Pavonia × *rufula* (Malvaceae): An Interspecific Hybrid between *Pavonia lasiopetala* and *Pavonia missionum*

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The genus *Pavonia* is one of the most abundant genera (more than 200 species) in the Malvaceae, and most of the species are native to North and South America (Fryxell, 1999). Although numerous species exist, only a few have been used as ornamental plants, such as *P. hastata* in Australia (Mitchell, 1982) and *P. lasiopetala* in Texas (Nokes, 1986). *Pavonia lasiopetala* is a small shrub native to Texas and northern Mexico, inhabiting dry, rocky landscapes (Fig. 1A). *Pavonia missionum* is a perennial shrub native to South America in Brazil, Argentina, and Paraguay (Fryxell, 1999) (Fig. 1B). *Pavonia lasiopetala* is native to central Texas; thus, plants can survive in U.S. Department of Agriculture (USDA) hardiness zone 7b (U.S. Department of Agriculture–Agricultural Research Service, 2012). *Pavonia missionum* has been observed to reseed and come back every year (John Ruter, personal observation) at Tifton, GA [USDA hardiness zone 8b (U.S. Department of Agriculture–Agricultural Research Service, 2012)].

Pavonia missionum belongs in subgen. *Pavonia*, sect. *Pavonia*, subsect. *Hastifoliae*, whereas *P. lasiopetala* is in subgen. *Pavonia*, sect. *Pavonia*, subsect. *Exsertae* (Fryxell, 1999). The base chromosome number for the genus *Pavonia* is $n = 7$. *Pavonia missionum* is an octoploid ($2n = 8x = 56$), whereas the chromosome number for *P. lasiopetala* is unknown (Fryxell, 1999). Both species prefer full sun to partial shade. The leaf bases are usually cordate with dentate margins for *P. lasiopetala* and cordate with crenate to serrate margins for *P. missionum*, and the apexes are acute for both species. The leaf blades are ovate to ovate-triangular with occasionally

three to five lobes (no lobe for *P. lasiopetala*) (Fryxell, 1999). The leaf of *P. lasiopetala* is moderate to heavily stellate pubescent (Fig. 2A and B). The adaxial leaf surface of *P. missionum* is heavily glandular-pubescent with appressed hairs, and the lower side of the leaf is sparsely pubescent with stellate and simple hairs (Fig. 2C and D). Both species have solitary flowers with staminal columns and five petals. *Pavonia lasiopetala* has five pink petals that slightly overlap, whereas *P. missionum* has five separate orange-red petals. The two species can be used as ornamental plants as a result of their heat and drought tolerance, and their attraction of pollinators (Quinn and Klym, 2009). A new interspecific hybrid with reduced fertility, which carries the two species' desired ornamental traits, would be ideal for developing new cultivars. Thus, the interspecific hybrid *P. × rufula* was developed by crossing *P. lasiopetala* (♀) and *P. missionum* (♂). *Pavonia × rufula* is an attractive perennial shrub with red or red-purple flowers and reduced fertility.

Origin

In Fall 2017, controlled crosses were made at the University of Georgia (UGA) Trial Garden in Athens, GA. In Spring 2018, 83 seeds from 60 initial crosses were treated with 15 min of acid scarification and were sown at the UGA Durham Horticulture Farm in 1020 trays (54 × 27 × 6 cm) (Landmark Plastic, Akron, OH) in Watkinsville, GA. In Summer 2018, 26 germinated plants were transplanted to 2.8-L pots at the UGA Durham Horticulture Farm. Only six of these seedlings showed novel traits, whereas the remaining seedlings appeared identical to the maternal parent. The six hybrid seedlings are intermediate morphologically to both parents. Five plants showed red [Royal Horticultural Society (RHS) 46B] flower color and one showed red-purple (RHS 57C) flower color (Royal Horticultural Society, 2001).

Terminal cuttings were collected on 5 Sept. 2018 for propagation, treated with indole-3-butyric acid potassium salt (Sigma-Aldrich Co., St. Louis, MO) at 3000 ppm for a

5-s quick dip at the Durham Horticulture Research Farm. Cuttings were placed in 8.8 × 8.8-cm plastic pots (Kord; The HC Companies, Inc., Twinsburg, OH) with a substrate of Pro PRO-MIX BX MYCORRHIZAE Pro (PRO-MIX, Quakertown, PA) and Aero-soil perlite (Dicalite, Bala Cynwyd, PA) (1:1, v:v). Mist was applied (8 s every 5 min from 7:00 AM to 7:00 PM) for 2 weeks. Rooting was 100% for all cuttings attempted. The six crosses were overwintered at the Durham Horticulture Research Farm in an overwinter house (set temperature, 7 °C) in 2018–19 and were planted to the field on 2 May 2019 for further evaluation. Plants were spaced 1.22 m apart in a row, 1.83 m between rows, with drip irrigation (emitters 30.5 cm apart) applied twice a week. Fertilizer (10N–4.4P–8.3K; Athens Seed Company, Watkinsville, GA) was incorporated into the soil at a rate of 84 kg nitrogen/ha before planting.

To observe the difference of leaf hairs precisely, a scanning electron microscope experiment was conducted at the UGA Electron Microscopy Laboratory. Three leaves of both parents and the hybrid were cut with a circular hole punch (circle diameter, 0.85 cm). Leaf samples (adaxial and abaxial) were coated using a Leica EM ACE600 Coater (Leica Microsystems Inc., Buffalo Grove, IL) and were then observed using an FE-SEM (Thermo Fisher Scientific, Precision, Waltham, MA).

Flow cytometry was used to determine the holoploid (2C) relative genome size for both species and the hybrid used in our research. The analysis was conducted at the UGA Center for Tropical and Emerging Global Diseases Cytometry Shared Resource Laboratory in Athens, GA, using a CytoFLEX Flow Cytometer (Beckman Coulter Life Sciences, Indianapolis, IN). Ten single leaf samples were collected and analyzed from each plant. Each leaf sample and internal standard of known genome size were included [*Pisum sativum* 'Ctirad' (2C = 9.19 pg)]. About 1 cm² of newly emerged leaf tissue from each plant was chopped using a razor blade into 1- to 2-mm² pieces. The leaf pieces were then mixed with 400 µL nuclei extraction buffer (CyStain ultraviolet Precise P; Sysmex Partec, Görlitz, Germany) for 30 s. Chopped particles were removed using 40-µm mesh filters (nonsterile CellTrics filters; Sysmex America, Inc., Mundelein, IL), and 1600 µL of nuclei staining buffer [CyStain Ultraviolet Precise P (4',6-diamidino-2-phenylindole), Sysmex Partec] was added. The holoploid (2C) genome size was calculated as 2C = [DNA content of the standard × (Mean fluorescence of sample ÷ Mean fluorescence of the sample)]. Means ± SE (n = 10) are presented.

Description

The description of the new interspecific hybrid species is as follows: *Pavonia × rufula* Yue and Ruter [*P. lasiopetala* (♀) Scheele × *P. missionum* (♂) Eckman] is an interspecific hybrid with intermediate or different morphological characteristics compared with the

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Fig. 1. Parents and new hybrids in the field at the UGA Durham Horticulture Farm. (A) *Pavonia lasiopetala*. (B) *Pavonia missionum*. (C) *Pavonia* $\times$ *rufula* (red). (D) *Pavonia* $\times$ *rufula* (red-purple).

Fig. 2. Leaf surface and pubescence viewed using a scanning electron microscope for (A) *Pavonia lasiopetala*, upper leaf surface; (B) *P. lasiopetala*, lower leaf surface; (C) *P. missionum*, upper leaf surface; and (D) *P. missionum*, lower leaf surface.

Fig. 3. Leaf surface and pubescence viewed using a scanning electron microscope for (A) *P. x rufula*, upper leaf surface; and (B) *P. x rufula*, lower leaf surface.

parents. The new hybrid's plant height is taller than either parent, the leaf area per leaf is less, the epicalyx lobes are intermediate to both parents, and the leaf shape and marginal serrations are similar to the paternal parent. The parent species' petal color is pink and orange-red, respectively, with the hybrid's petals being red to red-purple. The hybrid exhibits limited fertility.

The plants are small perennial shrubs with unique axillary red or red-purple flowers and five petals. The mericarps are reticulate-rugose, the adaxial leaf surfaces have simple trichomes on the lamina and stellate trichomes on prominent veins, and the abaxial surfaces have glandular and nonglandular simple trichomes. The leaf blades are usually 4 to 6 cm long and are slightly longer than wide, with an acute apex. The leaves have a cordate leaf base, dentate to crenate margins, and an overall cordate leaf shape. The leaf's upper surface is moderately stellate pubescent, and the lower surface is minutely stellate pubescent (Fig. 3). Petioles are green (RHS 144B) and mainly 2 to 3 cm long and 0.6 to 1.2 mm in diameter, with shorter simple hairs with glandular and nonglandular pubescence. Flowers are solitary and asymmetric, and are located in the leaf axis with 1- to 2.5-cm-long pedicels and five lanceolate involucellar bracts (7–9 $\times$ 2–2.5 mm). *Pavonia* $\times$ *rufula* has five separate red petals with a staminal column in the center. Flowers measure 35 to 45 mm in diameter, with 10 pistils and about 23 to 36 stamens. Styles are $\approx$ 1.75 cm long and 0.1 to 0.2 cm in width, with a light red color (RHS 49A) at the base, whereas the stigmas are a deeper red (RHS 46A). Styles exceed the column, and stigmas are usually villous. Stamens are white (RHS 155B), with yellow (RHS 16A) pollen in the anther. Pedicels are green (RHS 144C) and measure 1.5 to 4 cm in length, with simple and stellate pubescence. There are five sepals at the bottom of each flower $\approx$ 1.3 cm in length. The sepals are lanceolate in shape, hirsute with simple and stellate pubescence, and light green (RHS 44D) with deeper green veins. There are also five lanceolate epicalyces split into one-seeded mericarps. The mericarps of *P. x rufula* are very similar to *P. missionum*, except the number of mericarps per fruit is reduced. *Pavonia* $\times$ *rufula* is larger compared with both parents (Table 1). The leaf area of *P. x rufula* is smaller than both parents, and the leaf shape is more like *P. missionum* (Table 1, Fig. 4). The other traits, such as style length, calyx lobe length, epicalyx lobe length, and mericarp shape, are similar to one parent or intermediate between two parents (Table 1). *Pavonia* $\times$ *rufula* is a perennial shrub with a woody base that prefers full sun to partial shade and blooms during the long days of summer in Athens, GA (USDA hardiness zone 8a; Fig. 1C and D) (U.S. Department of Agriculture–Agricultural Research Service, 2012).

Although *P. x rufula* is larger at maturity than either parent, the flower diameter shows

Table 1. Morphological characteristics of *Pavonia lasiopetala*, *P. missionum*, and *P. ×rufula*.

Characteristics	<i>P. lasiopetala</i>	<i>P. missionum</i>	<i>P. ×rufula</i>
Plant height (cm)	78.97 b ^z	72.77 b	97.57 a
Plant width index (cm) ^y	127.23 b	135.53 ab	151.37 b
Leaf area (cm ²) ^x	15.99 a	15.59 a	12.63 b
Flower diameter (cm)	3.88 a	3.96 a	3.96 a
Sepal length (cm)	1.28 b	1.34 a	1.34 a
Epicalyx lobe length (cm)	1.11 a	0.93 c	1.01 b
Style length (cm)	1.72 a	1.71 a	1.75 a
Flower color	Pink (RHS 68A)	Orange-red (RHS 33A)	Red (RHS 46B) or red-purple (RHS 57C)
Mericaip shape	Reticulate-costate	Reticulate-rugose	Reticulate-rugose
Abaxial pubescence	Stellate hairs, lamina, and veins nonglandular	Different-length hairs on lamina and veins, veins simple and stellate	Simple hairs sparse on lamina, stellate hairs on veins
Adaxial pubescence	Some stellate hairs on lamina and veins	Short hairs mainly on veins	Multilength single and stellate hairs, mainly on veins
Petiole pubescence	Pubescent nonglandular, many stellates with 3–7 hairs	Long and short glandular hairs	Shorter simple hairs with glandular and nonglandular pubescence

^zMeans followed by a different letter in the same row are significantly different ($P < 0.05$).

^yPlant width index: (East–west width + North–south width)/2.

^xLeaf area was recorded using a Li-3100C leaf area meter (LI-COR Biosciences, Lincoln, NE) on fully expanded leaves for five leaves per plant.

RHS = Royal Horticultural Society.

Fig. 4. Leaf shape of *Pavonia lasiopetala*, *P. missionum*, and *P. ×rufula*. Fig. 5. Flower color of *Pavonia lasiopetala*, *P. ×rufula*, and *P. missionum*. RHS, Royal Horticultural Society. Fig. 6. Seed production of *Pavonia lasiopetala*, *P. ×rufula*, and *P. missionum*. Normal-size mericarps formed on *P. lasiopetala* and *P. missionum*. Mericarps on *P. ×rufula* were malformed and died at an early stage of development.

no difference from the parents (Table 1, Fig. 5). The fertility of *P. ×rufula* is reduced compared with both parents (Fig. 5). Previous research studies with other plants in the Malvaceae (*Hibiscus* sect. *Furcaria*) indicated that many interspecific hybrids were sterile or nearly so (Menzel et al., 1983). Partial or complete sterility is common in interspecific hybrids and is often caused by imperfect pairing of the chromosomes at meiosis (Fehr, 1991). Observations in 2018 of 100 flowers showed no seed was formed for all six *P. ×rufula* seedling selections, and all the

flowers aborted a few days after pollination (Fig. 6). In 2019, *P. ×rufula* plants at the UGA Durham Horticulture Farm produced a few seeds. If the seed was produced, only one viable seed was found in an individual fruit. Seeds were collected and sown in the UGA Horticulture Farm greenhouse for further evaluation. The resulting plants were uniform in appearance and had a phenotype like the hybrid parent.

The holoploid (2C) genome size for plants in this study were *P. lasiopetala* (5.55 ± 0.09 pg), *P. missionum* (4.00 ± 0.09 pg), and *P. ×rufula* (4.76 ± 0.03 pg). The intermediate genome size of the proposed new species provides further evidence for the hybrid nature of *P. ×rufula* and suggests interploid hybridization. Although two hexadecaploid ($2n = 16x = 112$) species are known in the genus *Pavonia*, there is no known intermediate ploidy series between $8x$ and $16x$ (Fryxell, 1999). Because there are no chromosome counts for *P. lasiopetala* or the entire subgen. *Pavonia*, sect. *Pavonia*, subsect. *Exsertae*, further research and chromosome count data may show *P. lasiopetala* and *P. ×rufula* to have ploidy levels greater than $2n = 8x$. Because no data for genome size exist for *Pavonia* in the Plant DNA C-values Database (2021), the values presented represent the first published holoploid (2C) genome sizes for this genus.

Controlled crosses between *P. lasiopetala* and *P. missionum* resulted in new interspecific hybrid seedlings with attractive red to red-purple flowers. *Pavonia ×rufula* Yue and Ruter (red rose mallow) is presented as a proposed name for this cross's hybrids and is validated with an appropriate diagnosis. Holotype: container-grown plant; UGA Durham Horticulture Farm, Watkinsville, GA; 24 Aug. 2018; Yue and Ruter 287572 (GA).

Availability

Information about the research and use of *P. ×rufula* can be obtained from Dr. John Ruter (e-mail: ruter@uga.edu) from the College of Agricultural and Environmental

Sciences, Department of Horticulture, University of Georgia, Athens, GA.

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