THE INHERITANCE OF PLANT AND FLOWER TRAITS IN ROSE

An Undergraduate Research Scholars Thesis

by

SARAH JONES

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Dr. David Byrne

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ABSTRACT

The Inheritance of Plant and Flower Traits in Rose (May 2013)

Sarah Jones Department of Horticultural Sciences Texas A&M University

Research Advisor: Dr. David H. Byrne Department of Horticultural Sciences

Limited data is available in the area of rose genetics making it difficult for rose breeders to efficiently develop improved rose cultivars. In order to improve efficiency of breeding programs, the patterns of genetic inheritance of important traits must be discovered through statistical genetic research. This genetic study focuses on valued traits including shrub growth type, flower color, flower form, flower diameter, the presence or absence of stem and petiole prickles, bloom habit, and proliferation in an interspecific diploid landscape population. Measurements and phenotypic observations were gathered by trait for each plant in the College Station, Texas, in the fall of 2012. Qualitative traits including bloom habit, flower color, flower form, and the presence of prickles were analyzed through chi square tests. Flower color, flower form, and stem prickles were inherited as supported in previous studies despite the overall observed deviation from the expected values common in interspecific rose crosses. The quantitative trait, flower diameter, was examined using mid-parent to progeny mean regression that showed a 59% additive

heritability. These statistical tests were used to quantify the inheritance patterns of aesthetically important characteristics in roses that will greatly aid plant breeders in decreasing the time and guesswork involved in breeding and improving successive generations of roses.

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CHAPTER I INTRODUCTION

Motivation

Knowing the inheritance patterns of aesthetically important characteristics in roses can greatly aid plant breeders by decreasing the time and guesswork involved in breeding and improving successive generations of roses. However, unlike agronomic crops with well documented, readily available information, there is little research available to the public because private breeding companies have little motivation to publish their work on rose genetics. The purpose of this experiment is to elucidate the inheritance of several important traits to improve the efficiency of rose breeding.

Breeding Improvements

Today over 60% of roses are grown on their own roots as compared to 35 years ago when most roses were grown on other root stocks to grow plants with aesthetically pleasing blooms and foliage with the added benefit of a vigorous root stock (Hutton 2012). This trend towards growing roses on their own roots will continue as breeders develop new varieties of roses with improved characteristics and disease resistance to create a plant worthy of consumer purchase (Hutton 2012). Roses grown on their own roots can be cultivated much faster, in about 12 months, without the inconvenience and additional labor costs of grafting the scion to a different root stock, which is desirable to growers responsible for production. (Hutton 2012). To make the development of the improved roses more efficient, knowledge of the inheritance patterns for important traits is necessary.

Roses, of the genus *Rosa*, come from one of the most economically important genera in ornamental horticulture (Gudin 2000). Roses are bred for increased productivity and better characteristics as garden roses, potted roses, and cut flowers (Debener 2009). But, despite the roses' high economic importance, little is known about their genetics, genome structure, and gene function due primarily to breeding and inheritance complications derived from polyploidy, limited public funding, and simple breeding methods still utilized by most breeders (Debener 2009). When examining genetic inheritance, classical genetic analysis is an integral part of the discovery process of gene function because molecular analysis alone will not identify the function of a particular gene, or sequence of DNA, without classical genetic tests to support the results of molecular analysis (Debener 2003). The utilization of these classical genetic tests can help determine the genetic inheritance of important physiological traits in roses such as shrub growth type, bloom diameter, flower form as a single or double bloom, and the presence or absence of stem and petiole prickles.

Growth Type

Inflorescence structure greatly determines value in ornamental roses, especially garden and landscape roses, because it determines the placement and number of flowers, and overall appearance of a plant (Kawamura 2011). Multiple traits such as internode elongation, axillary branching, and the timing of meristem differentiation all contribute to the final inflorescence structure, but even environmental conditions can have an effect on the inflorescence structure (Kawamura 2011). In a test of 98 F₁ hybrids from a cross of "The Fairy" and *R. wichurana*, the F₁ hybrids showed a broadened range of inflorescence trait values beyond values of the two parents, illustrating transgressive segregation (Kawamura 2011). In this same study, it was found

that inflorescence traits and flowering time are controlled by common genomic regions, but additional developmental components that further influence inflorescence structure such as node production, internode elongation, and axillary branching, are controlled by separate genomic regions (Kawamura 2011). In other studies, it has been found that the climbing growth type acts dominantly over non-climbing growth types (Morey 1954).

Bloom color

Flower color in *Rosa* is caused by carotenoids, flavonols, and anthocyanidins present in the flower petals (Debener 2003). Pink flower color has been shown to inherit codominantly with white being homozygous recessive, pink being heterozygous, and darker pink being homozygous dominant.

Flower form

The presence of double flowers was found to be inherited as a monogenic dominant character (Debener 2001). Double flowers have been selected for since the early history of rose breeding which seems to have caused intermediate physiological forms between stamens and petals. (Debener 2003). It appears that the number of stamens decreases in strongly double flowers as some of the inner petals may be morphologically related to the stamens and show intermediate structure between petals and stamens (Debener 1999). The inheritance of the double flower form is controlled by a dominant allele while the inheritance of single flower form is recessively inherited (Debener 1999, Lammerts 1945). This does not eliminate the possibility of two complementary genes governing flower form, but it is more likely that one gene determines if a

flower will be single or double and additive genes determine the quantity of petals in the double genotype (Zlesak 2006, Debener 2003).

Flower size

Not much information is available on the inheritance of flower size, however, in one study of *Rosa* genes and quantitative trait lock, QTL, mapping, rose flower size, flowering date, leaf size, and powdery mildew resistance were examined (Dugo 2005). Flower size and leaf size were found to have a significant positive correlation (Dugo 2005). In addition, flower size was shown to be largely affected by both female and male parents in addition to gene interactions (Dugo 2005).

Bloom Habit

Non-recurrent bloom habit has been shown to be determined by a single dominant gene with recurrent blooming caused by a homozygous recessive (Debener 1999, Debener 2003).

Prickles

Commonly called thorns, rose prickles that grow on stems and petioles are technically outgrowths of the epidermal layer of the stem, comparable to hairs (Rost 1998). Thorns can be modified stems or modified leaves but true thorns are actually modified stems, while the prickles present on rose stems are epidermal growths more closely related to hairs. (Rost 1998, Debener 2009). It has been shown that the presence of prickles is inherited by a single dominant gene (Debener 1999, Debener 2003, Shupert 2007).

CHAPTER II METHODS

Diploid rose population

The parental generation, with an ancestry containing *wichuriana* (Table 1), was pollinated in the spring of 2010 resulting in the diploid landscape population analyzed in this experiment .The seeds resulting from these pollinations were harvested in the fall of 2010 and subsequently planted in flats, in a peat based media, Metromix. The planted seeds were watered and allowed to drain before being covered with plastic to retain moisture during the stratification period. After covering, seed flats were placed in the cold room for 3 months at 4°C. In February to March of 2011, seed flats were removed from the cold room and relocated to a warm greenhouse where the seeds were allowed to germinate. Once seedlings were of a sufficient size, they were transplanted into 1 gallon pots and continued growing in their individual pots for the rest of year, and overwintered in the greenhouse. Next spring in May of 2012, the rose plants were planted in in the field. Parental phenotypic characteristics are as shown below in Table 2. Diploid landscape rose population is shown in Table 3.

Cultivar	Female	Pollen	% wichuriana in background
JO6-20-14-3	DD	DD	50%
JO6-28-4-6	WICH-THLESS	WOB26	75%
JO6-30-3-3	DD	M4-2	50%
JO6-30-3-6	DD	M4-2	50%
M4-4	WOB26	WOB26	50%
Old Blush	UNKNOWN	UNKNOWN	0%
Red Fairy	SIMON ROBINSON	SIMON ROBINSON	75%
Sweet Chariot	LITTLE CHIEF	VIOLETTE	12.50%
The Fairy	PAUL CRAMPEL	LADY GAY	25%

Table 1. % *Wichuriana* in Parental Generation's Background

*DD=Ducher x wichuriana

	Stem	Petiole	Average		Flower	Growth
Parent	Prickles	Prickles	Diameter (cm)	Color	Form	Туре
Red Fairy	Yes	Yes	3.6	Darker Pink	Semi-double	Intermediate
Sweet Chariot	Yes	Yes	4.1	Darker Pink	Semi-double/Double	Upright
JO6-20-14-6	Yes	Yes	4.9	White	Semi-double	Ground cover/Climbing
JO6-30-3-6	No	Yes	3.2	White	Single	Climbing
Old Blush	Yes	Yes	6.7	Lighter Pink	Semi-double/Double	Intermediate
JO6-28-4-6	No	Yes	2.5	Lighter Pink	Semi-double/Double	Intermediate
JO6-30-3-3	No	Yes	3.0	White	Single	Intermediate
M4-4	No	Yes	5.0	Lighter Pink	Single	Climbing
The Fairy	Yes	Yes	3.7	Lighter Pink	Double	Intermediate

Table 2. Parental Generation Characteristics

 Table 3. Diploid Landscape Rose Population

Cross Alias	Female Parent	Pollen Parent
10038	Old Blush	JO6-30-3-6
10039	The Fairy	JO6-30-3-6
10041	Old Blush	M4-4
10042	Vineyard Song	M4-4
10043	Sweet Chariot	M4-4
10061	JO6-28-4-6	Red Fairy
10066	JO6-30-3-3	Red Fairy
10067	JO6-30-3-6	Red Fairy
10068	Old Blush	Red Fairy
10074	JO6-20-14-3	Sweet Chariot
10075	M4-4	Sweet Chariot

Growing location information

The experimental field for this project is located about 2 miles from the Texas A&M University campus off of FM 2818. The individual plants were planted in rows oriented east to west in an open field that receives full sun. Raised beds were constructed in the field, and after planting, the roses were surrounded with a black cloth weed barrier for weed control. Irrigation water was supplied as needed.

Data collection

Prickles

Both stems and the underside of the petioles were evaluated for the presence of prickles. Occasionally prickles on the petioles were faint, but any detection of prickles was recorded as a positive. No prickles was recorded as "0" while the presence of prickles was recorded as "1". Example images of the presence and absence of prickles can be seen in the below Figures 1-3.



Figure 1. No prickles



Figure 2. Stem prickles



Figure 3. Petiole prickles

Bloom color

Observed basic color description was recorded from newly opened blooms. A variety of colors were observed in the field and then arranged gradually to allow numerical assignment. The color gradient with corresponding abbreviation and numerical representation is shown in Table 4.

12	Table 4. Bloom Color Gradient Cale						
1	White	wh					
2	White/ Light Pink	wh/lt pk					
3	Light Pink	lt pk					
4	Light Pink/ Purple	lt pk/pur					
5	Pink	pk					
6	Pink/ Purple	pk/pur					
7	Medium Pink	m pk					
8	Dark Pink	dk pk					
9	Red	red					

Table 4. Bloom Color Gradient Categories

Flower form

The number of petals for individual blooms was divided into categories of Single, Semi-Double, Semi-Double/ Double, and Double (see Figures 4-7). Blooms were then observed and placed into these categories, and petals were counted when the number of petals was near to the bounds of two categories to ensure correct placement. The number of petals in each category is as follows in Table 5.



Figure 4. Single flower



Figure 6. Semi-double/Double flower



Figure 5. Semi-double flower



Figure 7. Double flower

1	Single	8 or less petals
2	Semi-double	9-25 petals
3	Semi-double/Double	25-40 petals
4	Double	>40 petals

 Table 5. Flower Form Categories

Flower size

Flower size was measured in the field while the blooms were still on the plants using a clear, transparent ruler. Blooms were pressed up against the underside of the transparent ruler to spread petals to their full diameter. The diameters of three blooms per plant were recorded as blooms became available, but due to blooming time, recording at least 3 diameters per plant was not always achievable.

Growth Type

Growth type of the plants was determined in the field during data collection. Plant growth type ranges from Ground Cover, with stems clinging to the ground, Ground Cover/ Climbing, the plants with slightly raised stems close to the ground but not fully climbing, Climbing, with stems reaching outwards and upwardsoften bending back towards the ground, Intermediate, with branches not fully extending upright, to completely Upright. Growth type was determined by comparison to other plants in the diploid population and the plants were divided into the five different categories of Ground Cover, Ground Cover/ Climbing, Climbing, Intermediate, and Upright(see Figures 8-12). The groups were then numbered from 1-5 with Ground Cover being 1 and Upright being 5 (Table 6).



Figure 8. Ground Cover



Figure 10. Climbing



Figure 9. Ground Cover/ Climbing



Figure 11. Intermediate



Figure 12. Upright

Table 6. Growth Type Categories

- 1 Ground Cover
- 2 Ground Cover/Climbing
- 3 Climbing
- 4 Intermediate
- 5 Upright

Bloom habit

Bloom habit was recorded as "recurrent" if blooms were found on the plant in the summer and data could be taken. Bloom habit was recorded as "non-recurrent" (NR) if no indication of summer blooms was found on the plant. These plants bloomed once in the spring and did not have any subsequent cycles of flowering as did the recurrent types. If the remnants of blooms were noted, they were marked "missed" so that the plant could be revisited later to discover evidence of recurrent blooming.

Proliferation

An unusual characteristic observed in the field was the presence of proliferation on some rose bloom where the inner petals and stamens of the rose were deformed and packed together in the center of the rose (Figure 13). Some blooms had proliferation as well as budding from the center of the rose (Figure 13). Note of proliferation was recorded as "prolif" in the data sheet.



Figure 13. Proliferation Top: proliferation with budding Bottom: deformed stamens and central petals

CHAPTER III

RESULTS AND DISCUSSION

Prickles

Petiole prickles showed little to no segregation, however stem prickles did show segregation. The presence of stem prickles was inherited as a single dominant over the absence of prickles as supported by other studies (Debener 1999, Debener 2003, and Shupert 2007). However, three of the crosses examining stem prickles, (10041, 10038, and 10043 in Table 7), show probability values equal to or lower than 0.05. This means that the deviation from predicted segregation ratios may not be due solely to chance.

Table 7. Chi Square for Stem Prickles

Cross	Parent	Parent	Phenotype	Phenotype	Genotype	Genotype	Expected	Observed	Chi	Probability
	Female	Pollen	Female	Pollen	Female	Pollen	Ratio	Number	Square	
10038	Old Blush	JO6-30-3-6	prickles	no Prickles	PRpr	prpr	1:1	51:79	6.031	0.05
10041	Old Blush	M4-4	prickles	no Prickles	PRpr	prpr	1:1	5:14	4.26	0.05
10043	Sweet Chariot	M4-4	prickles	no Prickles	PRpr	prpr	1:1	8:35	17.36	0.001
10061	JO6-28-4-6	Red Fairy	no prickles	prickles	prpr	PRPR	0:1	0:98	-	-
10066	JO6-30-3-3	Red Fairy	prickles	no Prickles	PRpr	PRPR	0:1	0:6	-	-
10067	JO6-30-3-6	Red Fairy	no prickles	prickles	prpr	PRPR	0:1	0:4	-	-
10074	JO6-20-14-3	Sweet Chariot	prickles	prickles	PRpr	PRpr	1:3	8:25	0.009	0.95
10075	M4-4	Sweet Chariot	no prickles	prickles	prpr	PRpr	1:1	5:9	1.14	0.30

*Ratio = no prickles: prickles

Bloom habit

The cross 10061 was shown to segregate for recurrent and non-recurrent blooming, however the

segregation ration did not fit the expected 1:1 ratio and showed an excess of non-recurrent

seedlings (Table 8).

Cross	Parent	Parent	Phenotype	Phenotype	Genotype	Genotype	Expected	Observed	Chi	Probability
	Female	Pollen	Female	Pollen	Female	Pollen	Ratio	Number	Square	
10061	JO6-28-4-6	Red Fairy	non-recurrent	recurrent	Rr	rr	1:1	61:38	5.34	0.01
*Ratio	^k Ratio = non-recurrent: recurrent									

Table 8. Chi Square for Blooming Habit

Bloom color

Three crosses (10041, 10043, and 10061 as seen in Table 9) have a larger amount of deviation from the predicted 0:1:1 ratio of white:lighter pink:darker pink in the progeny. This deviation could be caused by inaccurate separation of bloom colors into the designated categories. Many of the white blooms may also be pale pink, as the pale pink blooms can easily bleach to white in the field which would cause mislabeling of the blooms. However the remaining crosses show 20% to 80% probability that the segregation ratios are due to chance alone. These crosses support the idea that flower color is governed by a single codominant gene (Debener 2003).

Cross	Parent	Parent	Phenotype	Phenotype	Genotype	Genotype	Expected	Observed	Chi	Probability
	Female	Pollen	Female	Pollen	Female	Pollen	Ratio	Number	Square	
10038	Old Blush	JO6-30-3-6	darker pink	white	PP	pp	0:1:0	16:96:0	2.286	0.2
10039	The Fairy	JO6-30-3-6	lighter pink	white	Рр	рр	1:1:0	4:1:0	1.8	0.5
10041	Old Blush	M4-4	darker pink	lighter pink	PP	Рр	0:1:1	0:17:0	17	0.001
10043	Sweet Chariot	M4-4	darker pink	lighter pink	PP	Рр	0:1:1	3:28:5	14.944	0.001
10061	JO6-28-4-6	Red Fairy	lighter pink	darker pink	Рр	PP	0:1:1	10:17:5	7.625	0.01
10066	JO6-30-3-3	Red Fairy	white	darker pink	pp	PP	0:1:0	1:2:0	0.333	0.8
10074	JO6-20-14-3	Sweet Chariot	white	darker pink	pp	PP	0:1:0	8:22:3	3.667	0.2
10075	M4-4	Sweet Chariot	lighter pink	darker pink	Рр	PP	0:1:1	0:10:4	2.571	0.3

Table 9. Chi Square for Bloom Color

*Ratio = white: lighter pink: darker pink

Flower form

Except for two crosses, (10038 and 10074 in Table 10) the observed segregating ratios support the hypothesis that double flower form is inherited as a single gene with additive genes contributing for different levels of doubleness. There is over 30% probability in 4 out of 6 crosses that the deviation from the predicted ratios is due to chance.

Cross	Parent	Parent	Phenotype	Phenotype	Genotype	Genotype	Expected	Observed	Chi	Probability
	Female	Pollen	Female	Pollen	Female	Pollen	Ratio	Number	Square	
10038	Old Blush	JO6-30-3-6	double	single	Dd	dd	1:1	45:74	7.067	0.01
10041	Old Blush	M4-4	double	single	Dd	dd	1:1	11:7	0.444	0.50
10043	Sweet Chariot	M4-4	double	single	Dd	dd	1:1	16:20	0.444	0.50
10061	JO6-28-4-6	Red Fairy	double	double	Dd	Dd	1:1	14:18	0.500	0.50
10074	JO6-20-14-3	Sweet Chariot	single	double	dd	Dd	1:1	5:28	16.030	0.001
10075	M4-4	Sweet Chariot	double	double	Dd	Dd	1:1	5:9	1.143	0.30

Table 10. Chi Square for Flower Form

*Ratio = single: double

Average flower diameter vs. single or double flower form

Average flower diameter and flower form were compared to discover if there was any relationship between flower diameter and flower (single or double) form. Average flower diameter was collected for each plant from each cross and then the diameters were divided into the diameters of single blooms and the diameters of double blooms (containing the categories semi-double, semi-double/double, and double). The range of diameters shown for single flower form matched the range shown for double blooms in each cross, as seen in Figure 14. Therefore, no bias was observed in flower diameter according to flower form. This indicates that flower diameter is primarily determined by petal size and not by petal number. See Appendix A for graphs of individual crosses in Figures 15-20.





Fig. 14. Average Diameter vs. Flower Form in College Station, TX, Fall 2012 Data was compiled from combined crosses.

Flower diameter

Mid-parent to progeny mean regression shows the relationship between parent and offspring traits and is used to quantify additive heritability. If a certain trait is additive, then the progeny should closely follow the mid-parent line, which averages the male and female parents' trait values. The mid-parent progeny mean of flower diameter indicates that 59% of the progeny data for flower diameter can be explained by additive heritability (Figure 15). Higher levels of additive heritability are desirable in selective breeding, because progeny will be closer to the mean value expected. With higher additive heritability, a breeder can direct a progeny more efficiently towards desired characteristics.



Figure 15. Mid-parent to progeny mean regression for flower diameter, College Station, TX, Fall 2012. Data was compiled from combined crosses.

Proliferation

Proliferation was observed in the progeny of 10039 (Old Blush X JO6-30-3-6), 10061 (JO6-28-4-6 X Red Fairy), and 10074 (JO6-20-14-3 X Sweet Chariot). In the crosses 10039 and 10074 the seedlings segregated 1:4 and 8:25 of seedlings with and without proliferation. These segregation ratios fit the expected 1:3 ratio (presence of proliferation: absence of proliferation) for a progeny derived from a cross between two heterozygous plants for a trait conditioned by a dominant/recessive allele combination. This would indicate that the parents of these two crosses (Old Blush, JO6-30-3-6, JO6-20-14-3 and Sweet Chariot) are heterozygous for the proliferation condition with the dominant allele conditioning no proliferation and the double recessive with proliferation of the flower. However, this explanation does not fit the other progeny (10061), so further crosses that segregate for proliferation would need to be studied to verify this hypothesis.

Growth type

Growth type was divided into ground cover, ground cover/climbing, climbing, intermediate, and upright. However, with these categories, the segregation ratios of the growth type did not fit any hypothesized segregation ratios. This trait needs to be further studied to quantify growth type inheritance pattern.

Segregation distortion

Some of the deviation from expected ratios could be explained by the interspecific nature of the crosses examined in this evaluation, because interspecific crosses can increase the distortion of the observed data in relation to the expected data (Shupert 2005). Mapping studies have shown that deviation from predicted values is seen in 15%-39% of the loci analyzed in rose crosses (Crespel 2002, Dugo 2005, Hibrand-Saint Oyant 2008, Zhang 2006). In addition, crosses involving R. *wichuriana* have also shown to increase distortion at loci (Crespel 2002, Dugo 2005, Hibrand-Saint Oyant 2008, Zhang 2006).

CHAPTER IV CONCLUSION

Phenotypic data from qualitative traits including bloom habit, flower color, flower form, stem and petiole prickles, shrub growth type and quantitative traits including flower diameter, were collected from an interspecific diploid landscape rose population in College Station, Texas in the fall of 2012. Qualitative traits were analyzed using chi square tests to discern deviations from expected progeny ratios.

Although some segregation distortion was observed in some progenies for all traits, the evidence supports that the presence of stem prickles is conditioned by a single dominant gene with the absence of stem prickles segregating as a homozygous recessive (Debener 1999, Debener 2003, and Shupert 2005). The double flower form is conditioned by a dominant allele and the single flower form is the homozygous recessive (Debener 1999). Flower color segregated as a codominant gene with white as the homozygous recessive, lighter pink as heterozygous, and darker pink as the homozygous dominant (Debener 2003). Bloom habit examined in one segregating cross yielded excessive deviation from expected values which seems to disprove the hypothesis that recurrent blooming is inherited as a homozygous recessive. However, due to the fact that interspecific crosses and crosses involving R. *wichuriana* have shown segregation distortion in 15% to 39% of the loci analyzed, the deviation observed may be due to the genetic background of the crosses (Byrne 2009). Further work needs to verify this hypothesis.

Flower diameter was analyzed with a mid-parent to progeny mean regression which showed a 59% additive heritability of flower diameter. Through a comparison of average flower diameters of single blooms with the average flower diameter of double blooms it was determined that a larger amount of petals does not increase the diameter of the flower. In selective breeding, flower diameter of the male and female parent has more of an effect on the progeny flower diameter while the number of petals has little to no effect on the flower diameter of the progeny population.

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APPENDIX A





Figure 16. 10038 Average Diameter vs. Flower Form in College Station, TX, Fall 2012

10038 = Old Blush X JO6-30-3-6





Figure 17. 10041 Average Diameter vs. Flower Form in College Station, TX, Fall 2012

10041 = Old Blush X M4-4





Figure 18. 10043 Average Diameter vs. Flower Form in College Station, TX, Fall 2012

10043 = Sweet Chariot X M4-4





Figure 19. 10061 Average Diameter vs. Flower Form in College Station, TX, Fall 2012 10061 = JO6-28-4-6 X Red Fairy





Figure 20. 10074 Average Diameter vs. Flower Form in College Station, TX, Fall 2012

10074 = JO6-20-14-3 X Sweet Chariot





Figure 21. 10075 Average Diameter vs. Flower Form in College Station, TX, Fall 2012

10075 = M4-4 X Sweet Chariot