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Flower Forms and Ploidy Levels Impact Fertility in Althea

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Abstract. **Althea (*Hibiscus syriacus*) is a popular shrub known for its vibrant summer blooms and winterhardiness; however, althea produces capsules with numerous seeds that germinate and cause a nuisance in production and the home landscape. Breeding for sterile forms has long been a goal of *Hibiscus* breeders, yet many popular "sterile" cultivars have been reported as weedy. The purpose of this study was to evaluate female and male fertility of tetraploid and hexaploid cultivars, and to evaluate the female fertility of pentaploid progeny resulting from 4*x* × 6*x* and 6*x* × 4*x* crosses. More than 600 self-pollinations were performed on 21 cultivars, yet only 24% of self-pollinations resulted in filled capsules, for an overall rate of four seeds per pollination. Significant differences were observed among taxa for seeds per capsule and seeds per pollination. Highest capsule set was observed on self-pollinated White Chiffon® and Pink Chiffon®. Anecdotally, we observed reduced vigor in the S_1 generation of most taxa. However, 'Woodbridge' produced vigorous seedlings through the S_2 generation. More than 2000 cross-pollinations were also performed, resulting in more than 15,000 seeds. To evaluate female fertility, 28 taxa were pollinated with a variety of male parents. Fertility was measured as seeds per capsule and seeds per pollination. Significant differences were found among taxa within and among flower forms (single, semi-double, and double) for seeds per capsule and seeds per pollination. Double-flowered forms had reduced female fertility. Taxa previously reported to be sterile were found to be fertile, including 'Aphrodite', 'Diana', 'Helene', and 'Minerva'. Two hexaploids, 'Pink Giant' and Raspberry Smoothie™, had reduced female fertility compared with tetraploids. Male fertility was estimated for 20 cultivars by pollinating between one and 23 cultivars. For male fertility, significant differences were found among taxa for seeds per capsule and seeds per pollination; however, no significant differences in male fertility were observed among flower forms. Four taxa had relatively high fertility with more than 10 seeds per capsule and seeds per pollination, including Blue Satin®, Lil' Kim™, Bali™, and Tahiti™. In addition to the significant differences among female and male fertility of each taxon, capsule set varied widely among individual cross combinations. Significant differences of female fertility were found in pairwise comparisons between almost all pentaploid taxa and the mean of tetraploid control cultivars. No difference in percent seed germination was observed between 4*x* × 6*x* and 6*x* × 4*x* crosses (45% and 45%, respectively) but both were significantly lower than seeds from open-pollinated tetraploids (89%). The reduced fertility of pentaploids will likely lead to new reduced fertility or sterile cultivars for the nursery industry, especially if combined with double flowers.**

Weediness or invasive potential is a constant concern for ornamental shrubs and trees such as *Lantana* (Czarnecki et al., 2014), *Buddleja* (Tallent-Halsell and Watt, 2009), *Berberis* (Brand et al., 2012), *Ligustrum* (Fetouh et al., 2016), and *Acer* (Wangen and Webster, 2006). The elimination or reduction of seeds, especially in taxa with heavy seed production, has been a primary goal for ornamental plant breeders. Even in taxa that do not pose an immediate risk to native forests, weediness creates a constant maintenance issue in nursery production and in the home landscape. Sterile cultivars will likely save money, save time, and reduce the use of pesticides by commercial growers and home gardeners.

Fertility estimates of elite cultivars of ornamental plants can be beneficial for breeders to design future crosses. For weedy and invasive species, fertility estimates have been shown to vary among genotypes. A fertility study on Japanese barberry (*Berberis thunbergii*), a plant considered invasive in ≈30 U.S. states, identified sterile cultivars already available in the nursery trade (Brand et al., 2012). Of the 46 cultivars investigated, they found that seed production varied from no seed to more than 12,000 seeds per plant, whereas the number of seeds per fruit ranged from 0.1 to 1.8 (Brand et al., 2012). However, mature plants that initially had low fertility were later evaluated and shown to be fertile, demonstrating that fertility can vary from year to year and demonstrating the necessity for long-term fertility tests on cultivars (Brand et al., 2012).

Environmental groups often advocate for complete bans on weedy or invasive species, including all cultivars of proscribed species (Gagliardi and Brand, 2007). However, many consumers (Kelley et al., 2006) and nursery and landscape professionals (Gagliardi and Brand, 2007) recognize that not all cultivars should be treated as invasive and banned. When surveyed about the best approach to reduce the sale of invasive plants, nursery and landscape professionals favored the creation of genetically altered sterile cultivars as one of their top choices (Gagliardi and Brand, 2007). Although natural mutations, induced mutations, and wide hybridization have been used to reduce or eliminate seed production, ploidy manipulation remains one of the more reliable tools for creating seedless or near-seedless clones, as illustrated by ornamental taxa such as *Hypericum* (Trueblood et al., 2010), *Buddleja* (Smith, 2010), and *Pyrus* (Phillips et al., 2015).

Fertility tests in plants with odd ploidy levels are useful for determining their fertility, as seen in tests of 10 triploid accessions of the weedy species *Hypericum androsaemum* (Trueblood et al., 2010). Among the triploid accessions, Trueblood et al. (2010) found a significant reduction in male fertility and a complete elimination of viable seed production in nine of the 10 triploids. The focus of developing sterile triploids is usually female fertility. However, in some cases, male sterility is also of concern, including in *Lantana*, in which the exotic ornamental *Lantana camara* outcrosses with the native *Lantana depressa* (Czarnecki et al., 2014). In a study of cultivars and breeding lines of *L. camara*, triploids were found to be the most male sterile of the ploidy levels, followed by hexaploids, pentaploids, tetraploids, and diploids (Czarnecki et al., 2014). In addition, elite cultivars were found to vary widely in male fertility based on pollen stainability (Czarnecki et al., 2014; Dehgan, 2006).

Although triploids are often sterile or nearly so, odd ploidy levels are not always a guarantee of seedlessness. Higher-level polyploids, such as pentaploids, vary in fertility levels, as observed in crops such as *Solanum* (Caruso et al., 2008), *Lantana* (Czarnecki et al., 2014), and *Vaccinium* (Laverty and Vorsa, 1991). For example, *Lantana* triploids had only 9.3% pollen stainability compared with 34.6% in pentaploids (Czarnecki et al., 2014). In *Solanum*, Caruso et al. (2008) found that several pentaploid hybrids were female fertile when crossed with the tetraploid *S. tuberosum*. In addition, they found that the number of extra chromosomes in their aneuploid accessions had a significant effect on most of their fertility parameters, including berry set, number of seeds per berry, and number of seeds per pollinated flower (Caruso et al., 2008). Their results agree with previous work in *Vaccinium* (Laverty and Vorsa, 1991), which showed a positive linear relationship between chromosome number and fertility in aneuploids. One theory is that the higher the number of chromosomes, the more opportunity there is to produce gametes that overcome the "triploid block" associated with endosperm balance number (Caruso et al., 2008).

Few guidelines exist to determine the acceptable rate of fertility for a cultivar of a potentially invasive plant. The only formal example known to the authors of a previously banned weedy ornamental plant allowing propagation and sale of sterile or near-sterile cultivars is the case of *Buddleja* in Oregon. The Oregon Department of Agriculture (ODA) approved cultivars for sale in the state that have a 98% reduction in viable seed compared with industry standards (Contreras and McAninch, 2013). The threshold of 2% provides a target for breeders seeking to create sterile forms of weedy or potentially invasive species.

Hibiscus syriacus is an important ornamental shrub grown for its vibrant summer blooms beginning in late June and lasting until fall (Dirr, 2009). *Hibiscus syriacus* is one of the few hardy species in one of the most diversified genera in the Malvaceae (Bae et al., 2015). This versatile shrub is tolerant of numerous environmental conditions, including a wide range of temperatures and soil conditions (Bae et al., 2015). In addition, *H. syriacus* can be a prolific seed producer, with part of its success due to herkogamous flowers. This type of pollination biology promotes outcrossing by the spatial separation of stigma and anthers, but reflexing stigmas allow seed production when pollinators are scarce (Cheng-Jiang et al., 2009). After pollination, capsules produce numerous seeds that readily germinate and can become a nuisance in production and in the landscape (Dirr, 2009).

Hibiscus syriacus occurs primarily as tetraploids ($2n = 4x = 80$), as reported by Skovsted (1941) and recently confirmed in a study to develop a draft genome (Kim et al., 2017). Although no reports exist on higher ploidy levels in the wild, numerous reports describe polyploid induction experiments in *H. syriacus* (Eeckhaut et al., 2004; Egolf, 1970, 1981, 1986, 1988; Lee and Kim, 1976; Shim et al., 1993; Van Huylenbroeck et al., 2000; Van Laere et al., 2006). Many of the cultivars produced from these studies have been reported as sterile or nearly so, including the U.S. National Arboretum (USNA) releases 'Aphrodite', 'Diana', 'Minerva', and 'Helene'. However, no comprehensive study on fertility among cultivars of *H. syriacus* exists. In addition, the fertility of odd ploidy level *H. syriacus* has not yet been studied. Therefore, the purpose of this study was to 1) evaluate the female and male fertility of tetraploid and hexaploid cultivars, and 2) evaluate the female fertility of pentaploid progeny resulting from interploid hybridization in *H. syriacus*.

Materials and Methods

Plant materials. To test fertility of 33 cultivars of *H. syriacus*, plants were collected from botanical gardens, arboreta, and nurseries (Table 1). Both potted plants as well as cuttings were acquired. Plants were grown at Oregon State University and mature plants were grown at the Lewis Brown Horticulture Farm (Corvallis, OR, USDA Zone 8b). For each taxon, original cultivar and trademark names were maintained from each source. However, for *H. syriacus* and many ornamental taxa, usually one name becomes common in the nursery trade as the market name. For simplicity, only market names (cultivar or trademark) will be used hereafter.

Intraploid cultivar crosses. Genome sizes and ploidy levels of each cultivar were determined using a combination of flow cytometry and root tip chromosome counts (Lattier et al., 2019). Taxa or newly acquired accessions not previously reported were analyzed using flow cytometry according to Lattier et al. (2019). From 2012 to 2014, a total of 204 combinations, representing both cross-pollinations and self-pollinations, were attempted among the tetraploid cultivars. Crosses were made in summer in a glasshouse kept free of pollinators with day/night temperatures of 25/20 °C and a 16-h photoperiod. Flowers were open for 2 days before stigmas reflexed to self-pollinate. Therefore, flowers were pollinated in the morning of their first flowering and stigmas were thoroughly covered with a dense layer of

Table 1. Source material for *Hibiscus syriacus* breeding at Oregon State University.

Cultivar[z]	Trade Name[y]	Accession[x]	Ploidy	Source[w]
American Irene Scott	Sugar Tip®	12-0019	4*x*	Bailey Nurseries
Antong Two	Lil' Kim™	12-0021	4*x*	Bailey Nurseries
Aphrodite[v]		11-0215	4*x*	Bailey Nurseries
Ardens		13-0050	4*x*	Blue Heron
Blue Bird		11-0219	4*x*	Monrovia
Blushing Bride		13-0059	4*x*	Monrovia
Bricutts	China Chiffon™	13-0060	4*x*	Monrovia
Buddha Belly		14-0128	4*x*	Yamaguchi Nursery
Collie Mullins		13-0061	4*x*	Monrovia
Diana		11-0211	4*x*	Bailey Nurseries
DS01BS	Blueberry Smoothie™	14-0092	4*x*	Greenleaf Nursery
DS02SS	Strawberry Smoothie™	14-0091	4*x*	Greenleaf Nursery
DS03RS	Raspberry Smoothie™	14-0094	6*x*	Greenleaf Nursery
DS04PS	Peppermint Smoothie™	14-0093	4*x*+8*x*	Greenleaf Nursery
DVPazurri	Azurri Satin®	14-0188	6*x*	Spring Meadow
Floru	Violet Satin®	13-0118	4*x*	JC Raulston Arboretum
Helene		13-0063	4*x*	Monrovia
Helene		13-0116	6*x*	JC Raulston Arboretum
Helene		13-0117	4*x*	JC Raulston Arboretum
JWNfour	Pink Chiffon®	13-0067	4*x*	Monrovia
Lucy		11-0216	4*x*	Bailey Nurseries
Marina	Blue Satin®	11-0210	4*x*	Bailey's Nursery
Mathilde	Blush Satin®	13-0058	4*x*	Monrovia
Mineru	First Editions® Tahiti™	12-0024	4*x*	Bailey Nurseries
Minerva		11-0213	4*x*	Bailey Nurseries
Minfren	First Editions® Bali™	12-0023	4*x*	Bailey Nurseries
Minrosa	Rose Satin®	13-0068	4*x*	Monrovia
Minspot	First Editions® Fiji™	12-0022	4*x*	Bailey Nurseries
Minsygrbl1	First Editions® Hawaii™	12-0020	4*x*	Bailey Nurseries
Notwoodone	Lavender Chiffon™	13-0046	4*x*	Blue Heron
Notwoodthree	Blue Chiffon™	13-0056	4*x*	Monrovia
Notwoodtwo	White Chiffon®	13-0044	4*x*	Blue Heron
Pink Giant		11-0217	4*x*	Bailey Nurseries
Red Heart		13-0049	4*x*	Blue Heron
Woodbridge		11-0214	4*x*	Bailey Nurseries

[z]Cultivar name.

[y]Trademark name.

[x]Accession number in research collection at the Ornamental Plant Breeding Laboratory, Oregon State University, Corvallis, OR.

[w]Container plant collected from the following sources: Bailey Nurseries, Yamhill, OR; Blue Heron Farm, Corvallis, OR; Forestfarm Nursery, Williams, OR; Greenleaf Nursery, Grants Pass, OR; JC Raulston Arboretum, Raleigh, NC; Monrovia Nursery, Dayton, OR; Spring Meadow Nursery, Grand Haven, MI; Yamaguchi Plantsman Nursery, Gifu, Japan.

[v]An additional clone with accession 13-0054 was obtained and used in some crosses but late in the study identified as a hexaploid. Most crosses conducted using 4*x* accession, but results may be confounded by alternate ploidy clone.

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pollen. Fresh pollen was collected from flowers for the crosses on the day of pollination. Pollen of *H. syriacus* is large (108- to 169-μm diameter), which prevents it from becoming airborne (Bae et al., 2015). It also produces numerous, long, sticky spines from its exine. There are 28 to 84 spines per grain with spine lengths of 8 to 25 μm, which cause the pollen to clump (Bae et al., 2015). Therefore, for pollination, clumps of pollen were placed on stigmas with forceps and forceps were sterilized in 70% ethanol between pollinations. When flowers were abundant, pollinations were performed directly using the monadelphous stamen of the male parent. Each pollinated flower was labeled with a jeweler's tag on which was recorded the parents and date, and observed daily for capsule development or flower abortion. Female fertility was evaluated for 28 cultivars used in combination with 20 male cultivars for which male fertility was also calculated. Number of combinations and pollinations varied across taxa, which was addressed by using a mixed model (GLIMMIX), which is described later in this article.

Tags were collected from aborted flowers throughout the summer, and failed crosses were recorded in the fall. Viable capsules were monitored daily, and capsules were collected 2 to 3 months post pollination, as they began to turn yellow, and sutures began to open. Data were collected on total number of pollinations, total number of filled capsules, and number of seeds per capsule. Filled capsules were those that remained on the plant to maturity and were found to contain at least one fully developed seed. Preliminary seed germination tests from several open-pollinated seed lots illustrated that fully developed seed germinated at a high percentage, regardless of parent genotype. Therefore, cross-compatibility and fertility estimates among cultivars were based on fruit and seed set. Nonstratified seeds from each cross were collected, cleaned, and sown into 1.3-L containers filled with growing medium (Metro-Mix; Sun Gro Horticulture, Agawam, MA) in lots of ≤30 seeds per container. Surviving seedlings were transplanted into 2.5-L containers filled with douglas fir–based potting substrate during the summer and grown under the conditions described previously.

Interploid crosses. Interploid crosses were designed to create pentaploid populations. A total of 47 combinations were attempted between tetraploid and hexaploid cultivars. Hexaploid taxa included 'Pink Giant', Azurri Satin®, and Raspberry Smoothie™. Genome sizes and ploidy levels for hexaploid cultivars were determined using a combination of flow cytometry and root tip chromosome counts (Lattier et al., 2019). Pollinations, data collection, and seed germination were carried out as described previously.

Pentaploid fertility testcrosses. In 2014, flow cytometry was performed on putative pentaploid seedlings created in 2012 and 2013, and cuttings were rooted for a subset of them. Cuttings were grown through the winter in a glasshouse under the conditions described previously. Fertility testcrosses were performed during 2015 and 2016, with proven male-fertile cultivars randomly selected to use as male parents. Each day, several randomly selected tetraploid flowers were used to pollinate all open flowers on pentaploid taxa. Tags were collected from aborted flowers throughout the summer, and the number of failed crosses was recorded in fall. Viable capsules were monitored daily, and capsules were collected 2 to 3 months post pollination, as the capsules began to yellow, and sutures began to open. Data were collected on total number of pollinations, total number of filled capsules, and number of seeds per capsule. Nonstratified seeds from each cross were collected, cleaned, and sown into 1.3-L containers filled with growing medium (Metro-Mix) in lots of ≤30 seeds per container. In addition, a positive control consisting of open-pollinated seed from proven-fertile female taxa was sown. Three taxa were chosen: Blue Satin®, White Chiffon®, and 'Woodbridge', and 10 seeds of each taxon were sown in five pots for a total of 150 seeds. Percent seed germination and number of albino seedlings were counted for all treatments.

Statistical analyses. Due to unequal variances and sample sizes, all analyses of variance were conducted using a generalized mixed model procedure (GLIMMIX) (SAS Studio, Cary, NC). For self-pollinations, taxon means were calculated for seeds per capsule and seeds per pollination using capsules and pollinations as replicates, respectively. Flower form means were calculated for seeds per capsule and seeds per pollination using taxon means as replicates. Mean comparisons were

Table 2. Fertility estimates from self-pollination of 20 cultivars of *Hibiscus syriacus* with single, semidouble, and double flowers.

Flower form[z]	Taxon	Ploidy[y]	Pollinations (no.)	Capsules (no.)	Capsule set (%)[x]	Seeds (no.)	Seeds per capsule (mean ± SE)[w]	Seeds per pollination (mean ± SE)[v]
Single							13 ± 1 A	4.0 ± 1.6 A
	Aphrodite	4*x*	70	22	31	374	17 ± 2 C	5.3 ± 1.1 C
	Blue Bird	4*x*	39	12	31	89	7 ± 1 D	2.3 ± 0.6 CD
	Blue Satin®	4*x*	39	22	56	186	8 ± 1 D	4.8 ± 0.8 C
	Buddha Belly	4*x*	27	0	0	0	0	0.0 ± 0.0 D
	Diana	4*x*	139	0	0	0	0	0.0 ± 0.0 D
	Hawaii™	4*x*	4	4	100	9	2 ± 1 D	2.3 ± 0.9 CD
	Lil' Kim™	4*x*	47	28	60	549	20 ± 1 BC	11.4 ± 1.6 B
	Minerva	4*x*	2	2	100	10	5	5.0
	Pink Giant	6*x*	29	0	0	0	0	0.0 ± 0.0 D
	Red Heart	4*x*	32	3	9	16	5 ± 1 D	0.5 ± 0.3 D
	Woodbridge	4*x*	30	20	67	411	21 ± 2 BC	13.7 ± 2.2 B
Semidouble							14 ± 4 A	6.1 ± 3.9 A
	Bali™	4*x*	23	0	0	0	0	0.0 ± 0.0 D
	Blue Chiffon™	4*x*	49	3	6	13	4 ± 0 D	0.3 ± 0.2 D
	China Chiffon™	4*x*	11	0	0	0	0	0.0 ± 0.0 D
	Fiji™	4*x*	37	6	16	24	4 ± 1 D	0.6 ± 0.3 D
	Lavender Chiffon™	4*x*	8	0	0	0	0	0.0 ± 0.0 D
	Pink Chiffon®	4*x*	20	20	100	439	22 ± 1 AB	21.9 ± 0.8 A
	Tahiti™	4*x*	10	1	10	5	5	0.5 ± 0.5 D
	White Chiffon®	4*x*	9	9	100	232	26 ± 1 A	25.8 ± 0.7 A
Double							0	0.0
	Blushing Bride	4*x*	6	0	0	0	0	0.0 ± 0.0 D

[z]Flower forms based on number of petaloid stamens.

[y]Ploidy estimates based on flow cytometry and root tip squashes. Ploidy series later discovered for 'Aphrodite' and 'Minerva' were used in crosses.

[x]Percent capsule set calculated per genotype as follows: [total filled capsules/total pollinations] × 100.

[w]Average seeds per capsule calculated among flower form (within boxes) and among genotypes. Among flower forms, replicates are the genotype means. Letters separating least squares (LS) means based on comparison lines test of the generalized linear mixed model procedure (GLIMMIX). Among genotypes, replicates are capsules. Letters separating LS means based on comparison lines test of GLIMMIX.

[v]Average seeds per pollination calculated among flower forms (within boxes) and among genotypes. Among flower forms, replicates are the genotype means. Letters separating LS means based on comparison lines test of GLIMMIX. Among genotypes, replicates are pollinations. Letters separating LS means based on comparison lines test of GLIMMIX.

performed using the comparison lines test of GLIMMIX (α = 0.05). For female fertility (seed per pollination), self-pollinations and interploid crosses were not included. The only exception was for female cross combinations using the hexaploids 'Pink Giant' and Raspberry Smoothie™, which were included to compare female fertility estimates with the tetraploid taxa. Taxon means were calculated for seeds per capsule and seeds per pollination by using means for each cross combination as a replicate. For example, 'Aphrodite' was used as a female in combination with nine male parents (Table 5) that were considered replicates to generate its female fertility (Table 3). Mean comparisons were performed using the comparison lines test of GLIMMIX (α = 0.05). For male fertility, self-pollinations and interploid crosses were excluded except for male cross combinations using 'Aphrodite', 'Pink Giant', and Raspberry Smoothie™. These taxa were included to compare male fertility estimates with the tetraploid taxa. Taxa means were calculated for seeds per capsule and seeds per pollination by using means for each male cross combination as replicates. For flower form means, replicates were the genotype means. Mean comparisons were performed using the comparison lines test of GLIMMIX (α = 0.05).

For seed set estimates of pentaploid progeny, means were compared for taxa using a control based on a hypothetical average fertile tetraploid. The control seeds per capsule and control seeds per pollination were calculated from female fertility estimates of single and semidouble 4*x* × 4*x* crosses with a minimum of five pollen parents and a minimum of 30 total pollinations. Taxa included as controls were Blue Satin®, 'Blue Bird', 'Buddha Belly', 'Diana', Lil' Kim™, 'Minerva', 'Red Heart', 'Woodbridge', Bali™, Blue Chiffon™, Fiji™, Tahiti™, and White Chiffon®. Taxon means were calculated for seeds per capsule and seeds per pollination using capsules and pollinations as replicates, respectively. Mean comparisons were performed using the comparison lines test of GLIMMIX (α = 0.05) with a Dunnett's adjustment for comparison with the controls. For seed germination estimates of pentaploid progeny, percent germination for each taxon was averaged for each interploid crossing group (4*x* × 6*x*) and (6*x* × 4*x*) and compared with that of open-pollinated seed from three proven-fertile taxa: Blue Satin®, White Chiffon®, and 'Woodbridge'. Mean comparisons were performed using the comparison lines test of GLIMMIX (α = 0.05) with a Dunnett's adjustment for comparison with the controls.

Results and Discussion

Self-pollinations. Among 21 cultivars, self-pollinations were attempted on 631 flowers resulting in 152 capsules and 2356 seeds (Table 2). This equated to 24% successful pollination and four seeds per pollination. Significant differences were found among individual taxa for seeds per capsule ($P < 0.0001$) and seeds per pollination ($P < 0.0001$). However, no significant differences were found between flower forms (single and semidouble flowers).

The highest capsule set following self-pollinations was observed in White Chiffon® and Pink Chiffon®, both of which had 100%

Table 3. Female fertility estimates for 28 cultivars of *Hibiscus syriacus* with single, semidouble, and double flowers.

Flower form[z]	♀ parent	Ploidy[y]	♂ parents (no.)	Pollinations (no.)	Capsules (no.)	Capsule set (%)[x]	Seeds (no.)	Seeds per capsule (mean ± SE)[w]	Seeds per pollination (mean ± SE)[v]
Single								17 ± 2 A	8.6 ± 1.4 A
	Aphrodite	4*x*	9	121	69	57	1406	20 ± 1 BC	11.6 ± 2.5 B–D
	Azurri Satin®	6*x*	2	2	0	0	0	0	0.0
	Blue Satin®	4*x*	11	107	75	70	1626	22 ± 1 B	14.1 ± 2.6 A–C
	Blue Bird	4*x*	14	240	109	45	1798	17 ± 1 D–F	8.3 ± 2.2 C–F
	Buddha Belly	4*x*	6	42	34	81	752	22 ± 1 B	19.2 ± 3.1 A
	Diana	4*x*	15	395	87	22	1302	15 ± 1 D–F	5.6 ± 1.5 D–F
	Hawaii™	4*x*	4	17	13	76	123	9 ± 1 F–H	7.8 ± 2.6 C–F
	Helene	4*x*	6	28	6	21	103	17 ± 7 B–F	5.7 ± 3.2 D–F
	Lil' Kim™	4*x*	10	74	28	38	440	16 ± 1 D–F	5.3 ± 2.4 D–F
	Minerva	4*x*	10	40	22	55	311	14 ± 2 EF	6.3 ± 2.5 D–F
	Pink Giant	6*x*	18	350	40	11	149	4 ± 0 H	0.4 ± 0.1 F
	Red Heart	4*x*	11	173	62	36	1720	28 ± 2 A	9.8 ± 2.9 C–E
	Woodbridge	4*x*	11	115	45	39	805	18 ± 1 B–E	8.5 ± 2.5 C–F
Semidouble								15 ± 2 AB	10.0 ± 1.7 A
	Bali™	4*x*	9	110	78	71	1443	19 ± 1 B–D	11.5 ± 2.6 B–D
	Blue Chiffon™	4*x*	9	127	76	60	1410	19 ± 1 B–D	16.7 ± 3.0 AB
	China Chiffon™	4*x*	2	11	6	55	59	10 ± 3 F–H	8.1
	Fiji™	4*x*	10	47	25	53	383	15 ± 1 D–F	9.3 ± 1.9 C–E
	Lavender Chiffon™	4*x*	3	36	19	53	133	7 ± 1 GH	3.9 ± 0.8 D–F
	Pink Chiffon®	4*x*	4	39	33	85	456	14 ± 1 E–G	10.4 ± 3.4 B–E
	Tahiti™	4*x*	8	39	6	15	29	5 ± 1 H	1.1 ± 0.5 F
	White Chiffon®	4*x*	6	40	25	63	690	28 ± 1 A	18.6 ± 4.8 AB
Double								9 ± 3 B	2.6 ± 2.0 B
	Ardens	4*x*	3	12	0	0	0	0	0.0 ± 0.0 F
	Blushing Bride	4*x*	6	43	18	42	302	17 ± 2 C–F	8.8 ± 2.5 C–F
	Collie Mullins	4*x*	2	18	0	0	0	0 ± 0	0.0
	Lucy	4*x*	12	60	5	8	30	6 ± 2 GH	0.4 ± 0.3 F
	Raspberry Smoothie™	6*x*	5	21	15	71	61	4 ± 1 H	2.7 ± 1.0 EF
	Strawberry Smoothie™	4*x*	3	15	4	27	33	8 ± 2 F–H	3.5 ± 1.9 D–F
	Sugar Tip®	4*x*	2	22	1	5	1	1	0.0

[z]Flower forms based on number of petaloid stamens.

[y]Ploidy estimates based on flow cytometry and root tip squashes (Lattier et al., 2019). 'Aphrodite' and 'Minerva' not in citation and ploidy determined later in the current study.

[x]Percent capsule set calculated per genotype as follows: [total filled capsules/total pollinations] × 100.

[w]Average seeds per capsule calculated among flower form (within boxes) and among genotypes. Self-pollinations were not included. Interploid crosses were not included, except for 'Aphrodite', 'Pink Giant', and Raspberry Smoothie™. Among flower forms, replicates are the genotype means. Letters separating least squares (LS) means based on comparison lines test of the generalized linear mixed model procedure (GLIMMIX). Among genotypes, replicates are capsules. Letters separating LS means based on comparison lines test of GLIMMIX.

[v]Average seeds per pollination calculated among flower form (within boxes) and among genotypes. Self-pollinations were not included. Interploid crosses were not included, except for 'Aphrodite', 'Pink Giant', and Raspberry Smoothie™. Among flower forms, replicates are the genotype means. Letters separating LS means based on comparison lines test of GLIMMIX. Among genotypes, replicates are means for each female cross combination. Letters separating LS means based on comparison lines test of GLIMMIX.

successful self-pollinations producing 25.8 ± 0.7 seeds per pollination and 21.9 ± 0.08 seeds per pollination, respectively (Table 2). Nearly half of the taxa investigated produced no capsules or seeds from self-pollinations, including Bali™, 'Blushing Bride', 'Buddha Belly', China Chiffon™, 'Diana', Lavender Chiffon™, and 'Pink Giant' (Table 2). In addition, the only two single-flowering taxa without an eye spot ('Diana' and 'Buddha Belly') set no seed when self-pollinated. Although many taxa with semidouble flowers were self-fertile, most double-flowered taxa could not be self-pollinated because of lack of pollen. Of the self-fertile taxa, eight were found to have <10 seeds per capsule and 10 seeds per pollination, including 'Blue Bird', Blue Chiffon™, Blue Satin®, Fiji™, Hawaii™, 'Minerva', 'Red Heart', and Tahiti™ (Table 2).

There are reports of self-incompatibility in *Hibiscus*, including *H. syriacus* (Yu and Youm, 1972). However, it appears to be a leaky system, as F2 plants have been reported among interspecific hybrids (Van Laere et al., 2007), and some cultivars in our study appear to have lost their self-incompatibility mechanism. However, some *Hibiscus* species (e.g., *Hibiscus laevis*) exhibit delayed autonomous self-pollination to ensure reproductive success, but with reduced seeds per flower (Klips and Snow, 1997). It is unclear why some taxa in our study maintain self-incompatibility, whereas others exhibit a gradient from modest self-compatibility to near fully fertile following self-pollination. Dhooghe et al. (2011) have reported induced polyploidy as a method to overcome one-locus gametophytic self-incompatibility, and although *H. syriacus* is a tetraploid, we have not observed a higher frequency of autonomous selfing among higher ploidy levels (5*x*, 6*x*, 8*x*) in our research plots (data not shown). Although we have not conducted systematic self-pollination studies among these cytotypes, we have generally observed a reduction in seed set, often to nil. Together, this does not necessarily support that the mechanism for breakdown of self-incompatibility in some cultivars of *H. syriacus* is related to ploidy.

After germination, an obvious reduction in vigor was observed in most self-pollinated (S_1) seedlings compared with cross-pollinated seedlings (J. Lattier, unpublished data). As such, development of inbred lines in *H. syriacus* may be limited by inbreeding depression, with the notable exception of 'Woodbridge', discussed later in this paragraph. Other exceptions were seen among S_1 seedlings of White Chiffon®, Pink Chiffon®, and 'Red Heart'. S_1 seedlings in White Chiffon® and 'Pink Chiffon' were vigorous, and flowered in their first year from seed. However, they appeared more compact and had a larger number of petaloid stamens than their parents. Therefore, self-pollination of taxa with semidouble flowers may be an approach for breeders seeking to develop more compact double-flowered cultivars. In contrast, S_1 seedlings of 'Red Heart' and 'Woodbridge' grew tall and vigorous, with large, single flowers during their first year. Further, 'Woodbridge' S_1 seedlings were self-pollinated, and the resulting S_2 seedlings also grew vigorously, indicating this cultivar has potential to develop inbred lines. Pedigree information is scant on cultivars and further work would be necessary to discover the level of inbreeding possible in various *H. syriacus* cultivars.

Female fertility. A total of 2342 cross-pollinations were attempted, resulting in 973 capsules and 15,565 seeds (Table 3) for 38% successful pollinations and seven seeds per pollination across all combinations. Significant differences were found among taxa for seeds per capsule ($P < 0.0001$) and seeds per pollination ($P < 0.0001$). In addition, significant differences were found among flower forms (single, semidouble, and double) for seeds per capsule ($P < 0.0001$) and seeds per pollination ($P = 0.027$). Of the filled capsules, taxa with single flowers produced 17 ± 2 seeds per capsule, whereas taxa with double flowers produced only 9 ± 3 seeds per capsule (Table 3). In addition, taxa with single and semidouble flowers produced more seeds per pollination (8.6 ± 1.4 and 10.0 ± 1.7, respectively) than double flowers (2.6 ± 2.0) (Table 3).

Cultivars previously reported to be sterile were found to be female fertile, including the USNA taxa, 'Aphrodite', 'Diana', 'Helene',

Table 4. Male fertility estimates for 20 cultivars of *Hibiscus syriacus* with single, semidouble, and double flowers.

Flower form[z]	♂ parent	Ploidy[y]	♀ parents (no.)	Pollinations (no.)	Capsules (no.)	Capsule set (%)[x]	Seeds (no.)	Seeds per capsule (mean ± SE)[w]	Seeds per pollination (mean ± SE)[v]
Single								16.0 ± 1.5 A	7.4 ± 0.9 A
	Aphrodite	4*x*	11	122	36	30	590	16.7 ± 1.5 B–D	6.9 ± 2.5 A–C
	Blue Satin®	4*x*	15	133	57	43	1203	20.7 ± 1.5 A	10.2 ± 2.5 AB
	Blue Bird	4*x*	14	124	43	35	763	17.2 ± 1.8 A–D	7.3 ± 2.7 AB
	Buddha Belly	4*x*	7	49	28	57	612	21.9 ± 1.3 A	7.3 ± 3.7 A–C
	Diana	4*x*	19	380	177	47	3534	20.0 ± 0.8 AB	9.2 ± 2.1 AB
	Hawaii™	4*x*	2	26	3	12	25	8.3 ± 3.4 DE	–
	Helene	4*x*	1	17	0	0	0	0.0	–
	Lil' Kim™	4*x*	13	114	77	68	1319	17.1 ± 0.7 B–D	12.7 ± 1.9 A
	Minerva	4*x*	15	96	39	41	516	13.4 ± 2.0 DE	5.6 ± 1.3 BC
	Pink Giant	6*x*	23	527	72	14	415	5.8 ± 0.6 E	1.6 ± 1.0 C
	Red Heart	4*x*	14	208	50	24	813	16.3 ± 1.5 CD	5.8 ± 2.5 BC
	Woodbridge	4*x*	13	119	50	42	939	18.9 ± 1.3 A–D	6.9 ± 2.5 A–C
Semidouble								17.1 ± 2.3 A	9.4 ± 0.7 A
	Bali™	4*x*	11	109	64	59	1174	18.3 ± 1.1 A–D	10.7 ± 2.5 AB
	Blue Chiffon™	4*x*	13	148	49	33	959	19.6 ± 1.3 A–C	7.3 ± 2.2 AB
	China Chiffon™	4*x*	2	27	5	19	128	25.6 ± 1.0 A	–
	Fiji™	4*x*	13	109	65	60	1040	16.0 ± 1.2 DE	9.0 ± 2.1 AB
	Pink Chiffon®	4*x*	1	3	3	100	16	5.3 ± 0.9 E	–
	Tahiti™	4*x*	10	75	50	67	917	18.3 ± 1.1 A–D	11.1 ± 2.1 AB
	White Chiffon®	4*x*	12	110	49	45	801	16.3 ± 1.0 CD	8.9 ± 2.5 AB
Double								–	–
	Blushing Bride	4*x*	1	4	1	25	6	6.0	–

[z]Flower forms based on number of petaloid stamen.

[y]Ploidy estimates based on flow cytometry and root tip squashes (Lattier et al., 2019). 'Aphrodite' and 'Minerva' not in citation and ploidy determined later in the current study.

[x]Percent capsule set calculated per genotype as follows: [total filled capsules/total pollinations] × 100.

[w]Average seeds per capsule calculated among flower form (within boxes) and among genotypes. Self-pollinations were not included. Among flower forms, replicates are the genotype means. Letters separating least squares (LS) means based on comparison lines test of the generalized linear mixed model procedure (GLIMMIX). Among genotypes, replicates are capsules. Letters separating LS means based on comparison lines test of GLIMMIX.

[v]Average seeds per pollination calculated among flower form (within boxes) and among genotypes. Self-pollinations were not included. Among flower forms, replicates are the genotype means. Letters separating LS means based on comparison lines test of GLIMMIX. Among genotypes, replicates are means for each female cross combination. Letters separating LS means based on comparison lines test of GLIMMIX.

Table 5. Fertility estimates for individual pairwise crosses among tetraploid cultivars of *Hibiscus syriacus*.

♀ parent	♂ parent	Pollinations (no.)	Capsules (no.)	Capsule set (%)[z]	Seeds (no.)	Seeds per capsule (mean ± SE)[y]	Seeds per pollination[x]
Aphrodite	Bali™	23	10	43	235	24 ± 3	10
	Blue Satin®	8	4	50	117	29 ± 4	15
	Blue Bird	5	4	80	143	36 ± 1	29
	Diana	31	22	71	470	21 ± 2	15
	Fiji™	21	14	67	195	14 ± 2	9
	Lil' Kim™	5	1	20	18	18	4
	Minerva	7	5	71	70	14 ± 6	10
	Red Heart	14	7	50	137	20 ± 4	10
	Woodbridge	7	2	29	21	11 ± 3	3
Ardens	Buddha Belly	9	0	0	0	0 ± 0	0
	Diana	2	0	0	0	0 ± 0	0
	White Chiffon®	1	0	0	0	0 ± 0	0
Bali™	Aphrodite	18	13	72	252	19 ± 2	14
	Blue Chiffon™	16	0	0	0	0 ± 0	0
	Blue Bird	20	18	90	329	18 ± 2	16
	Diana	17	16	94	325	20 ± 2	19
	Fiji™	4	2	50	4	2 ± 1	1
	Lil' Kim™	5	3	60	50	17 ± 4	10
	Minerva	5	3	60	24	8 ± 4	5
	Tahiti™	5	4	80	101	25 ± 2	20
	Woodbridge	20	19	95	358	19 ± 2	18
Blue Chiffon™	Aphrodite	5	5	100	133	27 ± 3	27
	Bali™	22	6	27	66	11 ± 2	3
	Diana	43	21	49	250	12 ± 1	6
	Fiji™	8	6	75	138	23 ± 2	17
	Lil' Kim™	5	5	100	86	17 ± 4	17
	Red Heart	22	17	77	339	20 ± 2	15
	Tahiti™	15	9	60	191	21 ± 2	13
	White Chiffon®	1	1	100	21	21	21
	Woodbridge	6	6	100	186	31 ± 2	31
Blue Satin®	Aphrodite	7	4	57	15	4 ± 1	2
	Bali™	11	9	82	202	22 ± 3	18
	Blue Bird	5	3	60	75	MV	15
	Buddha Belly	16	16	100	419	26 ± 1	26
	Diana	20	16	80	347	23 ± 2	17
	Lil' Kim™	7	6	86	145	24 ± 3	21
	Minerva	10	4	40	52	13 ± 6	5
	Red Heart	9	6	67	162	27 ± 2	18
	Tahiti™	2	2	100	17	9 ± 1	9
	White Chiffon®	8	8	100	187	23 ± 2	23
	Woodbridge	12	1	8	5	MV	0
Blue Bird	Aphrodite	6	2	33	21	MV	4
	Bali™	23	22	96	328	15 ± 2	14
	Blue Chiffon™	10	10	100	256	26 ± 1	26
	Blue Satin®	15	12	80	183	15 ± 3	12
	Buddha Belly	5	1	20	26	26	5
	China Chiffon™	6	4	67	103	26 ± 1	17
	Diana	47	3	6	10	3 ± 1	0
	Fiji™	26	13	50	124	10 ± 1	5
	Hawaii™	19	1	5	6	MV	0
	Lil' Kim™	29	21	72	381	18 ± 1	13
	Minerva	13	3	23	30	MV	2
	Red Heart	16	0	0	0	0 ± 0	0
	Tahiti™	19	16	84	329	21 ± 2	17
	Woodbridge	6	1	17	1	MV	0
Blushing Bride	Aphrodite	5	2	40	27	14 ± 7	5
	Blue Chiffon™	9	6	67	101	17 ± 4	11
	Diana	18	3	17	30	10 ± 4	2
	Fiji™	5	3	60	46	15 ± 1	9
	Minerva	6	4	67	98	25 ± 2	16
Buddha Belly	Blue Chiffon™	7	4	57	92	23 ± 6	13
	Blue Satin®	2	2	100	51	26 ± 7	26
	Diana	28	24	86	524	22 ± 1	19
	Lil' Kim™	2	2	100	25	13 ± 2	13
	Red Heart	1	1	100	31	–	31
	White Chiffon®	2	1	50	29	–	15
China Chiffon™	Blue Chiffon™	3	3	100	42	14 ± 5	14
	Blue Bird	8	3	38	17	6 ± 0	2
Collie Mullins	Blue Chiffon™	13	0	0	0	–	0
	Diana	5	0	0	0	–	0
Diana	Aphrodite	49	5	10	74	15 ± 3	2
	Bali™	5	4	80	37	9 ± 2	7

(Continued on next page)

Table 5. (*Continued*)

♀ parent	♂ parent	Pollinations (no.)	Capsules (no.)	Capsule set (%)[z]	Seeds (no.)	Seeds per capsule (mean ± SE)[y]	Seeds per pollination[x]
	Blue Chiffon™	39	4	10	104	26 ± 2	3
	Blue Satin®	28	11	39	198	18 ± 2	7
	Blue Bird	29	0	0	0	–	0
	Buddha Belly	10	8	80	138	17 ± 2	14
	China Chiffon™	21	1	5	25	–	1
	Fiji™	6	5	83	120	24 ± 4	20
	Helene	17	0	0	0	–	0
	Lil' Kim™	27	17	63	227	13 ± 1	8
	Minerva	12	5	42	70	20 ± 14	6
	Red Heart	101	13	13	115	9 ± 2	1
	Tahiti™	11	8	73	135	17 ± 2	12
	White Chiffon®	19	2	11	25	–	1
	Woodbridge	21	4	19	34	8 ± 3	2
Fiji™	Aphrodite	1	1	100	16	–	16
	Bali™	4	2	50	48	24 ± 3	12
	Blue Chiffon™	7	6	86	59	10 ± 3	8
	Blue Bird	7	2	29	16	8 ± 5	2
	Diana	5	0	0	0	–	0
	Hawaii™	7	2	29	19	–	3
Fiji™	Lil' Kim™	3	2	67	37	–	12
	Minerva	1	1	100	10	–	10
	White Chiffon®	6	5	83	110	22 ± 2	18
	Woodbridge	6	4	67	68	17 ± 2	11
Hawaii™	Fiji™	4	4	100	45	11 ± 3	11
	Minerva	7	6	86	51	9 ± 1	7
	Red Heart	4	1	25	3	–	1
	Tahiti™	2	2	100	24	12 ± 4	12
Helene	Blue Satin®	2	1	50	37	–	19
	Blue Bird	2	0	0	0	–	0
	Diana	18	3	17	25	8 ± 2	1
	Minerva	3	1	33	38	–	13
	Red Heart	2	1	50	3	–	2
	Woodbridge	1	0	0	0	–	0
Lavender Chiffon™	Diana	25	13	52	96	7 ± 1	4
	Pink Chiffon®	3	3	100	16	5 ± 1	5
	White Chiffon®	8	3	38	21	7 ± 2	3
Lil' Kim™	Bali™	4	2	50	30	–	8
	Blue Chiffon™	10	4	40	61	15 ± 3	6
	Blue Bird	6	2	33	23	12 ± 4	4
	Buddha Belly	3	0	0	0	–	0
	Diana	1	1	100	17	17	17
	Fiji™	1	0	0	0	–	0
	Red Heart	3	0	0	0	–	0
	Tahiti™	6	2	33	25	13 ± 9	4
	White Chiffon®	28	12	43	204	17 ± 1	7
	Woodbridge	12	5	42	80	16 ± 1	7
Lucy	Aphrodite	2	0	0	0	–	0
	Bali™	5	0	0	0	–	0
	Blue Chiffon™	1	0	0	0	–	0
	Blue Satin®	14	0	0	0	–	0
	Blue Bird	5	0	0	0	–	0
	Buddha Belly	1	0	0	0	–	0
Lucy	Diana	7	3	43	18	6 ± 2	3
	Fiji™	5	0	0	0	–	0
	Lil' Kim™	4	0	0	0	–	0
	Minerva	4	1	25	1	–	0
	Red Heart	5	1	20	11	–	2
	White Chiffon®	7	0	0	0	–	0
Minerva	Bali™	2	0	0	0	–	0
	Blue Satin®	2	1	50	17	–	9
	Blue Bird	3	3	100	20	7 ± 1	7
	Blushing Bride	4	1	25	6	–	2
	Diana	7	2	29	14	–	2
	Fiji™	8	8	100	185	23 ± 4	23
	Lil' Kim™	3	3	100	48	16 ± 1	16
	Red Heart	4	1	25	2	–	1
	Tahiti™	4	3	75	19	6 ± 1	5
	Woodbridge	3	0	0	0	–	0
Pink Chiffon®	Blue Satin®	10	6	60	44	7 ± 1	4
	Buddha Belly	5	3	60	29	10 ± 3	6
	Diana	13	13	100	250	19 ± 1	19
	White Chiffon®	11	11	100	133	12 ± 1	12

(Continued on next page)

Table 5. (*Continued*)

♀ parent	♂ parent	Pollinations (no.)	Capsules (no.)	Capsule set (%)^{z}	Seeds (no.)	Seeds per capsule (mean ± SE)^{y}	Seeds per pollination^{x}
Red Heart	Aphrodite	9	2	22	36	–	4
	Bali™	4	3	75	77	26 ± 1	19
	Blue Chiffon™	17	12	71	244	20 ± 3	14
	Blue Satin®	8	6	75	167	27 ± 4	21
	Blue Bird	15	1	7	5	–	0
	Diana	76	30	39	997	33 ± 2	13
	Lil' Kim™	1	1	100	27	–	27
	Minerva	9	0	0	0	–	0
	Tahiti™	7	0	0	0	–	0
	White Chiffon®	7	1	14	2	–	0
	Woodbridge	20	6	30	165	28 ± 4	8
Strawberry Smoothie™	Blue Satin®	8	0	0	0	–	0
	Minerva	5	3	60	20	7 ± 2	4
Strawberry Smoothie™	Woodbridge	2	1	50	13	13	7
Sugar Tip®	Blue Chiffon™	13	0	0	0	–	0
	Blue Satin®	9	1	11	1	–	0
Tahiti™	Aphrodite	3	1	33	8	–	3
	Blue Chiffon™	3	0	0	0	–	0
	Blue Satin®	8	0	0	0	–	0
	Blue Bird	10	2	20	3	–	0
	Fiji™	3	1	33	7	–	2
	Minerva	2	0	0	0	–	0
	Red Heart	7	1	14	3	–	0
	Woodbridge	3	1	33	8	–	3
White Chiffon®	Blue Satin®	10	9	90	267	30 ± 1	27
	Blue Bird	5	5	100	132	26 ± 3	26
	Diana	2	2	100	63	–	32
	Fiji™	10	5	50	135	27 ± 1	14
	Lil' Kim™	7	4	57	93	23 ± 3	13
	Red Heart	6	0	0	0	–	0
Woodbridge	Aphrodite	17	1	6	8	–	0
	Bali™	6	6	100	151	25 ± 2	25
	Blue Satin®	8	4	50	121	38 ± 1	15
	Blue Bird	4	0	0	0	–	0
	Diana	15	5	33	98	20 ± 3	7
	Fiji™	8	4	50	41	10 ± 4	5
	Lil' Kim™	16	12	75	182	15 ± 2	11
	Minerva	11	3	27	52	–	5
	Red Heart	14	1	7	7	–	1
	Tahiti™	4	4	100	76	19 ± 5	19
	White Chiffon®	12	5	42	69	14 ± 4	6

^{z}Percent capsule set calculated as follows: [capsules/pollinations] × 100.
^{y}Average seeds per capsule calculated using capsules for each combination as replicates.
^{x}Total seed per pollination calculated as follows: [total seed/total pollination] × 100.

and 'Minerva'. Their rates of successful pollinations were 57%, 22%, 21%, and 55%, respectively (Table 3). Four hexaploids included taxa previously identified by flow cytometry, including 'Pink Giant', Raspberry Smoothie™, Azurri Satin®, and a single "clone" of 'Aphrodite' (Lattier et al., 2019). Azurri Satin® was acquired near the end of the study. It had already produced numerous open-pollinated (OP) fruit, but produced few new flowers for cross-pollinations (Table 3). However, OP fruit and seeds were collected, and germinated seedlings were recovered that exhibited pentaploid genome sizes (data not shown). The variation in genome size for "clones" of 'Aphrodite' were identified near the end of the pollination study. Therefore, the fertility estimates for 'Aphrodite' likely represent the combined fertility for tetraploid and hexaploid cytotypes of 'Aphrodite' (Table 3, 5). Similarly, the flow cytometry results for 'Helene' indicated that it exists both as a tetraploid and hexaploid. Our results primarily point to this clone breeding as a tetraploid. Field observations suggest that seedlings produced around the base of stock blocks may be a source of such ploidy variation.

'Pink Giant' and Raspberry Smoothie™, both hexaploids, had 371 pollinations (Table 3). Most crosses focused on combinations with 'Pink Giant', identified as a hexaploid at the beginning of the study. None of the hexaploids yielded more than 4 ± 1 seeds per capsule, compared with the most prolific tetraploid, White Chiffon®, at 26 ± 1 seeds per capsule (Table 3). 'Pink Giant' produced filled capsules from 11% of pollinations compared with 71% in Raspberry Smoothie™. In addition, 'Pink Giant' had some of the lowest fertility estimates among the cross-pollinations, with 4 ± 0 seeds per capsule and 0.4 ± 0.1 seeds per pollination (Table 3). In contrast, Raspberry Smoothie™ had relatively high fertility estimates at 4 ± 1 seeds per capsule and 2.7 ± 1.0 seeds per pollination. Therefore, the relatively high female fertility of the double-flowered Raspberry Smoothie™ appears to make it a good parent for breeders to create double-flowered, pentaploid seedlings of *H. syriacus* (Table 3).

Of the taxa investigated, nine produced double flowers with all (or nearly all) of the stamens producing petals, including 'Ardens', 'Blushing Bride', 'Collie Mullins', 'Lucy', Sugar Tip®, Raspberry Smoothie™, Strawberry Smoothie™, Blueberry Smoothie™, and Peppermint Smoothie™. Although these flowers produced only petaloid stamens, most produced normal or slightly contorted pistils. After many pollinations, several taxa yielded no seed set, including 'Ardens', 'Collie Mullins', and Sugar Tip®. Although Sugar Tip® did produce a single fruit and a single seed, no seedlings were recovered. Peppermint Smoothie™ and Blueberry Smoothie™ were considered completely sterile because their pistils were converted to petals on all flowers. Therefore, selection for increased petaloid stamens and pistils may be a reliable approach for breeders to reduce fertility in *H. syriacus*.

However, several double-flowered taxa had normal pistils and produced viable offspring, including the hexaploid Raspberry Smoothie™ (mentioned previously) and tetraploids 'Blushing Bride' and Strawberry

Smoothie™ (Table 3). Of these double-flowered taxa, Raspberry Smoothie™ was most fertile and produced filled capsules from 71% of pollinations, followed by 'Blushing Bride' with 42%, and Strawberry Smoothie with 27% (Table 3). Among the remaining tetraploid single-flower and semidouble forms, all taxa were found to be female fertile with six taxa producing more than 10 seeds per pollination: Blue Satin®, 'Buddha Belly', Bali™, Blue Chiffon™, Pink Chiffon®, and White Chiffon®.

Male fertility. Significant differences for male fertility were found among taxa based on seeds per capsule ($P < 0.0001$) and seeds per pollination ($P = 0.035$); however, no significant differences in male fertility were observed among flower forms (Table 4).

USNA taxa, including 'Aphrodite', 'Diana', and 'Minerva', proved to be male-fertile with capsules resulting from 30%, 47%, and 41% of pollinations, respectively (Table 4). Only one cross was attempted with 'Helene' as the pollen parent, and further work will be necessary to determine if it is male-fertile. Most double-flowered taxa produced only petaloid stamens with no pollen and were therefore male sterile. However, 'Blushing Bride' proved to be a useful male parent in a few pollinations with 'Minerva', yielding a single capsule containing six seeds (Table 4). Of more than 500 pollinations, the hexaploid 'Pink Giant' developed capsules from 14% of pollinations (Table 4). In addition, 'Pink Giant' had low male fertility, at only 5.8 ± 0.6 seeds per capsule and 1.6 ± 1.0 seeds per pollination (Table 4). Of the remaining single and semidouble tetraploids, four proved to have high fertility with more than 10 seeds per capsule and 10 seeds per pollination: Blue Satin®, Lil' Kim™, Bali™, and Tahiti™ (Table 4).

Individual crosses. Despite the significant differences in female and male fertility for each taxon, individual cross combinations varied widely. For instance, one of the most female fertile taxa, Bali™, had 94% successful pollination when pollinated with 'Diana', with 16 capsules from 17 pollinations (Table 5). However, Bali™ produced no capsules following pollination of 16 flowers with Blue Chiffon™. Blue Chiffon™ had an overall pollination success of 33% when used as a

Table 6. Fertility estimates for 47 pairwise interploid (tetraploid and hexaploid) crosses of *Hibiscus syriacus*.

Ploidy[z]	♀ parent	♂ parent	Pollinations (no.)	Capsules (no.)	Capsule set (%)[y]	Seed (no.)	Seeds per capsule (mean ± SE)[x]	Seeds per pollination[w]
$4x \times 6x$								
	Aphrodite	Pink Giant	10	7	70	31	4 ± 1	3
	Ardens	Pink Giant	45	0	0	0	–	0
	Bali™	Pink Giant	11	3	27	6	2 ± 1	1
	Blue Chiffon™	Pink Giant	19	0	0	0	–	0
	Blue Satin®	Pink Giant	25	15	60	87	6 ± 1	3
	Blue Bird	Pink Giant	17	5	29	27	5 ± 2	2
	Blushing Bride	Pink Giant	45	12	27	81	7 ± 1	2
	Buddha Belly	Pink Giant	6	0	0	0	–	0
	China Chiffon™	Pink Giant	27	0	0	0	–	0
	Collie Mullins	Pink Giant	19	0	0	0	–	0
	Diana	Pink Giant	42	2	5	7	–	0
	Fiji™	Pink Giant	22	4	18	8	2 ± 1	0
	Helene	Pink Giant	1	1	100	23	–	23
	Lil' Kim™	Pink Giant	48	4	8	28	7 ± 2	1
	Lucy	Pink Giant	17	0	0	0	–	0
	Minerva	Pink Giant	9	3	33	4	1 ± 0	0
	Pink Chiffon®	Pink Giant	71	5	7	36	7 ± 2	1
	Red Heart	Pink Giant	14	0	0	0	–	0
	Strawberry Smoothie™	Pink Giant	14	0	0	0	–	0
	Sugar Tip®	Pink Giant	7	0	0	0	–	0
	Tahiti™	Pink Giant	4	1	25	1	–	0
	White Chiffon®	Pink Giant	42	3	7	41	14 ± 6	1
	Woodbridge	Pink Giant	31	7	23	35	5 ± 3	1
$6x \times 4x$								
	Azurri Satin®	Blue Satin®	1	0	0	0	–	0
	Azurri Satin®	Minerva	1	0	0	0	–	0
	Pink Giant	Bali™	25	3	12	8	3 ± 1	0
	Pink Giant	Blue Chiffon™	32	4	13	11	3 ± 1	0
	Pink Giant	Blue Satin®	33	2	6	2	–	0
	Pink Giant	Blue Bird	5	3	60	5	2 ± 1	1
	Pink Giant	Blushing Bride	33	3	9	21	7 ± 2	1
	Pink Giant	China Chiffon™	3	0	0	0	–	0
$6x \times 4x$								
	Pink Giant	Diana	28	2	7	13	–	0
	Pink Giant	Fiji™	18	4	22	22	6 ± 2	1
	Pink Giant	Hawaii™	7	0	0	0	–	0
	Pink Giant	Lavender Chiffon™	3	1	33	1	–	0
	Pink Giant	Lil' Kim™	32	8	25	34	4 ± 1	1
	Pink Giant	Minerva	5	0	0	0	–	0
	Pink Giant	Pink Chiffon®	1	0	0	0	–	0
	Pink Giant	Red Heart	13	5	38	16	3 ± 0	1
	Pink Giant	Tahiti™	15	4	27	11	3 ± 1	1
	Pink Giant	White Chiffon®	80	0	0	0	–	0
	Pink Giant	Woodbridge	7	0	0	0	–	0
	Raspberry Smoothie™	Blue Satin®	4	3	75	5	2 ± 1	1
	Raspberry Smoothie™	Blue Bird	3	0	0	0	–	0
	Raspberry Smoothie™	Diana	5	3	60	27	9 ± 6	5
	Raspberry Smoothie™	Minerva	4	4	100	18	5 ± 1	5
	Raspberry Smoothie™	Woodbridge	5	5	100	11	2 ± 1	2

[z]Interploid combinations included $4x \times 6x$ and $6x \times 4x$.

[y]Percent capsule set calculated as follows: [capsules/pollinations] × 100.

[x]Average seeds per capsule calculated using capsules for each combination as replicates.

[w]Total seed per pollination calculated as follows: [total seed/total pollination] × 100.

male in multiple cross combinations (Table 4). To aid future breeders of *H. syriacus*, cross-compatibility data have been reported for all attempted tetraploid crosses (Table 5). In addition, cross-compatibility data are reported on all 4*x* × 6*x* and 6*x* × 4*x* combinations (Table 6).

Pentaploid testcrosses. Pentaploid accessions resulting from crosses with hexaploid 'Pink Giant' grew slowly in their first 2 years and flowered sporadically. Most pentaploid accessions produced capsules and seeds from daily controlled crosses with randomly collected flowers from fertile male parents. Two novel floral phenotypes were observed among the pentaploid seedlings from the fertility testcrosses. One seedling (H2013-129-08) from the cross 'Pink Giant' (6*x*) × Fiji™ (4*x*) exhibited pink, bicolor flowers that never fully opened, and were reminiscent of a rose. Both parents produce flowers that fully open, and it is unclear whether the semiclosed flowers of H2013-129-08 were inherited from one of its parents or is a product of gigas effects from the odd ploidy level; however, no other pentaploid accession exhibited semiclosed flowers. The tetraploid male parent, Fiji™, is one of the only available taxa of *H. syriacus* with bicolor petals, with red-pink pigment present on the abaxial petal surface. One drawback of Fiji™ is that the pigment is most striking on the expanding flower bud, but less striking on the adaxial petal surface when the flower is fully open. Producing semiclosed, rose-like flowers in *H. syriacus* may be a novel way to enhance this ornamental characteristic derived from Fiji™, as illustrated in H2013-129-08 (Fig. 1A) compared with the more standard phenotype of fully opened flowers in half-sib relatives (Fig. 1B).

Another seedling (H2013-131-06) produced large, petaloid male and female whorls, eliminating any possibility for fertility. Although pollinations could not be performed on this accession, observations were made on longevity of the flowers. Flowering was sporadic and inhibited by high levels of flower bud abortion, yet open flowers were observed to last up to 2.5 weeks, compared with 2 days in a typical flower of *H. syriacus* (J. Lattier, personal observation). This striking flower longevity may indicate a longer bloom time as a byproduct of

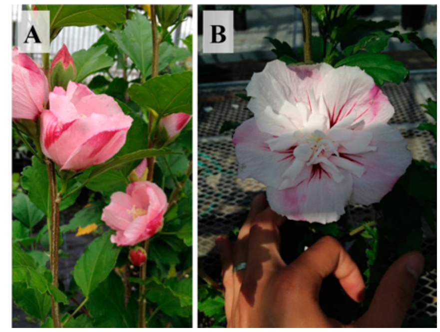

Fig. 1. Bicolor Fiji™ floral phenotype in *Hibiscus syriacus* expression in pentaploid and tetraploid seedlings. (**A**) Pentaploid hybrid (H2013-129-08) resulting from the cross 'Pink Giant' × Fiji™ exhibiting bicolor petals on semiclosed flowers. (**B**) Tetraploid hybrid (H2013-059-09) from the cross Fiji™ × White Chiffon® exhibiting the standard, bicolor Fiji™ phenotype on fully opened flowers.

Table 7. Pollination, capsule, and seed estimates from testcrosses on progeny resulting from combinations of tetraploid and hexaploid *Hibiscus syriacus*.

Treatment[z]	♀ pedigree[y]	♀ 5*x* accession no.	♀ 2C genome size (pg)[x]	Pollinations (no.)	Capsules (no.)	Capsule set (%)[w]	Seeds (no.)	Seeds per capsule (mean ± SE)[v]	Seeds per pollination (mean ± SE)[u]
4*x* × 6*x*									
	Aphrodite × PG	H2012-005-01	6.4	51	34	67	188	5.5 ± 0.4 A	3.7 ± 0.5 A
		H2013-017-21	5.7	12	9	75	27	3.0 ± 0.7 A	2.3 ± 0.7 A
		H2013-017-16	6.1	58	23	40	40	1.7 ± 0.3 A	0.7 ± 0.2 A
	Bluebird × PG	H2012-011-02	5.8	2	0	0	0	–	–
		H2012-011-04	6.3	3	2	67	0	–	0.0 ± 0.0 A
		H2012-011-07	6.1	24	7	29	4	0.6 ± 0.3 A	0.2 ± 0.1 A
	Diana × PG	H2013-049-01	5.7	3	1	33	0	–	0.0 ± 0.0 A
	Helene × PG	H2013-124-13	6.1	81	77	95	552	7.1 ± 1.7 A	6.8 ± 1.8 A
		H2013-124-19	5.9	14	9	64	5	0.6 ± 0.4 A	0.4 ± 0.3 A
		H2013-124-03	6.3	29	27	93	64	2.3 ± 0.4 A	2.2 ± 0.4 A
	Woodbridge × PG	H2012-041-01	6.8	1	1	100	1	–	–
6*x* × 4*x*									
	PG × Aphrodite	H2013-077-05	5.6	38	23	61	289	12.6 ± 1.4 A	7.6 ± 1.3 B
	PG × Bali™	H2013-078-03	5.7	21	4	19	5	1.3 ± 0.5 A	0.2 ± 0.1 A
	PG × Bluebird	H2012-030-01	5.9	6	5	83	28	5.6 ± 0.8 A	4.7 ± 1.1 A
	PG × Fiji™	H2013-129-08	5.6	30	21	70	76	3.6 ± 0.4 A	2.5 ± 0.4 A
	PG × Lil' Kim™	H2013-084-21	6.0	31	4	13	3	0.8 ± 0.3 A	0.1 ± 0.1 A
	PG × Red Heart	H2013-085-01	6.1	29	6	21	6	1.0 ± 0.4 A	0.2 ± 0.1 A
Control – 4*x* × 4*x* ♀ fertility estimates								18 ± 2 B	9.9 ± 1.4 B
	Blue Satin®	–	–	107	75	70	1626	22 ± 1	14.1 ± 2.6
	Blue Bird	–	–	240	109	45	1798	17 ± 1	8.3 ± 2.2
	Buddha Belly	–	–	42	34	81	752	22 ± 1	19.2 ± 3.1
	Diana	–	–	395	87	22	1302	15 ± 1	5.6 ± 1.5
	Lil' Kim™	–	–	74	28	38	440	16 ± 1	5.3 ± 2.4
	Minerva	–	–	40	22	55	311	14 ± 2	6.3 ± 2.5
	Red Heart	–	–	173	62	36	1720	28 ± 2	9.8 ± 2.9
	Woodbridge	–	–	115	45	39	805	18 ± 1	8.5 ± 2.5
	Bali™	–	–	110	78	71	1443	19 ± 1	11.5 ± 2.6
	Blue Chiffon™	–	–	127	76	60	1410	19 ± 1	11.5 ± 2.6
	Fiji™	–	–	47	25	53	383	15 ± 1	9.3 ± 1.9
	Tahiti™	–	–	39	6	15	29	5 ± 1	1.1 ± 0.5
	White Chiffon®	–	–	40	25	63	690	28 ± 1	18.6 ± 4.8

[z]Tetraploid (4*x*) and hexaploid (6*x*) cross combinations. Controls are represented by single and semidouble female fertility estimates from 4*x* × 4*x* crosses with 5+ male parents and 30+ pollinations.

[y]Pedigree of interploid hybrids between hexaploid Pink Giant (PG) and tetraploid taxa. Controls include open-pollinated seed from three fertile tetraploid taxa.

[x]Estimate of holoploid 2C genome size.

[w]Percent capsule set calculated per accession as follows: [total filled capsules/total pollinations] × 100.

[v]Seedlings per pollination calculated for each treatment (within boxes) and per accession. For each accession, seedlings per pollination was calculated as follows: [total seedling/total pollinations] × 100. For each treatment, average seedlings per pollination were calculated from accession estimates with at least three pollinations. Letters separating least squares means based on comparison lines test of the generalized mixed model procedure (GLIMMIX). MV = missing value.

[u]Percent germination calculated for each treatment (within boxes) and per accession. For each accession, percent germination was calculated as follows: [total seedlings/total seeds] × 100. For each treatment, average percent germination was calculated from accession estimates with 10+ seed.

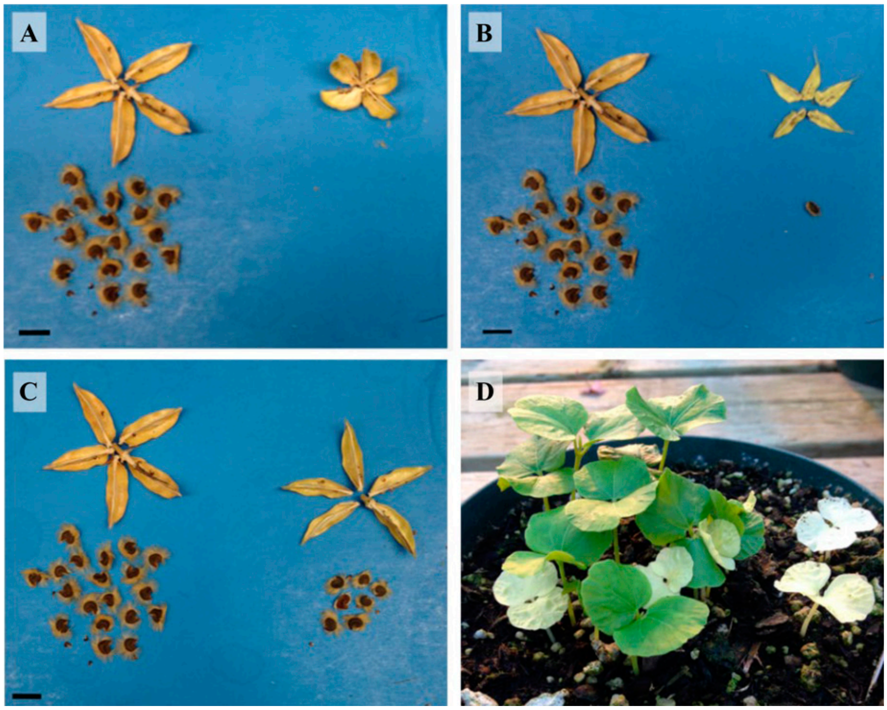

Fig. 2. Seed and seedling development from testcrosses of pentaploid *Hibiscus syriacus*. Scale bar = 1 cm. (**A**) Fruit from open-pollinated *H. syriacus* 'Woodbridge' (left) and pentaploid hybrid *H. syriacus* 'Blue Bird' × 'Pink Giant' (right) pollinated with *H. syriacus* 'Red Heart'. (**B**) Fruit from open-pollinated *H. syriacus* 'Woodbridge' (left) and pentaploid hybrid *H. syriacus* 'Helene' × 'Pink Giant' (right) pollinated with *H. syriacus* Lil' Kim™. (**C**) Fruit from open-pollinated *H. syriacus* 'Woodbridge' (left) and pentaploid hybrid *H. syriacus* 'Helene' × 'Pink Giant' (right) pollinated with *H. syriacus* 'Woodbridge'. (**D**) Germinating seeds from pentaploid hybrid *H. syriacus* 'Pink Giant' × 'Aphrodite' pollinated with *H. syriacus* 'Diana' exhibiting albino seedlings.

sterility in *H. syriacus*. Further work will be necessary to determine differences in flower duration and bloom time among accessions with different female fertility. All other pentaploid accessions produced large, single, pink flowers.

Of the 17 pentaploid accessions of >20 attempted pollinations, four yielded <30% filled capsules per pollination, including H2012-011-07 ('Bluebird' × 'Pink Giant') at 29%, H2013-078-03 ('Pink Giant' × Bali™) at 19%, H2013-084-21 ('Pink Giant' × Lil' Kim™) at 13%, and H2013-085-01 ('Pink Giant' × 'Red Heart') at 21% (Table 7). Significant differences were found in pairwise comparisons between pentaploid accessions and tetraploid controls for seeds per capsule ($P < 0.0001$) and seeds per pollination ($P < 0.0001$). Female fertility of most pentaploid accessions was significantly reduced compared with the average of the tetraploid taxa, which produced 18 ± 2 seeds per capsule and 9.9 ± 1.4 seeds per pollination (Table 7). The most striking difference was observed as capsules began to dehisce, with many of the capsules producing no seed (Fig. 2A) or relatively few seeds (Fig. 2B and C) compared with fertile tetraploids. Because of the low flower production and low seed set, percent seed germination estimates were obtained only from plants that produced at least 10 seeds. Significant differences in percent seed germination were observed between pentaploids and

Table 8. Pollination, germination, and seedling estimates from testcrosses on progeny resulting from combinations of tetraploid and hexaploid *Hibiscus syriacus*.

Treatment[z]	♀ pedigree[y]	♀ 5*x* accession no.	♀ 2C genome size (pg)[x]	Pollinations (no.)	Capsules (no.)	Capsule set (%)[w]	Seeds (no.)	Seedlings (no.)	Seedlings per pollination (mean ± SE)[v]	Germination (%)[u]
5*x* progeny from 4*x* × 6*x*									0.8 ± 0.3 A	45 ± 4 A
	Aphrodite × PG	H2012-005-01	6.4	51	34	67	188	71	1.4	38
		H2013-017-21	5.7	12	9	75	27	14	1.2	52
		H2013-017-16	6.1	58	23	40	40	22	0.4	55
	Bluebird × PG	H2012-011-02	5.8	2	0	0	0	0	–	–
		H2012-011-04	6.3	3	2	67	0	0	0.0	–
		H2012-011-07	6.1	24	7	29	4	0	0.0	0
	Diana × PG	H2013-049-01	5.7	3	1	33	0	0	0.0	–
	Helene × PG	H2013-124-13	6.1	81	77	95	552	253	3.1	46
		H2013-124-19	5.9	14	9	64	5	1	0.1	20
		H2013-124-03	6.3	29	27	93	64	22	0.8	34
	Woodbridge × PG	H2012-041-01	6.8	1	1	100	1	0	–	0
5*x* progeny from 6*x* × 4*x*									1.4 ± 0.9 A	45 ± 14 A
	PG × Aphrodite	H2013-077-05	5.6	38	23	61	289	206	5.4	71
	PG × Bali™	H2013-078-03	5.7	21	4	19	5	2	0.1	40
	PG × Bluebird	H2012-030-01	5.9	6	5	83	28	12	2.0	43
	PG × Fiji™	H2013-129-08	5.6	30	21	70	76	17	0.6	22
	PG × Lil' Kim™	H2013-084-21	6.0	31	4	13	3	2	0.1	67
	PG × Red Heart	H2013-085-01	6.1	29	6	21	6	3	0.1	50
4*x* control – OP Seeds										89 ± 1 B
	Blue Satin®	11-0210	4.7	–	–	–	50	43	–	86
	White Chiffon®	13-0044	4.7	–	–	–	50	45	–	90
	Woodbridge	11-0214	4.7	–	–	–	50	45	–	90

[z]Tetraploid (4*x*) and hexaploid (6*x*) cross combinations. Controls represent open-pollinated (OP) seed from tetraploid cultivars.

[y]Pedigree of interploid hybrids between hexaploid Pink Giant (PG) and tetraploid taxa. Controls include OP seed from three fertile tetraploid taxa.

[x]Estimate of holoploid 2C genome size.

[w]Percent capsule set calculated per accession as follows: [total filled capsules/total pollinations] × 100.

[v]Seedlings per pollination calculated for each treatment (within boxes) and per accession. For each accession, seedlings per pollination was calculated as follows: [total seedling/total pollinations] × 100. For each treatment, average seedlings per pollination were calculated from accession estimates with at least three pollinations. Letters separating least squares means based on comparison lines test of the generalized mixed model procedure (GLIMMIX).

[u]Percent germination calculated for each treatment (within boxes) and per accession. For each accession, percent germination was calculated as follows: [total seedlings/total seeds] × 100. For each treatment, average percent germination was calculated from accession estimates with 10+ seed.

the OP seed from tetraploid controls ($P < 0.005$). An average percent germination of 45% was observed for pentaploids from both 4*x* × 6*x* combinations and 6*x* × 4*x* combinations compared with 89% among OP tetraploids (Table 8). In addition to reduced germination, increased production of albino seedlings was observed in the pentaploid progeny (Fig. 2D). No albino seedlings were observed in the tetraploid controls.

Holoploid (2C) genome size was analyzed for a subset of seedlings resulting from the testcrosses (data not shown). From the analysis of single leaf samples from each seedling, most had tetraploid genome sizes; however, one seedling was found to be hexaploid (6.80 pg) from the cross H2013-124-13 ('Helene' × 'Pink Giant') × 'Diana'. We speculate this plant resulted from a 5*x* female parent producing a 4*x* gamete that was fertilized by a normally reduced 2*x* sperm cell. In addition, four seedlings were found to be heptaploid (7.21 pg to 7.60 pg) from the cross H2012-005-01 ('Aphrodite' × 'Pink Giant') × 'Minerva' and the cross H2013-124-13 ('Helene' × 'Pink Giant') × 'Diana'. Our hypothesis for the origin of these 7*x* plants is that unreduced female gametes (5*x*) combined with normally reduced 2*x* male gametes. To our knowledge, this is the first report of heptaploid *H. syriacus,* and these novel odd ploidy seedlings may show reduced fertility in future testcrosses. In addition, these seedlings expand the ploidy series of Lattier et al. (2019) to five cytotypes for future research. Although only a single accession, the (near) decaploid (12.22 pg) seedling recovered from a previous polyploid induction experiment (Lattier et al., 2019) expanded the ploidy series to six cytotypes: 4*x*, 5*x*, 6*x*, 7*x*, 8*x*, and 10*x*.

The combination of reduced capsule development, reduced number of seeds per capsule, and thus few seeds per pollination for nearly all pentaploids illustrates their reduced fertility compared with fertile tetraploids. These reduced fertility estimates, combined with reduced germination percentage, place many of the pentaploid taxa below the 2% relative fertility threshold outlined by the ODA (Contreras and McAninch, 2013).

Future work will include a continuation of female and male fertility tests as pentaploid plants mature. Although less important, male fertility of pentaploids will also be evaluated by a combination of pollen staining and fertility testcrosses. Some crosses warrant repeating to produce more novel phenotypes, including interploid crosses with 'Blushing Bride' and Fiji™. Hexaploids Azurri Satin® and Raspberry Smoothie™ will be used in further interploid combinations, especially the proven-fertile, double-flowered Raspberry Smoothie™. Future work will also include flow cytometry of the seedlings resulting from fertility testcrosses to develop new novel cytotypes of *H. syriacus*. These new seedlings could provide material to determine fertility rates among more interploid combinations. The combination of low-fertility interploid hybrids with double flowers may lead to the production of new generations of novel, sterile *H. syriacus* for the nursery industry and home landscapes.

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