

## Effects of Inbreeding and Out-crossing on Measures of Fitness In an Isolated Population of *Cornus florida*

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### ABSTRACT

Plant populations that are small and isolated may become inbred as a result of self-fertilization or breeding with genetic relatives. The result of such inbreeding is a reduction in fitness (i.e., inbreeding depression). Out-crossing is an effective way to reduce inbreeding depression in small, isolated populations. I investigated potential benefits of out-crossing for a remnant, natural population of *Cornus florida* at Hartwick College. The population is currently small ( $\approx 100$  trees) and shows little recruitment, characteristics of populations exhibiting inbreeding. To study the effects of out-crossing, I pollinated dogwood inflorescences on campus with pollen from multiple donors growing (1) within the population ('inbred'), (2) in neighborhoods in Oneonta ('out-crossed'), or (3) in both populations ('mixed'). I measured the effect of each treatment on measures of fitness (e.g., seed weight and germination). Inflorescences from the inbred treatment produced more fruit than inflorescences in the out-crossed, mixed, and control treatments. In contrast, there was no difference among treatments for fruit or seed weights, percent germination, or growth rates of seedlings. Higher measures of fitness in the inbred treatment suggest incompatibility between the campus population and the horticultural trees used for out-crossing. Inbreeding may not be occurring in the campus population; instead, the lack of recruitment may result from low levels of pollination or lack of seed dispersal.

### INTRODUCTION

In most natural populations, genetic variation among individuals is considerable (Keller and Waller, 2002). Often, the exchange of genes between populations allows for the retention of heterozygosity within the population. If a population decreases in size and becomes isolated from other natural populations, genetic diversity within the population may decrease because of inbreeding between genetic relatives (Paschke et al., 2002). Inbreeding causes both a loss of heterozygosity and an increase in the rate of expression of deleterious recessive alleles, which can lead to declines in the average fitness of the population (Keller and Waller, 2002; Paschke et al., 2002).

In small plant populations, inbreeding causes reductions in measures of fitness such as seed set, germination, seedling survival, and resistance to stress (Keller and Waller, 2002). For example, Fischer and Mathies (1998) found fewer seeds per fruit and per plant, and more rapid population declines as the population size of *Gentianella germanica* decreased. In populations of less than 100 flowering plants, Heschel and Paige (1995) found a reduction in seed size, germination success, and an overall resistance to stress in *Ipomopsis aggregata*.

Inbreeding may also be reflected in stage-specific gene expression associated with seedling growth and development (Morgan, 2001). As inbreeding increases, genetic variability decreases which allows for an increase in the rate at which deleterious mutations become fixed in the population due to genetic drift (Keller and Waller, 2002).

In populations of less than 100 individuals, “mutational meltdown” has been shown to occur due to the decrease in genetic variability (Keller and Waller, 2002).

If inbreeding depression is occurring in a plant population, one way to increase the fitness of individuals and the persistence of the population is to promote out-crossing (Paschke et al., 2002). Pollen from another population may restore heterozygosity and increase genetic variation. Hauser and Siegismund (2000) found that *Silene nutans* zygotes from out-crossed pollinations survived better and were more developed than zygotes from inbred pollinations. Also, Herschel and Paige (1995) found that seed mass and percent germination was higher in a small population of scarlet gilia when it was out-crossed than when it was inbred.

Flowering dogwood (*Cornus florida*) is a highly self-incompatible (Reed, 2004), understory tree that is found in well-drained soil from coastal northern Massachusetts to the Gulf of Mexico. Although flowering dogwood is common in southeastern New York, it is uncommon elsewhere in the state, except for regions with warm microclimates such as the Finger Lakes and the southern coast of Lake Ontario (Boyd and Pitzel, 1991; Rossel et al., 2001). Each dogwood inflorescence can contain up to 30 perfect flowers surrounded by four large white bracts, but on average only 1–5 drupes typically mature (Carr and Banas, 2000). Despite its self-incompatibility, *C. florida* may still be able to mate with relatives (Reed, 2004), which can result in inbreeding depression.

Recently, flowering dogwoods have come under the threat of an anthracnose disease *Discula destructiva*. This fungal pathogen causes a severe disease with high mortality rates of adult trees (Carr and Banas, 2000). Santamour et al. (1989) showed that anthracnose can also be detrimental to seedlings; 73.3% of seedlings died after two growing seasons when exposed to dogwood anthracnose. Dogwood anthracnose is mainly characterized by purple-brown spotting on leaves, leaf necrosis, branch dieback, and ultimately, death to the host tree (Brown et al., 1994; Carr and Banas, 2000; Rossel et al., 2001; Santamour et al., 1989). Dogwood anthracnose might further reduce fitness of small populations of flowering dogwood.

I studied a small, isolated, natural population of flowering dogwood growing in ‘natural’ areas on the Hartwick College campus. There are approximately 100 mature trees, but only about 80 individuals produced substantial floral displays and seed sets in 2003 (pers. obs.). In addition, there is little recruitment in the population, although one edge of the population contains many immature trees and seedlings (pers. obs.). The overall poor recruitment in the population may result from the effects of inbreeding on seed development, germination, or seedling growth. Poor recruitment could also result from low fertilization rates if self-incompatibility causes abortion of pollen tubes from pollen donated by close relatives. Assuming that inbreeding has prevented recruitment in this population of flowering dogwood, I propose to test the hypothesis that out-crossing with pollen from local horticulture trees will increase measures of fitness in this small, natural population of flowering dogwood.

## MATERIALS AND METHODS

### Study Population

I studied the effects of inbreeding and out-crossing on an isolated, natural population of flowering dogwood (*Cornus florida*) on the Hartwick College campus in Oneonta, Otsego

County, New York. This natural population of approximately 100 adult trees is growing on a rocky, south-facing slope in the northern portion of *C. florida*'s natural range, approximately 1–5 km from the nearest cultivated dogwoods in Oneonta. The nearest natural population is unknown, but may be as far as 70 km away.

### Experimental Design

I pollinated flowers from the isolated population on campus (hereafter, 'wild' population) with pollen collected within the 'wild' population or with pollen from cultivated trees found within the city of Oneonta (hereafter, 'cultivar' population). The cultivar population consists of 8 mature trees of unknown cultivars of *Cornus florida*. I conducted pollinations during the first week of June 2004.

I randomly assigned 3 inflorescences (within reach of a 2.5 m step ladder) on 15 'wild' trees to 5 different pollination treatments (15 inflorescences per tree x 15 trees = 225 total inflorescences). The treatments are summarized in Table 1. For non-control treatments, I randomly selected and completely removed inflorescences with mature flowers (open anthers) from pollen donors, and haphazardly assigned each inflorescence to a receiving tree in the wild population. Donor inflorescences were stored in plastic bags during transport. Pollinations were completed within 12 hours of collecting pollen to maintain pollen viability, and thus achieve the highest rate of fertilization (Reed et al., 1996). Anthers of donor inflorescences were rubbed over the open stigma of receiving inflorescences. To increase rate of successful pollination, I pollinated flowers twice approximately 6 days apart, with first pollinations approximately 6 days after inflorescence opening. I used this pollination scheme because it is an efficient means of pollinating flowering dogwood (Reed 1999). Different donor trees were used for the first and second pollinations to minimize individual tree effects, especially for the out-crossing treatments in which pollen could be from different cultivars.

I attached waxed-paper pollination bags to each hand-pollinated inflorescence with twist-ties to ensure that no other pollen reached the stigmas of experimental inflorescences. I attached pollination bags prior to maturity of inflorescences and removed them after flowers were no longer receptive.

I included two control treatments in the experiment: an 'open' control in which I used no pollination bags and did not hand pollinate inflorescences, and a 'closed' control in which I placed pollination bags over inflorescences but did not hand pollinate the inflorescences. The 'open' control was used to determine the effects of natural pollination on seed production and fitness. The 'closed' control was used to verify that pollinations within the other treatments were due to my hand-pollinations and not a result of self-fertilization within the pollination bag or transfer of unwanted pollen during application of the treatments.

On September 18, 2004, I collected mature fruits (showing at least some red color) from each treated inflorescence, and recorded fruit and seed number and mass. After 10 days, I removed all pulp from the seeds, and stratified them in moistened sphagnum moss at 4°C for 140 days. Once stratification was complete, I planted seeds in MetroMix 560 Scotts Coir® Growing Medium, and allowed them to germinate at 25°C in 16:8 photoperiod. I recorded percent germination per treatment 21 days post-planting and I recorded shoot length to the nearest mm on days 10 and 21 post germination.

## Statistical Methods

To determine if there was a difference in measures of fitness (fruit size, seed size) among treatments, I performed ANOVA tests in SPSS. I tested for the effect of tree, treatment, and tree\*treatment interaction. Because inflorescences on a tree were not independent, I used the mean value for the three replicates in each treatment for ANOVA analyses. I used a 2 x 4 chi-square contingency test to determine if percent germination was independent of treatment. I excluded the closed control treatment from statistical analyses because only one fruit was produced in this treatment (indicating my success in preventing unwanted pollinations).

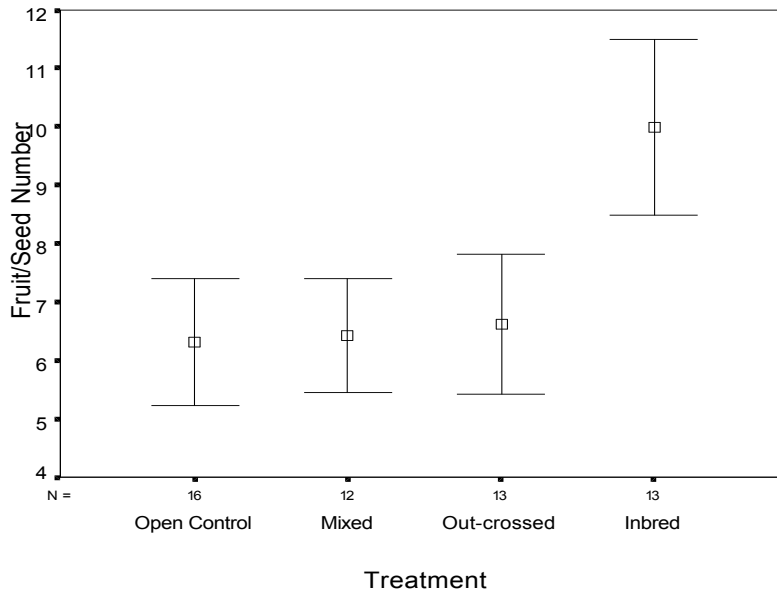
**Table 1.** Pollination treatments used to determine the effects of an outside source of pollen on the fitness of an isolated population of *Cornus florida* in Otsego County, NY. ‘Wild’ refers to the natural population at Hartwick College, and ‘Cultivar’ refers to the horticulture trees used as pollen donors from the Oneonta area.

Treatment	No. and source of pollen donor	Pollination Bags Used?
‘Open’ control	No Donor – Natural Pollination	No
‘Closed’ control	No Donor – No Pollination	Yes
‘Inbred’	2 ‘Wild’ Donors	Yes
‘Out-Crossed’	2 ‘Cultivar’ Donors	Yes
‘Mixed’	1 ‘Wild’ + 1 ‘Cultivar’ Donors	Yes

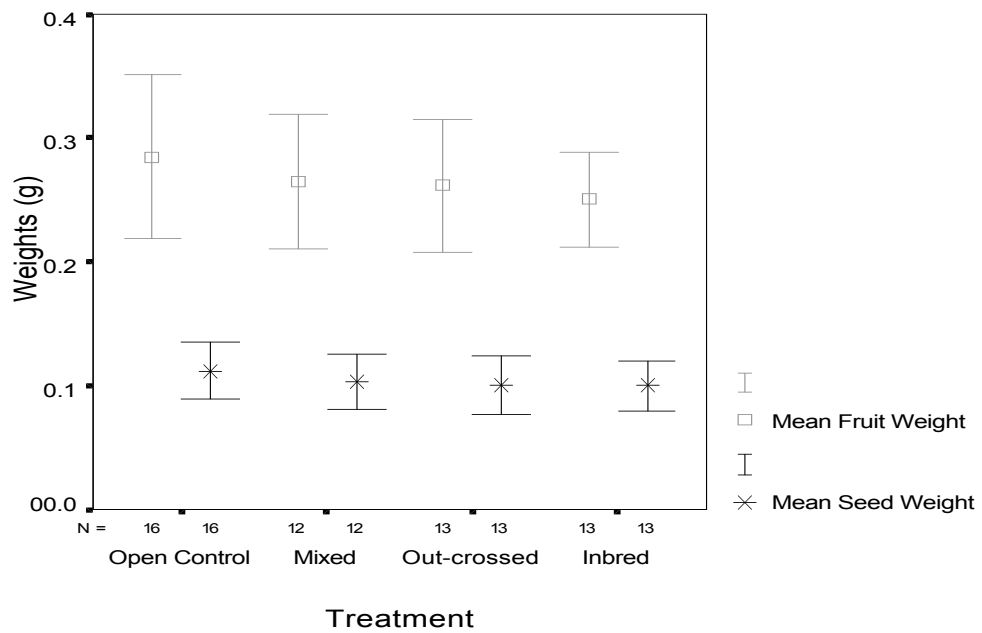
## RESULTS

Overall, 55.6% of the ‘inbred’, ‘out-crossed’, and ‘mixed’ inflorescences produced fruit. In total, including the ‘open’ control, 388 fruits were produced, and 214 (55.1%) of the seeds germinated.

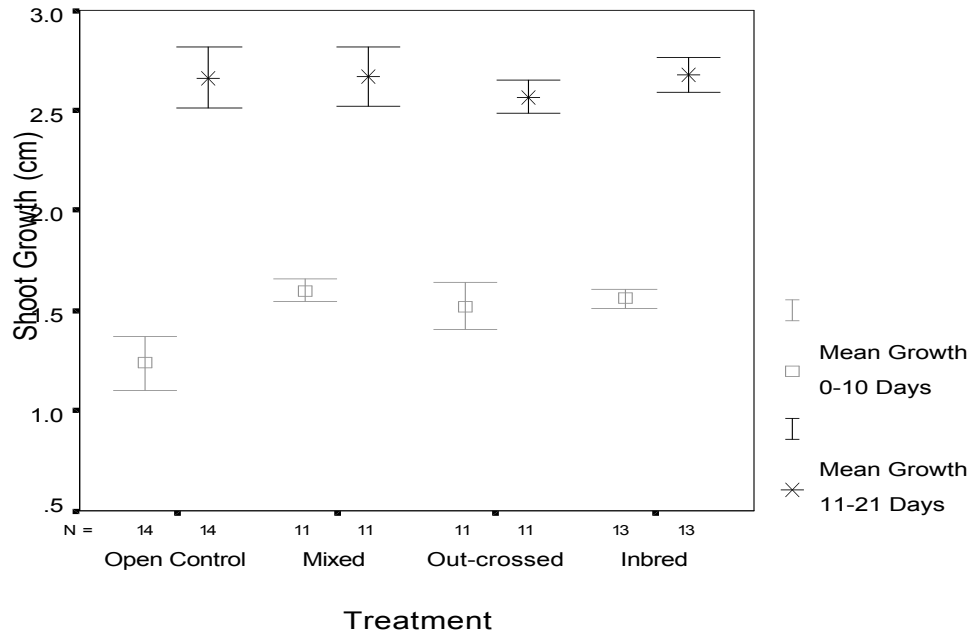
The only measure of fitness with significant treatment effects was the number of fruit (or seeds) per treatment (ANOVA:  $F=3.947$ ,  $df=3$ ,  $P=0.016$ ; Fig. 1). Based on a Tukey HSD *post-hoc* test, the inbred treatment produced significantly more fruit than all other treatments ( $P<0.06$ ). There was no significance difference between treatments for mean fruit weight (ANOVA  $F=0.378$ ,  $df=3$ ,  $P=0.769$ ; Fig. 2), mean seed weight (ANOVA  $F=1.116$ ,  $df=3$ ,  $P=0.356$ ; Fig. 2), mean growth between 0-10 days (ANOVA  $F=2.563$ ,  $df=3$ ,  $P=0.073$ ; Fig. 3), or mean growth between 11-21 days (ANOVA  $F=0.862$ ,  $df=3$ ,  $P=0.471$ , Fig. 3). In addition, the number of seeds that germinated was independent of treatment ( $P=0.407$ ,  $\chi^2=3$ ; Fig. 4).



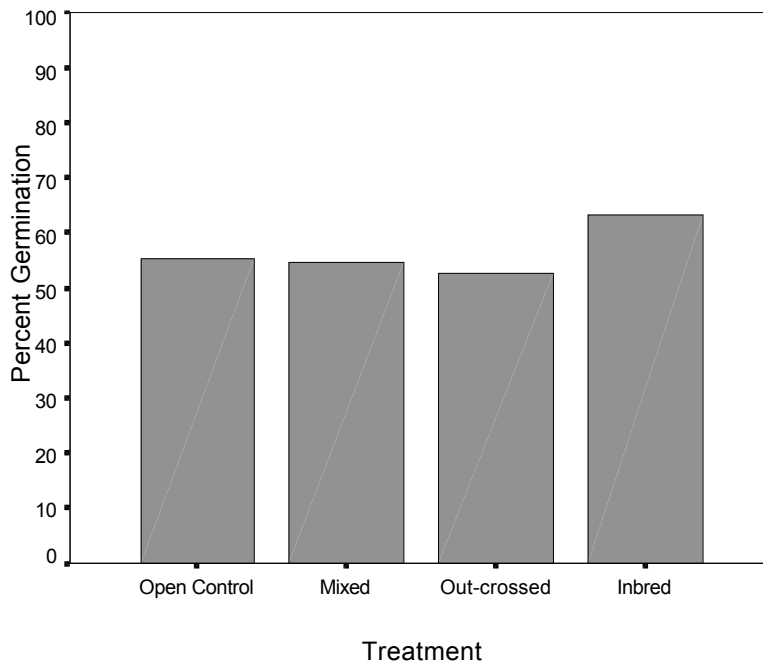
**Figure 1.** Mean fruit or seed number ( $\pm$  1 standard deviation) for *C. florida* exposed to different pollination treatments at Hartwick College, Oneonta, New York. See table 1 for a description of the pollination treatments.



**Figure 2.** Mean fruit and seed weights in grams ( $\pm$  1 standard deviation) per pollination treatment for *C. florida* trees at Hartwick College, Oneonta, NY. See table 1 for a description of the pollination treatments.



**Figure 3.** Mean shoot growth in centimeters (+/- 1 standard deviation) for days 0-10 and 11-21 post germination for *C. florida* seeds produced at Hartwick College, Oneonta, NY. See table 1 for a description of the pollination treatments.



**Figure 4.** Mean percent germination after 21 days post-planting for *C. florida* seeds produced at Hartwick College, Oneonta, NY. See table 1 for a description of the pollination treatments.

## DISCUSSION

Out-crossing was not an effective way to increase measures of fitness in an isolated population of flowering dogwood. If out-crossing were effective, the inflorescences in the out-crossing treatment would have produced fruit with significantly higher measures of fitness than inflorescences in the other treatments, especially the inbred treatment. Instead, the inflorescences in the inbred treatment produced significantly more fruit than those in all other treatments (Fig. 1). Measures of fitness related to seedling growth, in contrast, were not different among treatments.

Inflorescences in the inbred treatment produced more fruit and seeds than inflorescences of other treatments because of either a difference in pollen load between treatments, or a genetic incompatibility between the cultivar donors and the wild population. The pollen load for each hand-pollination treatments was probably greater than that which is naturally occurring, and as a result, I observed fewer fruit produced in the open control than in the inbred treatment. The higher fruit production in the inbred treatment than in the out-crossed and mixed treatments, however, cannot be explained by differences in pollen load. Instead, the increased fruit production in the inbred treatment appears to be caused by genetic incompatibility or outbreeding depression between the natural trees and the cultivated trees (Paschke et al., 2002). For example, Hauser and Siegismund (2000) suggest that maternal plants may stop fertilization or seed development because of some incompatibility with pollen from sources outside a population. Indeed, Fisher and Matthies (1997) observed outbreeding depression in *Gentianella germanica*; inter-population pollinations resulted in lower germination rates and lower plant size than intra-population pollinations. I believe that out-crossing with another natural population may increase fitness in the remnant, natural population of flowering dogwood at Hartwick College, but using pollen from horticultural trees is not effective because of outbreeding depression. Unfortunately, I could not use trees from a natural population of *C. florida* as pollen donors because they were too far away to collect pollen and perform pollinations on the same day, a restriction caused by pollen viability (Reed et al., 1996).

Because 'inbred' pollinations successfully produced seedlings, I believe the observed lack of recruitment observed in this isolated population is due to a lack of seed dispersal by birds. Flowering dogwood requires birds or other animals to eat the fruit and then regurgitate or excrete the seeds, thusly removing the pulp and stimulating germination (Rossell et al., 2001). Alternatively, a reduction in pollination could account for the lack of recruitment. *C. florida* is pollinated by a wide range of insects, but is mainly pollinated by bees in the Andrenidae and Halictidae families (Mayor et al., 1999). Inflorescences in my hand-pollinated 'within' treatment produced more fruit than inflorescences pollinated naturally (i.e., inflorescences in the 'open' control). Thus, fewer fruit are produced naturally than is possible. Either a sparse pollinator community or a lack of particularly effective pollinators could cause the reduction in fruit production in the 'open' controls. Production of fewer fruit can reduce recruitment directly (i.e., fewer total seeds available to produce seedlings). In addition, the production of fewer fruit can reduce recruitment indirectly; if fewer pollinations result in fewer fruit, then birds may not be attracted to the area to disperse the seeds. Carr and Banas (2000) state that on average, 1-5 drupes are produced per inflorescence. On average, there were 3.2 fruits per inflorescence in my 'open' control

treatment and 4.6 fruits per inflorescence in my 'inbred' treatment, within the range of fruits typically produced by flowering dogwoods.

In order to determine if the trees within in this isolated flowering dogwood population are genetically related, molecular techniques such as DNA fingerprinting should be done. Given the genetic incompatibility observed between cultivar donors and natural trees, I suggest repeating this experiment using another natural population to verify genetic incompatibility between the horticultural and natural trees observed in this study and to exclude the possibility of geographic outbreeding depression due to local adaptations even among natural populations. Because lack of recruitment in this natural population is important to the long-term persistence of this isolated flowering dogwood population, I suggest that further research be conducted on type and abundance of pollinators and fruit dispersers. Lastly, this population of flowering dogwood is showing symptoms of dogwood anthracnose and should be tested for the fungal pathogen. Anthracnose has been shown to decrease the probability of flowering as severity of the disease increases (Rossell et al. 2001). If this isolated population is infected, regrettably, the probability of increasing recruitment and maintaining the population into the distant future is low.

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