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Karyotype Analysis and Genome-size Evaluation of Native Hydrangea Taxa in China

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Abstract. ***Hydrangea*** **is a widely used ornamental plant, with its commercial cultivars mainly derived from** ***H. macrophylla*****,** ***H. paniculata*****, and** ***H. arborescens*****. Although China exhibits rich genetic diversity in** ***Hydrangea*** **germplasms, the evaluation and breeding utilization of native Chinese** ***Hydrangea*** **resources were limited. To evaluate the unique** ***Hydrangea*** **germplasms in China, the karyotype and genome size of 29 resources were initially disclosed, along with additional 11 reported hydrangeas. The results revealed remarkable differences in the 2C DNA content, with values ranging from 1.98 pg in** ***H. arborescens*** **to 6.89 pg in** ***H. obovatifolia*****. Additionally, the karyotypes of all 40 germplasms revealed two distinct chromosomal groups: 30 taxa exhibited 2n = 2x = 36, and 10 taxa were 2n = 2x = 34. Notably, the majority of taxa with a basic chromosome number other than x = 18 belong to the Hydrangea subsection, suggesting a potentially higher level of evolutionary advancement within this group. The findings provide valuable insights for future identification and breeding using local** ***Hydrangea*** **germplasms.**

The genus *Hydrangea* is a diverse group of flowering plants with significant research value in both horticulture and ecology. Certain taxa were cultivated as ornamental plants due to their large, vividly colored flowers and diverse range of flower hues. The circumscription of *Hydrangea* remains contentious, with recognized species numbers varying between 23 (McClintock 1956) and 73 (Lu and Huang 1995), primarily distributed in temperate regions of East Asia and eastern North America, with some species extending into tropical regions of both hemispheres. China is a major distribution center for *Hydrangea* germplasms, with wild resources comprising 73% of the total worldwide. The *Flora of China* (Lu and Huang 1995) recorded 46 species and 10 varieties of *Hydrangea* in China, with a wide range of wild habitats and ecological conditions. However, foundational research on the genetic resources of *Hydrangea* in China remains undeveloped, with many naturally occurring populations inadequately protected, developed, and used, leading to a delay in breeding efforts.

Assessing the genome size of species not only aids in species identification but also reveals differences among genera and within the genus, making it an indispensable tool for studying genetic diversity (Bennett and Leitch 2001; Greilhuber et al. 2005; Pellicer et al. 2018). Furthermore, variations in genome size and chromosome number inform phylogenetic relationships and plant classification (Jang et al. 2016; Suda et al. 2003; Zonneveld 2001). Flow cytometry, a rapid and reliable method, has been widely used to measure genome size in plants (Doležel et al. 2007). Genome size variation is a common characteristic across different species and subspecies of *Hydrangea*. According to the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/), genome size had been recorded for 14 species of *Hydrangea*, with reported values ranging from ~1.95 pg (*H. quercifolia*) to 7.27 pg (*H. macrophylla* ssp. *macrophylla* 'Enziandom') (Cerbah et al. 2001; Demilly et al. 1998; Jones et al. 2007; Mortreau et al. 2010).

Karyotype analysis is a traditional cytogenetic step in the comparison of genomes among related species and can reveal the number, size, and morphology of chromosomes in a plant, providing information on its genetic diversity, evolutionary history, and potential breeding capabilities. Determination of chromosome number is a key step in hybrid breeding (Sattler et al. 2016), whereas precise ploidy identification is crucial for overcoming breeding obstacles such as hybrid sterility (Doležel and Bartoš 2005; Mason and Batley 2015). Chromosome size further correlates with evolutionary divergence (Schubert and Lysak 2011), underscoring the significance of cytogenetic data in angiosperm systematics (Bennett and Leitch 2011; Guerra 2008; Jang et al. 2013, 2018). Some *Hydrangea* species had been reported as euploids with chromosome numbers ranging from 2n = 2x = 30, 2n = 2x = 34, 2n = 2x = 36, 2n = 2x = 38, 2n = 3x = 54, 2n = 4x = 72 to 2n = 6x = 108 (Cerbah et al. 2001; Jones et al. 2007; Mortreau et al. 2010; Sax 1931; Van Laere et al. 2008). Additionally, using fluorescence in situ hybridization (FISH), the karyotype formulas of different *Hydrangea* species, including *H. paniculata*, *H. quercifolia*, *H. involucrata*, and *H. aspera*, had been determined (Mortreau et al. 2010; Van Laere et al. 2008). Although some *Hydrangea* species had been identified as diploids through flow cytometry, karyotype observations had yet to be conducted, and such analyses in future studies hold significant importance.

Challenges in the classification of *Hydrangea* arise from limited available resources, impeding comprehensive analysis and comparison of *Hydrangea*, especially those indigenous to China. Moreover, many studies indicated that *Hydrangea* does not form a strictly natural group. Variations in pollen, leaf, and seed traits among *Hydrangea* taxa overlap significantly with those of other genera (Samain et al. 2010). Various classification systems for *Hydrangea* had been supported by macro-morphological traits. However, some research has reported differing systematic placements for certain species. Thus, clarifying the evolutionary relationships of various *Hydrangea* taxa with their closely related genera remains an area for further investigation. In this study, we adopted *Flora of China* (FOC) classification system (Lu and Huang 1995), which divides the genus into five distinct categories: Petalanthae, Heteromallae, Hydrangea, Calyptranthe, and Cornidia.

Despite existing some research on genome size and karyotype analysis for related plant species, comprehensive studies specifically targeting Chinese *Hydrangea* germplasms are still lacking. To fill this gap, cytological techniques, including flow cytometry and chromosome observation were employed in this research, the 40 wild hydrangeas were

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evaluated by determining their chromosome numbers and estimating their genome size. Our findings not only contribute to the understanding of the evolutionary history and genetic diversity of *Hydrangea* but also provide a scientific basis for the rational conservation and utilization of *Hydrangea* resources indigenous genetic resources in China.

Materials and Methods

Origin of samples. Apart from *H. arborescens*, which was obtained from North America, the other 39 accessions were collected from the wild areas in China and carefully identified based on the *Flora of China* (Table 1). The investigated accessions were classified into four distinct sections based on the FOC classification system: Hydrangea, Heteromallae, Petalanthae, and Calyptranthe. All materials (Supplemental Fig. 1) were cultivated in the South Tropical Garden located in Kunming, Yunnan, China (24.86°N, 102.98°E).

Determination of nuclear DNA content by flow cytometry. Of the 40 *Hydrangea* taxa, three to five young plants were collected from the wild areas, planted in a greenhouse, and subsequently analyzed to estimate their nuclear DNA content. Three replicates of each sample were analyzed. The total DNA amount in nuclei was assessed by flow cytometry using *Zea mays* B73 (reference genome size 2.3G) (Schnable et al. 2009) or tomato (reference genome size 900Mb) (Tomato Genome Consortium 2012) as an internal reference.

To prepare the cell suspension, we placed the sample in 0.8 mL precooled MGb dissociation solution [45 mM $MgCl_2 \cdot 6H_2O$, 20 mM MOPS, 30 mM sodium citrate, 1% (W/V) PVP 40, 0.2% (v/v) Triton X-100, 10 mM Na_2EDTA, 20 μL/mL β-mercaptoethanol, pH 7.5] (Tian et al. 2011). A knife with a sharp blade was used to quickly chop the tissue vertically, which was placed in the dissociation solution on ice for 10 min, then filtered with a 400 mesh filter (Aperture size of 30 μm). The cell nucleus suspension was obtained, and appropriate volumes of prechilled propidium iodide (PI) (stock solution concentration 1 mg/mL) and RNase solution (stock solution concentration 1 mg/mL) were added to the suspension. The mixture was then incubated on ice in the dark for 0.5 to 1 h for staining (Doležel and Bartoš 2005; Doležel et al. 2007).

The suspension of the test sample and the internal reference were mixed in a 1:1 ratio by volume. BD FACScalibur flow cytometer (Franklin Lakes, NJ, USA) was used to detect the stained cell nucleus suspension sample on the machine, using 488-nm blue light excitation to detect the fluorescence intensity of the emitted light of propidium iodide, and 10,000 particles were collected for each detection. The coefficient of variance (*CV*; %) was controlled within 5% (Jang et al. 2018). The nuclear genome size of the samples was determined using the formula provided by Doležel et al. (2007). Modifit 3.0 analysis software was used for graphing analysis.

Determination of chromosome number. The method was adapted from Li and Zhang (1991) with minor modifications. The detailed procedure is as follows: stem tips were immersed in 2 mM 8-hydroxyquinoline for 6 h at room temperature (22 °C) in the dark, then rinsed in distilled water, fixed in a solution of ethanol: acetic acid (3:1) for 24 h at 4 °C, and finally transferred to 70% ethanol at –20 °C until needed. Before examination, stem tips were hydrolyzed in 1 M HCl at 55 °C for 12 min, rinsed with distilled water, and soaked in 1% aceto-orcein. The meristematic region of the stem tip was crushed in aceto-orcein, and chromosomes were counted. For each genotype, at least 10 cells with good chromosomal dispersion were selected, counted, photographed, and analyzed. Photoshop CC 2019 was used for basic image processing and MATO for chromosome measurement and pairing.

Results

Genome size. All 40 collected taxa were analyzed using flow cytometry, among which the 2C DNA amount of 29 *Hydrangea* species native to China was first reported. The results (Table 1 and Supplemental Fig. 2) demonstrated that 2C DNA amount ranged from 1.98 pg in *H. arborescens* to 6.89 pg in *H. obovatifolia*. The majority of taxa exhibited between 3.0 and 4.5 pg, with 27 of the 40 taxa falling within this range. Only two species, *H. lingii* and *H. obovatifolia*, had 2C DNA content larger than 4.5 pg, with sizes of 6.34 and 6.89 pg, respectively. Eleven taxa exhibited genome size (2C) smaller than 3.0 pg, including *H. arborescens*, *H. longipes*, *H. longipes* var. *fulvescens*, *H. rosthornii*, *H. sargentiana*, *H. villosa*, *H. dumicola*, *H. mandarinorum*, *H. davidii*, *H. stenophylla*, and *H. glancophylla* var. *scricea*. Among these, only *H. arborescens* from North America had a 2C DNA amount smaller than 2.0 pg.

In the Hydrangea subsection, genome size (2C) ranged from 1.98 pg of *H. arborescens* to 4.25 pg of *H. coacta*. In the Heteromallae subsection, genome size (2C) ranged from 2.29 pg of *H. mandarinorum* to 4.13 pg of *H. hypoglauca*. In the Petalanthae subsection, genome size (2C) ranged from 2.92 pg of *H. stenophylla* to 6.89 pg of *H. obovatifolia*. The 2C DNA amount of *H. glancophylla* var. *scricea* in the Calyptranthe subsection was 2.74 pg. Analysis of variance revealed that the average 2C DNA content of taxa in the Petalanthae subsection was 3.71 pg—significantly higher than that of the Hydrangea (3.11 pg) and the Heteromallae subsection (3.09 pg).

Chromosome number. The karyotypes of all 40 *Hydrangea* taxa were examined. Although it was difficult to determine the exact chromosome type for each taxa, we recorded the number of chromosomes in each karyotype (Table 1 and Supplemental Fig. 3).

The results indicate that all analyzed *Hydrangea* taxa were diploid, with most taxa having a chromosome number of $2n = 2x = 36$, and a basic chromosome number of $x = 18$, consistent with previous studies (Cerbah et al. 2001; Cai et al. 2015). However, 10 taxa were diploid with 34 chromosomes ($2n = 2x = 34$), including *H. coacta*, *H. glabripes*, *H. longipes*, *H. longipes* var. *fulvescens*, *H. longipes* var. *lanceolata*, *H. rotundifolia*, *H. sargentiana*, *H. strigosa*, *H. villosa*, and *H. glancophylla* var. *scricea*.

In the Heteromallae and Petalanthae subsections, all tested taxa had a chromosome number of $2n = 2x = 36$. In the Hydrangea subsection, seven species have a chromosome number of $2n = 2x = 36$, and nine taxa have a basic chromosome number of $x = 17$ ($2n = 2x = 34$), including *H. coacta*, *H. glabripes*, *H. longipes*, *H. longipes* var. *fulvescens*, *H. longipes* var. *lanceolata*, *H. rotundifolia*, *H. sargentiana*, *H. strigosa*, and *H. villosa*.

Discussion

Genome size variations in hydrangea taxa. Genome size of 40 *Hydrangea* taxa were quantified, encompassing 11 accessions that had been previously analyzed using flow cytometry. The amount of 2C DNA ranged from 1.98 to 6.89 pg, with notable differences observed, particularly between *H. arborescens* (1.98 pg), *H. lingii* (6.34 pg), and *H. obovatifolia* (6.89 pg). Such interspecific differences in genome size have also been widely documented in other plants (Bennet and Leitch 2011; Pellicer and Leitch 2020). These discrepancies may reflect multifactorial influences. First, intraspecific genetic diversity leads to differences in genome size. Additionally, ecological specialization and geographical isolation could drive adaptive genomic adjustments, as exemplified by the reduced DNA content in the American-native *H. arborescens* (Cerbah et al. 2001).

Comparative analysis revealed that the genome size of *Hydrangea* taxa observed in this study were generally lower than literature-reported values (Cerbah et al. 2001; Mortreau et al. 2010). The North American native species *H. arborescens* demonstrated a 2C DNA amount of 1.98 pg, significantly smaller than the previously reported 2.31 pg. Parallel reductions were observed in Asian native taxa, including species such as *H. paniculata* (3.07 vs. 3.77 pg), *H. serrata* (3.22 vs. 3.85 pg), and *H. macrophylla* (4.11 vs. 4.30 pg), whereas the interspecific genome size relationships remained consistent. This discrepancy may arise from inconsistencies in experimental methods, where variations in fluorochrome selection for flow cytometry (Doležel and Bartoš 2005), choice of internal reference standard, and differences in the values assigned to internal reference standards can collectively influence the 2C DNA amount.

In flow cytometry, the selection of reference standards is a critical factor influencing the accuracy of genome size estimation. This study uses *Zea mays* B73 and tomato as internal references, both of which are widely used standard species in plant genomics research. However, their distant phylogenetic relationship to *Hydrangea* might introduce potential errors (Greilhuber et al. 2005). For example,

Table 1. Ploidy level and 2C DNA of hydrangeas in the present study in comparison with published ploidy levels compiled from the literature.

SS	Taxon name	Sampling location	ISFI	SFI	(*CV*)/%	2C DNA (pg) Taxon mean	Taxon SD	Previous reports	Ref	Chrom. no.
Hy	*H. arborescens*	America	66.43	28.03	1.18	1.98	0.01	2.31	Cerbah et al. 2001	2n = 2x = 36
Hy	*H. aspera*	Hubei	16.51	29.11	0.51	3.17	0.01	4.74	Cerbah et al. 2001	2n = 2x = 36
Hy	*H. coacta*	Guizhou	12.06	28.45	1.51	4.25	0.04	NA	NA	2n = 2x = 34
Hy	*H. discocarpa*	Hubei	9.55	16.52	0.47	3.11	0.01	NA	NA	2n = 2x = 36
Hy	*H. glabripes*	Sichuan	16.31	31.99	0.42	3.54	0.01	NA	NA	2n = 2x = 34
Hy	*H. kawakamii*	Guizhou	16.99	33.21	1.28	3.52	0.02	4.63	Mortreau et al. 2010	2n = 2x = 36
Hy	*H. longialata*	Yunnan	19.39	35.77	1.79	3.31	0.03	NA	NA	2n = 2x = 36
Hy	*H. longifolia*	Hubei	22.35	40.01	1.58	3.21	0.03	NA	NA	2n = 2x = 36
Hy	*H. longipes*	Shaanxi	52.06	33.04	1.48	2.97	0.03	NA	NA	2n = 2x = 34
Hy	*H. longipes.* var. *fulvescens*	Shaanxi	22.03	30.91	0	2.49	0.02	NA	NA	2n = 2x = 34
Hy	*H. longipes.* var. *lanceolata*	Shaanxi	20.13	37.62	0	3.44	0.01	NA	NA	2n = 2x = 34
Hy	*H. rosthornii*	Hubei	19.71	29.9	0.34	2.72	0.04	NA	NA	2n = 2x = 36
Hy	*H. rotundifolia*	Xizang	12.01	21.46	0.7	3.21	0.01	NA	NA	2n = 2x = 34
Hy	*H. sargentiana*	Hubei	12.54	19.82	0.3	2.84	0	2.98	Mortreau et al. 2010	2n = 2x = 34
Hy	*H. strigosa*	Guizhou	12.59	21.75	0.28	3.11	0.08	3.47	Cerbah et al. 2001	2n = 2x = 34
Hy	*H. villosa*	Guizhou	24.89	39.57	2.52	2.92	0.04	3.39	Mortreau et al. 2010	2n = 2x = 34
He	*H. dumicola*	Xizang	23.84	32.58	0	2.52	0	NA	NA	2n = 2x = 36
He	*H. heteromalla*	Shaanxi	17.75	33.85	0.25	3.44	0	2.95	Cerbah et al. 2001	2n = 2x = 36
He	*H. hypoglauca*	Guizhou	24.94	55.97	1.56	4.13	0.03	NA	NA	2n = 2x = 36
He	*H. mandarinorum*	Hubei	59.25	28.83	0.97	2.29	0.01	NA	NA	2n = 2x = 36
He	*H. paniculata*	Guizhou	12.31	20.92	1.66	3.07	0.03	3.77	Cerbah et al. 2001	2n = 2x = 36
Pe	*H. macrophylla*	Guizhou	22.81	52.07	0.37	4.11	0.01	4.30	Cerbah et al. 2001	2n = 2x = 36
Pe	*H. serrata*	Zhejiang	24.72	43.22	0.29	3.22	0.01	3.85	Cerbah et al. 2001	2n = 2x = 36
Pe	*H. caudatifolia*	Guizhou	20.45	39.61	0.24	3.48	0	NA	NA	2n = 2x = 36
Pe	*H. chungii*	Anhui	20.05	42.23	0.24	3.89	0	NA	NA	2n = 2x = 36
Pe	*H. coenobialis*	Jiangxi	58.43	39.46	1.85	3.17	0.03	NA	NA	2n = 2x = 36
Pe	*H. davidii*	Yunnan	20.37	33.77	0.78	2.99	0.01	NA	NA	2n = 2x = 36
Pe	*H. gracilis*	Jiangxi	25.03	43.68	0.29	3.21	0	NA	NA	2n = 2x = 36
Pe	*H. kwangsiensis*	Hunan	19.93	34.84	0.64	3.15	0.01	NA	NA	2n = 2x = 36
Pe	*H. kwangsiensis* var. *hedyotidea*	Hunan	18.77	30.95	2.06	3.99	0.03	NA	NA	2n = 2x = 36
Pe	*H. kwangtungensis*	Jiangxi	20.41	39.73	0	3.58	0	NA	NA	2n = 2x = 36
Pe	*H. lingii*	Fujian	23.03	79.24	0	6.34	0	NA	NA	2n = 2x = 36
Pe	*H. linkweiensis*	Guizhou	20.21	36.74	0	3.27	0	3.82	Cerbah et al. 2001	2n = 2x = 36
Pe	*H. linkweiensis* var. *subumbellata*	Guizhou	20.13	35.46	0	3.15	0.02	NA	NA	2n = 2x = 36
Pe	*H. mangshanensis*	Hunan	20.25	34.14	0.57	3.05	0.04	NA	NA	2n = 2x = 36
Pe	*H. obovatifolia*	Sichuan	49.87	73.28	0	6.89	0.03	NA	NA	2n = 2x = 36
Pe	*H. stenophylla*	Guizhou	13.78	22.43	0.76	2.92	0.01	NA	NA	2n = 2x = 36
Pe	*H. vinicolor*	Guizhou	12.66	21.82	0.27	3.11	0.01	NA	NA	2n = 2x = 36
Pe	*H. zhewanensis*	Zhejiang	58.59	40.64	0.68	3.33	0.06	NA	NA	2n = 2x = 36
Ca	*H. glancophylla* var. *scricea*	Chongqing	23.76	35.59	2.67	2.74	0.04	NA	NA	2n = 2x = 34

ISFI = internal sample fluorescence intensity; SFI = sample fluorescence intensity; Chrom = chromosome. Subsection (SS) is classified according to the *Flora of China* (FOC) system. Hy = Hydrangea subsection; He = Heteromallae subsection; Pe = Petalanthae subsection; Ca = Calyptranthe subsection.

Temsch et al.'s (2022) study showed that using distantly related references could cause genome size errors of 5% to 8% due to differences in staining efficiency. Furthermore, the accuracy of genome size estimation heavily relies on the variability in genome size assignment for the internal reference standard. If there is a discrepancy between the actual genome size of the reference and the assigned value, the calculated genome size of the sample will be proportionally skewed from the true value. For instance, the reported reference genome size of *Zea mays* B73 varies between 2.3 Gb (Schnable et al. 2009) and 2.39 Gb (Liu et al. 2025), which could introduce an error of ~3.8%. To minimize these effects, this study employs a dual-reference cross-validation approach, along with three biological replicates, ensuring that the *CV* is ≤5% to reduce random error (Doležel et al. 2007).

Significant genome size variation among *Hydrangea* subsections was found, with the Petalanthae subsection exhibiting a markedly larger average 2C DNA content than Hydrangea and Heteromallae. Molecular phylogenetics indicates higher evolutionary complexity in the Hydrangea subsection compared with Heteromallae and Petalanthae (Zhang et al. 2021). Notably, although the Hydrangea subsection demonstrates greater evolutionary advancement, it paradoxically harbors smaller genomes, whereas the genomically larger Petalanthae subsection appears less evolved. This contradicted the view that the evolutionary tendency of plant genome size was toward an increase (Hawkins et al. 2008; Leitch et al. 2005) but aligned with the core characteristics of the C-value paradox, which states that genome size was not directly related to organismal complexity or evolutionary status (Gregory 2001, 2011). Some studies suggest that genome size may reflect distinct evolutionary strategies: genome streamlining facilitates adaptive radiation (Soltis and Soltis 1997), while genome expansion enhances ecological adaptability without significantly increasing morphological complexity (Gregory 2011; Samain et al. 2010). Future research should integrate repeat sequence analysis, functional genomics, and ecological data to elucidate the evolutionary significance of genome size dynamics in *Hydrangea*. Additionally, certain species in the Petalanthae subsection, such as *H. obovatifolia*, exhibit exceptionally large genomes. Although these species are currently diploid, further investigation is needed to explore potential hidden genome duplication events (Hufford et al. 2001).

Chromosome number and ploidy of hydrangea. H. arborescens was found to exhibit the lowest 2C DNA content at 1.98 pg, whereas *H. obovatifolia* had the highest 2C DNA content at 6.89 pg. Both species, however, share the same chromosome number of 36 (2n = 2x = 36). Previous studies had demonstrated variations in DNA content among subspecies of *H. aspera*, as well as within groups of the same subspecies. Notably, these differences in DNA content were not correlate with chromosome number (Mortreau et al. 2010). In the case of *H. involucrata*, for instance, the DNA content was ~4.99 pg, while it possessed only 30 chromosomes (Mortreau et al. 2010). These findings indicated that DNA content and chromosome number may vary independently within *Hydrangea* taxa.

A notable finding from chromosomal analysis was that all taxa tested in the Petalanthae

subsection exhibited a chromosome number of 2n = 2x = 36. Similarly, species from the Heteromallae subsection also displayed a chromosome count of 2n = 2x = 36. Most of species with a basic chromosome number other than x = 18 belong to the Hydrangea subsection. Variations in chromosome numbers were a part of biological evolution and diversity, serving as a potential mechanism for organisms to adapt to new environments and ecological niches, which suggested that taxa within the Hydrangea subsection exhibit greater chromosomal variability, indicating a higher likelihood of chromosomal rearrangements.

Accurate determination of parent ploidy and genome size is significant for improving hybrid affinity and breeding efficiency. Smaller genome size, for instance, are advantageous traits that facilitate molecular approaches. In addition, taxa with the same ploidy can be effectively used in hybrid breeding programs to mitigate hybridization incompatibility. This study reported the chromosome numbers of 40 *Hydrangea* taxa, among which 29 were reported for the first time. The chromosome numbers of the remaining 11 taxa had been previously documented, which were consistent with the results presented in this article. The findings indicated that all tested hydrangeas are diploid, with karyotypes of 2n = 2x = 34 and 2n = 2x = 36, most of which are 2n = 2x = 36. These findings provided valuable guidance for selecting hybridization parents within the *Hydrangea* genus.

In conclusion, this study assessed the genome size and chromosome numbers of 40 *Hydrangea* taxa, providing fundamental genetic resource data for plant breeding applications. Future research using fluorescent DNA probes for in situ hybridization, coupled with molecular phylogenetics, is crucial to expand the genus and understanding the evolution of cytological and morphological traits. Subsequent studies could aim to integrate plant morphological characteristics and cytogenetic data with modern molecular biology, enabling a more accurate and objective evaluation of the systematic evolution and relationships among *Hydrangea* taxa, ultimately contributing to the development of high ornamental qualities and adaptable *Hydrangea* varieties.

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