Scarlet rosemallow (Hibiscus coccineus Walter) is a diploid, perennial, erect, and woody shrub. The species is a desirable inclusion in home landscapes because it is a native plant with attractive flowers and unusual foliage. The objective of these experiments was to determine the number of loci, number of alleles, and gene action controlling flower color (red vs. white) in scarlet rosemallow. Three white-flowered and 1 red-flowered parental lines were used to create \( S_1 \) and \( F_1 \) populations, which were self-pollinated or back-crossed to generate \( S_2 \), \( F_2 \), and \( BC_1 \) populations. Evaluation of these generations showed that flower color in these populations was controlled by a single diallelic locus with red flower color completely dominant to white. I propose that this locus be named "white flower" with alleles \( W \) and \( w \).

**Key words:** complete dominance, hibiscus, native plant, single diallelic locus

Scarlet rosemallow (Hibiscus coccineus Walter) is a perennial, deciduous, erect, and woody shrub that inhabits swamps, marshes, sloughs, lake banks, and ditches from southeastern Georgia, Alabama, and Texas to southern Florida (Godfrey and Wooten 1979). Growth of the species is typically vase-like with multiple erect stems that can grow to 3 m in height. Juvenile leaves are deltoid and mostly unlobed, whereas mature leaves are strongly palmate, with deep clefts separating each leaf into 5 lobes (Godfrey and Wooten 1979). Scarlet rosemallow is classified as an obligate wetland plant (USDA NRCS 2011), but performs well in less inundated areas provided soil moisture is maintained at adequate levels. Plants begin to flower in May in northern Florida and bear brilliant red 5-petaled flowers that may be as broad as 20 cm. Flowering continues until plants die back in November or December. Plants remain dormant throughout the winter and begin to resprout from underground rhizomes as early as March. Some sources list the species as being hardy in United States Department of Agriculture (USDA) Zones 7–11, whereas others suggest that the plant can survive in regions as cold as Zone 5 (Odenwald and Turner 2006; Gilman 2007; Anonymous 2011).

The perennial nature, native status, wide geographic range, and showy flowers of scarlet rosemallow have made the species a popular garden plant that is sometimes available locally from selected native plant nurseries. In the absence of local outlets, plant and seeds can easily be purchased from any number of nurseries and private individuals on the Internet. Although feral populations of scarlet rosemallow almost exclusively bear red flowers, white-flowered specimens are available in the nursery trade and on the Internet. Variations in flower color are frequently transmitted and inherited in a simple Mendelian fashion (Durbin et al. 2003). For example, flower color variation is controlled by a single diallelic locus in pickerelweed (Gettys and Wofford 2007), stokes aster (Gaus et al. 2003), morning glory (Zufall and Rauscher 2003), Chinese houses (Lankinen and Wofford 2006; Gilman 2007; Anonymous 2011).

There is no published information outlining the genetic control and inheritance of flower color in scarlet rosemallow. The objective of these experiments was to identify...
the type of gene action and number of loci controlling flower color in these populations of scarlet rosemallow.

Materials and Methods

Plants used in these experiments were part of a population maintained for breeding and genetic studies at the University of Florida Center for Aquatic and Invasive Plants in Gainesville. Parent, \( S_1 \), and \( F_1 \) populations were grown in 4-l nursery containers, whereas \( F_2 \), \( S_2 \), and \( BC_1 \) populations were grown in 1-l nursery containers. All containers were filled with a commercial potting mix amended with 10 or 5 g (for 4-l and 1-l containers, respectively) of controlled-release fertilizer per container. Plants were maintained in pollinator-free greenhouses under ambient temperature conditions, with supplemental lighting used to artificially increase day length to 16 h. Young plants were irrigated using an overhead mist system and were later transferred to a subirrigation regime to ensure that plants received adequate water. Plants were supported with bamboo stakes as needed throughout the growing period.

Three white-flowered parents (coded W1, W2, and W3) and 1 red-flowered plant (coded R) were utilized in these experiments. All white-flowered parents were started from seeds purchased from a hobbyist gardener in Texas via the Internet and are presumably derived from the same population. The red-flowered parent was a seedling from a plant originally collected from a native population of scarlet rosemallows at Lake George in central Florida and maintained as part of an aquatic ornamentals collection in Gainesville. Given the geographic separation of the sources of the white-flowered parents and the red-flowered parent, it is unlikely that the color morphs used as parents in these experiments share close common ancestors. Each parent was self-pollinated to create the \( S_1 \) families W1 \( \emptyset \), W2 \( \emptyset \), W3 \( \emptyset \), and R \( \emptyset \). Cross- and reciprocal pollinations were performed between parents to create the \( F_1 \) families W1 \( \times \) R, W2 \( \times \) R, and W3 \( \times \) R. Subsamples were selected from each \( S_1 \) and \( F_1 \) family and were self-pollinated to produce \( S_2 \) and \( F_2 \) families, respectively. In addition, subsamples of \( F_1 \) families were backcrossed to white-flowered parents to generate \( BC_1 \) families. Self, cross-, and reciprocal pollinations to create \( S_1 \) and \( F_1 \) populations were performed between June and September 2008, whereas self-pollinations of \( S_1 \) and \( F_1 \) plants (to create \( S_2 \) and \( F_2 \) populations, respectively) and backcrosses (to create \( BC_1 \) populations) took place between August and November 2009.

Flowers of scarlet rosemallow remain open and stigmas are receptive for a single day, so pollinations were performed as soon as flowers were fully expanded (usually between 7:30 and 9:30 am). Self-pollination of parents to create \( S_1 \) families revealed that plants were highly self-fertile, so seed parents in \( F_1 \) crosses were emasculated prior to pollen transfer to avoid self-pollination. Parental information and date of pollination were recorded on a jewelry tag, which was then hung gently on the pedicel of the pollinated flower. Capsule formation was monitored throughout the maturation process and ripe capsules were collected 30–40 days after pollination. Seeds were manually removed from each capsule and placed in a labeled manila coin envelope until seed set was completed in all pollinations. All seeds in each envelope were sown on the surface of a common container filled with a commercial potting mix and placed under mist irrigation (irrigation events every 2 h from 8 AM to 8 PM; duration of each event 3 min) in a greenhouse with temperature maintained between 25 and 30 °C. Germinated seeds were transplanted into individual labeled cells in 612 cell flats and maintained under mist until seedlings were approximately 15 cm tall. Seedlings were then transplanted into 4-l (\( S_1 \) and \( F_1 \) populations) or 1-l containers (\( S_2 \), \( F_2 \), and \( BC_1 \) populations), subirrigated and moved to the greenhouses described above.

All plants were grown to reproductive maturity and scored for flower color. No maternal effects were noted, so data for each family were pooled within each cross/reciprocal set. Data from \( F_1 \) and \( S_1 \) families were used to develop a proposed model to explain the type of gene action and number of loci controlling flower color in scarlet rosemallow. This model was used to assign likely genotypes to parents used in these experiments and the model was then verified by analyses of \( F_2 \) and \( BC_1 \) populations. All data were analyzed using goodness-of-fit (chi-square or \( \chi^2 \) tests (Steel et al. 1997). A test for heterogeneity of data collected from \( F_2 \) families from different crosses was employed to determine whether it was appropriate to pool data for all \( F_2 \) progeny; the same test was applied to data from \( BC_1 \) families.

Results and Discussion

All \( S_1 \) and \( S_2 \) progeny in the families W1 \( \emptyset \), W2 \( \emptyset \), and W3 \( \emptyset \) bore white flowers, whereas all \( S_1 \) and \( S_2 \) progeny from the family R \( \emptyset \) bore red flowers. In addition, all progeny in the 3 \( F_1 \) families bore red flowers. The simplest model that explains the progeny types recovered in these \( S_1 \) and \( F_1 \) families is one with a single diallelic locus, with red flower color completely dominant to white. Genotypes were assigned to all 4 parents using the proposed model and segregation of \( S_1 \) and \( F_1 \) progeny; all white-flowered parents were homozygous recessive (\( ww \)) and the red-flowered parent was homozygous dominant (\( WW \)).

Sixteen \( F_1 \) plants (3, 5, and 8 \( F_1 \) plants from the families W1 \( \times \) R, W2 \( \times \) R, and W3 \( \times \) R, respectively) were self-pollinated to produce 16 \( F_2 \) families. Each \( F_2 \) family produced progeny that segregated in a manner that was not significantly different from a 3 red:1 white ratio (data not shown). Heterogeneity chi-square analysis showed that all \( F_2 \) progeny within each cross/reciprocal set were from populations with identical genotypic constitutions (data not shown); therefore, data for \( F_2 \) progeny within each cross/reciprocal set were pooled. These pooled progeny segregated in a manner that was not significantly different from the expected 3 red:1 white ratio (Table 1). Heterogeneity chi-square analysis was performed on progeny from all
Table 1 Classification of plants of scarlet rosemallow in the F1, F2, and BC generations for red or white flower color

<table>
<thead>
<tr>
<th>Parents</th>
<th>Generation</th>
<th>Genotype</th>
<th>Number of plants observed</th>
<th>Expected ratio</th>
<th>Number of plants expected</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1 $\times$ R</td>
<td>$F_1$</td>
<td>$ww \times WW$</td>
<td>11</td>
<td>3</td>
<td>84.00</td>
<td>0.76</td>
<td>0.38</td>
</tr>
<tr>
<td>W1 $\times$ R</td>
<td>$F_2$</td>
<td>$Ww \otimes$</td>
<td>88</td>
<td>3</td>
<td>177.00</td>
<td>0.20</td>
<td>0.65</td>
</tr>
<tr>
<td>W1 $\times$ R</td>
<td>BC1</td>
<td>$ww \times Ww$</td>
<td>44</td>
<td>1</td>
<td>194.25</td>
<td>1.40</td>
<td>0.24</td>
</tr>
<tr>
<td>W2 $\times$ R</td>
<td>$F_1$</td>
<td>$ww \times WW$</td>
<td>40</td>
<td>3</td>
<td>38.0</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>W2 $\times$ R</td>
<td>$F_2$</td>
<td>$Ww \otimes$</td>
<td>180</td>
<td>3</td>
<td>194.25</td>
<td>1.40</td>
<td>0.24</td>
</tr>
<tr>
<td>W3 $\times$ R</td>
<td>BC1</td>
<td>$ww \times Ww$</td>
<td>49</td>
<td>1</td>
<td>38.0</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>W3 $\times$ R</td>
<td>$F_2$</td>
<td>$Ww \otimes$</td>
<td>186</td>
<td>3</td>
<td>194.25</td>
<td>1.40</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Cross- and reciprocal pollinations are pooled within each parental set and are listed by the cross (e.g., observations attributed to W1 $\times$ R include data from W1 $\times$ R and from R $\times$ W1).

$F_2$ families and the test was not significant; therefore, data for all segregating $F_2$ progeny from all $F_1$ families were pooled and subjected to chi-square analysis. These pooled progeny segregated in a manner that was not significantly different from the expected 3 red:1 white ratio ($P = 0.31$).

Thirteen $F_1$ plants (2, 2, and 9 $F_1$ plants from the families W1 $\times$ R, W2 $\times$ R, and W3 $\times$ R, respectively) were backcrossed to white-flowered parents to produce 13 BC1 families. Each BC1 family produced progeny that segregated in a manner that was not significantly different from a 1 red:1 white ratio (data not shown). Heterogeneity chi-square analysis showed that all BC1 progeny within each backcross/reciprocal set were from populations with identical genotypic constitutions (data not shown); therefore, data for BC1 progeny within each backcross/reciprocal set were pooled. These pooled progeny segregated in a manner that was not significantly different from the expected 1 red:1 white ratio (Table 1). Heterogeneity chi-square analysis was performed on progeny from all BC1 families and the result was not significant; therefore, data for all segregating BC1 progeny were pooled and subjected to chi-square analysis. These pooled progeny segregated in a manner that was not significantly different from the expected 1 red:1 white ratio ($P = 0.36$).

Conclusion

The results of these experiments suggest that flower color in these populations of scarlet rosemallow is controlled by 2 alleles at a single locus. Gene action is completely dominant, with white flower color recessive to red. All progeny in these experiments segregated as expected when tested against this model. Self-pollination of white-flowered plants (genotype $ww$) produced exclusively white-flowered progeny, whereas self-pollination of red-flowered plants resulted in progeny that only bore red flowers (parent genotype $WW$; source of $S_1$ and $S_2$ populations) or progeny with red and white flowers in a 3:1 ratio (parent genotype $Ww$; source of $F_2$ populations). I propose that this locus controlling flower color in scarlet rosemallow be named "white flower" with alleles $W$ and $w$.

The mechanism responsible for this novel flower color is unknown and beyond the scope of this paper. However, it seems likely that white flowers in scarlet rosemallow are the result of a loss-of-function mutation affecting the anthocyanin pathway such as that described by Rauscher (2008). Red flower color in wild-type scarlet rosemallow results from a combination of the flavonoid aglycones quercetin and cyanidin in an approximate 1:2 ratio (Puckhaber et al. 2002). Quercetin and cyanidin represent alternate pathways that branch from DH-quercitin, an intermediate substrate that is converted to one of these two aglycones (Figure 1).

DH-quercitin is converted to quercetin by the enzyme flavonol synthase, whereas the substrate becomes cyanidin in a 2-step process mediated by the enzymes dihydroflavonol 4-reductase and anthocyanidin synthase (Rauscher 2008). Although I did not investigate the biochemical composition of floral pigments in this study, Puckhaber et al. (2002) found that levels of quercetin were highest in white-flowered species of Hibiscus.

Figure 1. Simplified diagram of the portion of the anthocyanin pathway that is likely responsible for flower color in scarlet rosemallow. The precursor DH-quercitin is converted to either quercetin or cyanidin via opposing pathways.
Most transitions from pigmented to white flowers are the result of loss-of-function mutations, which can arise in a variety of ways. For example, mutations in regulatory regions may damage or render inoperable transcription factors and other elements that regulate and control gene function. Coding regions may be disrupted through insertions, deletions, or point mutations, which cause frameshift mutations and alter the reading frame. The end result of mutations that affect the components of the anthocyanin pathway is loss of function in a branch of the pathway. In scarlet rosemallow, it seems likely that the branch responsible for conversion of the intermediate substrate DH-quecitin to cyanidin is inoperable due to a mutation in the gene coding for the enzyme dihydroflavonol 4-reductase. This renders the branch leading to cyanidin synthesis nonfunctional and excess DH-quecitin is diverted to the alternate branch that produces quercetin instead.

Scarlet rosemallow is a desirable native perennial plant that has great utility in wetland areas and in home gardens and landscapes. The species has a number of attractive characteristics that appeal to those searching for an attractive, unusual, and woody perennial plant to include in vegetation plans. Although red-flowered specimens of scarlet rosemallow can be locally common, white-flowered plants are not always accessible to growers who wish to offer this color variant of the species. This research revealed that white flowers are the result of a recessive condition at the white flower locus, so white-flowered plants of scarlet rosemallow are inherently true breeding for flower color. Nurseries and growers of native, ornamental, and wetland plants can exploit this information to produce white-flowered plants for their customers simply by maintaining white-flowered plants that can be used as parents in seed production.

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References

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