik Jena, Jena, Germany) attached to a microscope (Axio Scope, A1; Carl Zeiss, Oberkochen, Germany) and chromosome numbers were counted.

DATA ANALYSIS. For each genotype, at least 10 cells with good chromosomal dispersion were selected, counted, photographed, and analyzed. Images were processed with VideoTest-Karyo (Version 3.1; VideoTest, Saint-Petersburg, Russia), Image-Pro Plus (Version 6.0; Media Cybernetics, Rockville, MD) and Photoshop (Version CS2; Adobe, San Jose, CA). The centromeric position was classified as median (m), submedian (sm), or subterminal (st) according to Levan et al. (1964). The symmetry of karyotype was classified strictly according to the method of Stebbins (1971). The ratio of the sum of the length of

Fig. 2. Flowers and hips of some studied wild *Rosa* species in Xinjiang, northwestern China: (A) flower of *R. berberifolia* (sample 1), (B) hip of *R. berberifolia* (sample 1), (C) flowers of *R. spinosissima* var. *altaica* (sample 3), (D) hip of *R. spinosissima* var. *altaica* (sample 3), (E) flowers of *R. platyacantha* (sample 4), (F) hip of *R. platyacantha* (sample 4), (G) flower of *R. beggeriana* var. *beggeriana* (sample 5), (H–I) hips of *R. beggeriana* var. *beggeriana* (samples 5 and 7), (J) hips of *R. laxa* var. *laxa* (sample 11), (K) flowers of *R. laxa* var. *laxa* (sample 11).

Fig. 3. Metaphase chromosomes of four wild *Rosa* samples in Xinjiang, northwestern China. (A) *R. berberifolia* (sample 1), (B) *R. Platyacantha* (sample 4), (C) *R. beggeriana* var. *beggeriana* (sample 5), (D) *R. laxa* var. *laxa* (sample 11).

Fig. 4. Chromosome karyotypes of studied *Rosa* species in Xinjiang, northwestern China (bar = 5 μm): (A) *R. berberifolia* (sample 1), (B) *R. spinosissima* var. *spinosissima* (sample 2), (C) *R. spinosissima* var. *altaica* (sample 3), (D) *R. platyacantha* (sample 4), (E-G) *R. beggeriana* var. *beggeriana* (samples 5, 6, 7), (H) *R. acicularis* (sample 8), (I) *R. laxa* var. *laxa* (sample 9), (J) *R. laxa* var. *mollis* (sample 10), (K-M) *R. laxa* var. *laxa* (samples 11, 12, 13).

the long arms of individual chromosomes to the total haploid length of the chromosome complement (AS.K%) was measured on the basis of Arano (1963), and index of relative length (IRL) and relative length composition of genome (RLC) were calculated in accordance with Kuo et al. (1972).

Results

Four spread metaphase of the studied *Rosa* taxa are shown in Fig. 3. Karyotypes of all studied *Rosa* species are shown in Fig. 4. Parameters of chromosomes of studied species are listed in Table 3. No aneuploidy and no odd ploidy were observed in this study.

KARYOTYPE ANALYSIS OF *R. BERBERIFOLIA*. This morphologically distinctive species was diploid with a karyotype formula of $12m + 2sm$. The genome were composed of all four types of chromosomes [i.e., long (L), medium long (M2), medium short (M1), and short (S)], which made the constitution of relative length $2n = 2L + 6M2 + 4M1 + 2S$. The centromere index ranged from 35.43% to 46.16%, and the asymmetry index was 59.82%. The length ratio of longest to shortest chromosome (Lt/St) was 1.68. With none of the chromosomes' arm ratios larger than 2, the karyotype of *R. berberifolia* belonged to 1A.

KARYOTYPE ANALYSIS OF *R. SPINOSISSIMA*. In all metaphase plates analyzed in *R. spinosissima*, a tetraploid chromosome number ($2n = 4x = 28$) was observed. However, chromosome morphology differed between *R. spinosissima* var. *spinosissima* and *R. spinosissima* var. *altaica*. All chromosomes of var. *spinosissima* were metacentric, and the constitution of relative length was $2n = 2L + 10M2 + 10M1 + 4S$ for *R. spinosissima* var. *spinosissima* and $2n = 2L + 6M2 + 16M1 + 2S$ for *R. spinosissima* var. *altaica*. In addition, *R. spinosissima* var. *spinosissima* had the largest Lt/St of 2.69, whereas *R. laxa* var. *laxa* (sample 10) had the smallest Lt/St of only 1.49. The asymmetry index of *R. spinosissima* var. *spinosissima*, 52.60%, was the smallest from all the studied species.

KARYOTYPE ANALYSIS OF *R. PLATYACANTHA*. The chromosome number for *R. platyacantha* was $2n = 4x = 28$. The genome of this tetraploid species was composed of all four types of chromosomes, which made the constitution of relative length $2n = 4L + 10M2 + 12M1 + 2S$. Although no satellites were

observed in the genomes of most of the wild *Rosa* species in this study, for *R. platyacantha*, the tenth chromosome pair had a pair of satellites.

KARYOTYPE ANALYSIS OF *R. BEGGERIANA* VAR. *BEGGERIANA*. All three tested samples were diploid with exclusively metacentric and submetacentric chromosomes. The constitutions of relative length of samples 5, 6, and 7 were $2n = 2L + 6M2 + 6M1$, $2n = 2L + 2M2 + 10M1$, and $2n = 2M2 + 10M1 + 2S$, respectively. Besides, the asymmetry index of 63.77% from sample 5 was the largest among all the tested samples.

KARYOTYPE ANALYSIS OF *R. ACICULARIS*. *R. acicularis* was tetraploid with 28 exclusively metacentric and submetacentric chromosomes ($2n = 4x = 28$). The genome was also composed of all four types of chromosomes, making the constitution of relative length $2n = 4L + 8M2 + 12M1 + 4S$. The Lt/St ratio was 2.24. Therefore, with the arm ratio larger than two for one pair of chromosomes, the karyotype of this taxon was 2B.

KARYOTYPE ANALYSIS OF *R. LAXA*. The five *R. laxa* samples were divided into diploids ($2n = 2x = 14$) and tetraploids ($2n = 4x = 28$) with values of the asymmetry index from 55.14% to 60.11%. The karyotypes of all five samples were made up of m and sm chromosomes, which included 1A, 2A, and 1B. The ideograms of the five tested samples for this species are illustrated in Figure 5.

Discussion

The basic chromosome number is relevant to the systematic position of a taxon at high taxonomic levels (Raven, 1975). Wild *Rosa* species comprise a polyploid complex with a base chromosome number of seven. Not surprisingly, the present investigations regarding the base chromosome number agree completely with previous reports.

As shown in Table 3, *R. berberifolia* and *R. beggeriana* var. *beggeriana* were found to be diploid, whereas *R. spinosissima* var. *spinosissima*, *R. spinosissima* var. *altaica*, *R. platyacantha*, and *R. acicularis* were found to be tetraploid. Because previous chromosome counts of *R. platyacantha* were diploid (Ku and Robertson, 2003), this species has two ploidy levels in its area of distribution. The aforementioned tetraploid species had more advanced karyotypes of 1B or 2B than the diploids had. There is no direct evolutionary relationship between the variations of the

Table 3. Parameters of chromosomes for 13 tested wild *Rosa* samples in Xinjiang, northwestern China.

Code	Species	Chromosome no.	Karyotype formula	Arm ratio	Lt/St ratio ^z	Asymmetry index (%)	Centromere index (%)	Karyotype group
1	<i>R. berberifolia</i>	$2n = 2x = 14$	$12m + 2sm$	1.17–1.82	1.68	59.82	35.43–46.16	1A
2	<i>R. spinosissima</i> var. <i>spinosissima</i>	$2n = 4x = 28$	28 m	1.03–1.91	2.69	52.60	42.47–49.43	1B
3	<i>R. spinosissima</i> var. <i>altaica</i>	$2n = 4x = 28$	28 m	1.09–1.68	2.05	57.90	37.33–47.88	1B
4 ^y	<i>R. platyacantha</i>	$2n = 4x = 28$	28 m (2 sat, 10)	1.03–1.60	2.36	55.61	38.42–49.23	1B
5	<i>R. beggeriana</i> var. <i>beggeriana</i>	$2n = 2x = 14$	$6m + 8sm$	1.29–2.60	1.66	63.77	27.75–43.72	2A
6	<i>R. beggeriana</i> var. <i>beggeriana</i>	$2n = 2x = 14$	$12m + 2sm$	1.08–1.73	1.62	56.52	36.61–48.14	2A
7	<i>R. beggeriana</i> var. <i>beggeriana</i>	$2n = 2x = 14$	$10m + 4sm$	1.15–2.59	1.79	58.18	27.82–46.60	2A
8	<i>R. acicularis</i>	$2n = 4x = 28$	$22m + 6sm$	1.01–2.21	2.24	56.39	31.12–49.61	2B
9	<i>R. laxa</i> var. <i>laxa</i>	$2n = 4x = 28$	28 m	1.12–1.51	1.86	57.20	39.82–47.15	1A
10	<i>R. laxa</i> var. <i>mollis</i>	$2n = 2x = 14$	$12m + 2sm$	1.10–1.82	1.49	57.96	35.51–47.73	1A
11	<i>R. laxa</i> var. <i>laxa</i>	$2n = 2x = 14$	14 m	1.03–1.43	1.74	55.14	41.07–49.23	1A
12	<i>R. laxa</i> var. <i>laxa</i>	$2n = 2x = 14$	14 m	1.15–1.44	2.14	56.39	40.95–46.42	2A
13	<i>R. laxa</i> var. <i>laxa</i>	$2n = 4x = 28$	$20m + 8sm$	1.22–1.82	2.51	60.11	35.45–44.96	1B

^zLt/St ratio = ratio of chromosome length, the longest chromosome to the shortest one.

^yThe length of satellites is not included in the chromosome length.

Fig. 5. Ideograms of five tested samples of *Rosa laxa* from different location in Xinjiang, northwestern China (A–E represent samples 9–13, respectively).

ploidy level and the species, as we can see from the tetraploid sample 9, which possessed a very symmetric karyotype. This phenomenon was also observed by Luo et al. (2009) in traditional Chinese rose cultivars.

It is believed that wild *Rosa* species in China and Chinese traditional rose cultivars are all supposed to exhibit karyological diversity (Jian et al., 2010b, 2012; Luo et al., 2009; Yang, 2009; Yang et al., 2008), and our study confirmed those previous reports. However, in strong contrast to the present study, previous authors (Jian et al., 2012; Song and Li, 1989) have reported that only two types of karyotypes (1A, 2A) occur in the wild *Rosa* species, whereas four types of karyotypes (1A, 2A, 1B, 2B) were found in our research. However, among the studied samples, all of the karyotypes consisted of metacentric or metacentric

with submetacentric chromosomes. Meanwhile, lack of satellites implies terminal position of the nucleolar organizer region, not absence of at least one nucleolar organizer region.

Many species occur at only one ploidy level, but a few form a polyploid series (Wissemann, 2003). Available results have divided *R. laxa* on the basis of ploidy (Cairns et al., 2000; Yang, 2009; Yang et al., 2008; Zhu, 2011). In this study, we found diploid and tetraploid *R. laxa* in different floristic regions. Meanwhile, *R. laxa* is obviously variable in morphology. Guo (2010) studied the natural populations of *R. laxa* in the Tianshan Mountains and believed the genetic diversity of *R. laxa* was abundant both within populations and among populations. In this study, the tetraploid karyotype of sample 13 was distinct in having the highest asymmetry index and the most evolved arm

Fig. 6. Morphological differences of *Rosa laxa* between sample 10 and sample 13: (A) hip of sample 10, (B) hip of sample 13, (C–D) comparison of the single flower and flower bud (left = sample 10, right = sample 13).

ratios among all taxa within *R. laxa*. Besides, this taxon differs from typical *R. laxa* (sample 10) in the presence of the even bigger flower and hip size as well as the solitary flower rather than three to six flowers in a corymb (Fig. 6). These obvious morphological differences were reported by Liu Shixia (2000), who recognized this taxon as a new botanical variety of *R. laxa*, namely *R. laxa* var. *tomurensis*. Taking into consideration this description and the karyological data, along with the precedent that Lewis (1959) recognized the two morphologically distinct ploidies in *R. acicularis* as distinct subspecies, we support the previous view by Liu Shixia that this tetraploid taxon, *R. laxa* var. *tomurensis*, should be treated as a distinct botanical variety of *R. laxa*.

However, sample 12, which has leathery leaflets, was regarded as another botanical variety, *R. laxa* var. *kaschgarica*, which had already been recognized in *Flora Xinjiangensis* (Han, 1995). However, considering only one morphological difference as well as the same pattern of their karyological differentiation, it seems that further study is needed to ascertain whether it should be treated as a botanical variety of *R. laxa* or promoted to a specific rank. Because the karyotypes differed little, the hip color of *R. beggeriana* (Fig. 2G–H) was not regarded as a species diagnostic morphological trait in this study.

The classification of the genus *Rosa* has been disputed for a long time. Previous studies mainly focused on morphological characters, germplasm collection, and germplasm preservation. Moreover, the morphological descriptions of wild *Rosa* species are always complicated and often present problems in the precise identification of the species and especially their botanical varieties. The chromosome counts of genus *Rosa* have varied and can be used in its taxonomy (Ghanbari et al., n.d.). From our point of view, the chromosome morphology or karyotype could be a useful tool for identifying species, botanical varieties, and populations, investigating their genetic relationships and better understanding their evolutionary history. However, it is unlikely that further, more detailed karyotype analysis will change the major sectional alignments in *Rosa*.

The increase in chromosome number and consequent genome redundancy created by polyploidy has long been recognized as a prominent feature in the evolution of flowering plants (Levin, 2002; Stebbins, 1971). In *Plant Speciation*, Grant (1981) devoted five chapters (15% of the total text) to polyploidy, reflecting the importance of the topic both to the author and to plant biologists. Alpine and arctic floras have been regarded to have high frequencies of polyploidy (Brochmann et al., 2004; Ohba, 1988). Species in these restricted areas had to face harsh environmental

conditions that led to the creation of new adaptive traits. The Tomur National Nature Reserve lies on the southern flank of the Tianshan Mountains (lat. 41°40' N to 42°02' N, long. 80°07' E to 80°52' E) in Wensu County, which is one of the biggest alpine nature reserves in China. The mean elevation of the reserve is 4000 m and its climate is cold and arid with a mean annual rainfall 600 to 700 mm. *R. laxa* in Tomur exhibits tetraploidy, in accordance with the aforementioned generalization that extreme habitats promote the evolution of polyploidy.

Similarly, other species of *Rosa* section *Cinnamomeae* are polyploidy at high altitude in Yunnan Province, southwestern China (Jian et al., 2012). Thus, a comprehensive study would require a greater number of population samples that represent all the variations and the full range of habitats in which *R. laxa* occurs. What is more, the results indicate that different ploidy levels are associated with different regions and habitats where the germplasm resources grow.

Unfortunately, there is little information in the literature focused on the use of wild *Rosa* species in Xinjiang, although these species were found to have many desirable traits (Chen, 1997; Muller et al., 2008), partly because of their complex genetic background. This information gap should be addressed as early as possible. Although not discussed in this article, attention must be given to the relationship between the ploidy difference and the success of distant hybridization. Detailed analysis of the *Solanum* polyploid series, where the endosperm behavior has been mapped out over numerous species in the primary and secondary gene pools of potato (Carputo et al., 2003), suggests that the same evolutionary path may account for the origin and evolution of polyploidy taxa in *Rosa*. We infer from the text and our hybridization data (unpublished) that *Rosa* has an endosperm balance requirement of two maternal genomes to one paternal genome for successful growth of the endosperm early in seed development. Further research in the likely existence of endosperm balance numbers in *Rosa* should also be conducted.

The cytological data would supply chromosomal proofs not only for the taxonomy and evolution of genus *Rosa*, but also facilitate the exploitation of useful traits of wild *Rosa* species in breeding strategies. As we know, polyploids are often associated with particular characteristics such as increased heterozygosity and gene redundancy, proportional increases in gene expression, increase in cell and body size, changes in stomata size, and environmental stress tolerance (Coughlan, 2012; Levin, 1983).

Different ploidy levels among the *R. laxa* var. *laxa* (samples 9, 10, 11) and the botanical varieties with desirable traits (samples 12, 13) could provide diverse breeding materials. For example, with leathery leaflets, sample 12 could provide potential resistance against the powdery mildew and downy mildew (*Botrytis cinerea*). Tetraploid samples 9 and 13, which have the same ploidy level with most of the modern rose cultivars, will expectedly improve the success rate of distant hybridization. In sum, a better understanding of the cytological data that contribute to breeding systems and desired traits will definitely lead to improved modern rose cultivars.

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