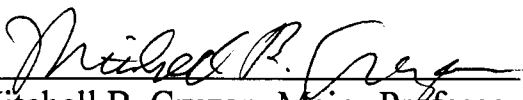


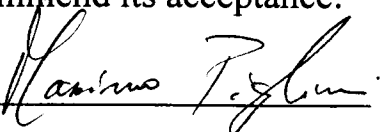
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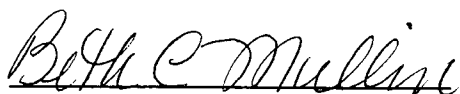
I am submitting herewith a thesis written by Jiongqian Wang entitled "Reproductive Interactions Between Hybridizing Species of *Piriqueta*." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.



Mitchell B. Cruzan, Major Professor

We have read this thesis and
recommend its acceptance:





Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

Reproductive Interactions Between Hybridizing Species of *Piriqueta*

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Jiongqian Wang

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Abstract

Post-pollination mechanisms can play an important role in limiting or promoting natural hybridization in plants. I used controlled hand pollinations with mixtures of genetically marked pollen to study these mechanisms in two species of *Piriqueta* that hybridize in central Florida: *Piriqueta caroliniana* and *P. viridis*. More F₁ hybrids were produced by *P. viridis* plants than by *P. caroliniana*. Relative pollen-tube growth rates were significantly different between the two species, with *P. caroliniana* tubes outperforming *P. viridis* tubes in both intra-specific and inter-specific styles. Although pollen competition may not limit the formation of hybrids in *P. viridis*, pre-fertilization gamete attrition apparently acts against the formation of hybrids. Pollen competition acts as an inter-specific insulating mechanism in *P. caroliniana*, but there may also be selective abortion of intra-specific ovules after fertilization in this species. Experimental crosses show that there is asymmetric hybridization in *Piriqueta*, with *P. caroliniana* being more successful in siring hybrid seeds and a more selective mother than *P. viridis*. Backcross seeds were formed at higher frequencies in *P. viridis* than in *P. caroliniana* indicating introgression is likely to occur in the

direction of *P. viridis* populations after initial F_1 establishment. Further work is needed to compare the results predicted from mating interactions to field experiments.

Table of Contents

Chapter	Page
I. Introduction	1
II. Materials and Methods	4
Pollen Viability.....	5
Pollen Production	7
Pollinations	8
Starch Gel Electrophoresis	10
Experiments	11
III. Results	17
Pollen Viability	17
Pollen Production.....	17
Pollen Replacement Series	18
Pollen Dosage	22
Patterns of Fecundity	25
Advanced-Generation Hybrid Formation	27
IV. Discussion	46
F ₁ Hybrid Formation.....	46
Advanced Generation Hybrid Formation.....	52
V. Conclusion	55
References	59
Vita	70

List of Tables

Table	Page
1. The number of pollen grains per anther for <i>P. caroliniana</i> , <i>P. viridis</i> and F_1 and the adjustment of anther ratio for the 50:50 pollen mixture.....	9
2. Analysis of variance in pollen viability in <i>P. caroliniana</i> , <i>P. viridis</i> and F_1 plants.....	18
3. Analysis of variance in pollen production per anther of <i>P. caroliniana</i> , <i>P. viridis</i> and F_1 plants.....	19
4. The results of pollen replacement experiment. The observed and expected (in parenthesis) number of seeds are given for each pollination treatment.....	20
5. Analysis of variance in the deviation of the frequency of intra-specific seeds from the expected (F'_{intra}) for <i>Piriqueta viridis</i> ..	21
6. Analysis of variance in the deviation of the frequency of the intra-specific seeds from the expected (F'_{intra}) for <i>Piriqueta caroliniana</i>	23
7. Regression analysis of pollen load size on fertilization success of five pollen mixtures on <i>P. caroliniana</i> and <i>P. viridis</i>	24
8. Summary of the fertilization results from the experiments of formation of advanced-generation hybrids.....	28
9. Regression analysis of pollen load size on fertilization success of <i>P. caroliniana</i> in the experiment of advanced-generation hybrid formation.....	32
10. Analysis of the seed set data in the experiment of advanced-generation hybrid formation.....	34

11. Regression analysis of pollen load size on fertilization success of <i>P. viridis</i> in the experiment of advanced- generation hybrid formation.....	40
12. Regression analysis of the pollen load size on fertilization success of F_1 in the experiment of advanced-generation hybrid formation.....	42

List of Figures

Figure	Page
1. Natural habitats of <i>P. caroliniana</i> and <i>P. viridis</i>	6
2. The deviation of the frequency of intra-specific fertilization from the expected for <i>P. viridis</i> . F'_{intra} is the deviation of intra-specific fertilization from the expected frequency.....	20
3. The deviation of intra-specific fertilization from the expected frequency of <i>P. caroliniana</i> . F'_{intra} is the deviation of intra-specific fertilization from the expected frequency.....	22
4. Patterns of fecundity of <i>P. viridis</i> . G/O is the ratio of the number of pollen grain to the number of ovule, S/O is the number of seed to the number of ovule.....	26
5. Patterns of fecundity of <i>P. caroliniana</i> . G/O is the ratio of the number of pollen grain to the number of ovule, S/O is the number of seed to the number of ovule.....	26
6. Formation of parental genotypes, F1 hybrid, and advanced-generation hybrid seeds on <i>P. caroliniana</i>	29
7. Formation of parental genotypes, F1 hybrid, and advanced-generation hybrid seeds on <i>P. viridis</i>	30
8. Formation of parental genotypes, F1 hybrid, and advanced-generation hybrid seeds on F1 plants.....	31
9. Fertilization success of <i>P. caroliniana</i> in the experiments of advanced-generation hybrid formation.....	33
10. Fertilization success on <i>P. caroliniana</i> in the experiments of advanced-generation hybrid formation.....	35

11. Fertilization success of <i>P. viridis</i> in the experiments of advanced-generation hybrid formation.....	37
12. Fertilization success on <i>P. viridis</i> in the experiments of advanced-generation hybrid formation.....	39
13. Fertilization success of F_1 in the experiments of advanced-generation hybrid formation.....	43
14. Fertilization success on F_1 in the experiments of advanced-generation hybrid formation.....	45

Chapter I

Introduction

Many reviews and studies have implicated reticulations (natural hybridization between different evolutionary lineages) as an important mechanism in the evolution for a diverse set of plant and animal groups (Anderson, 1949; Stebbins, 1859, 1969; Grant, 1981; Arnold 1992; Rieseberg and Wendel 1993; Arnold 1994). The impact of hybridization on plant evolution apparently encompasses both contemporary and ancient plant populations (Arnold, 1994). An estimated 70% of all flowering plants may have resulted from reticulate events (Whitman et al. 1991). Numerous contemporary examples of gene transfer between species, subspecies and genera of plants have been detected (Knobloch 1972; reviewed in Arnold 1992, Rieseberg and Wendel, 1993). Because natural hybridization is phylogenetically and temporally so widespread (Stebbins 1959; Grant 1981; Ehrich and Wilson 1991; Whitman et al 1991; Masterson 1994) and may have a number of proposed consequences, it plays a major role in evolution of many plant taxa (Reiseberg and Wendal, 1993). Thus, to

define the mechanisms that control natural hybridization is to define a major factor in plant evolution (Arnold 1994). Central to the characterization of natural hybridization is an understanding of the processes of plant reproduction that may limit or promote inter-specific mating (Carney et al, 1994).

Many aspects of reproduction have potential influences on inter-specific hybridization including pre-pollination factors such as flowering time (Cruzan and Arnold 1994), pollinator behavior (Levin 1978) and post-pollination factors such as pollen/pistil interactions, pollen competition and ovule abortion (Carney et al. 1994). Although Charles Darwin recognized that reproductive processes may act as barriers to crosses between different species as early as in 1859, to date there have been few analyses testing for barriers to hybridization that exist between naturally hybridizing plant species (Smith 1970; Kiang and Hamrick 1978; Arnold et al. 1993; Carney et al. 1994; Rieseberg et al., 1995; Carney et al. 1996).

In this study I test for post-pollination reproductive mechanisms that affect inter-specific compatibility between two hybridizing taxa of the *Piriqueta* complex (Turneraceae), *Piriqueta caroliniana* Urban (= *P. caroliniana* var *tomentosa*) and *Piriqueta viridis* Small (Small 1933: = *P.*

caroliniana var *glabra*). Species of *Piriqueta* are perennial herbs that are found along the Coastal Plain from South Carolina to southern Florida. Plants of *Piriqueta* are distylous, with individuals either bearing flowers with long styles and short stamens (Long morph) or bearing flowers with short styles and long stamens (Short morph). Species of *Piriqueta* not only have interesting patterns of intra-specific mating and incompatibility (Ornduff and Perry 1964), but also have been found to be inter-compatible (Ornduff 1970). Observations indicate *Piriqueta caroliniana* and *P. viridis* hybridize and form a broad hybrid zone in central Florida (Ornduff, 1970; L. J. Martin and M. B. Cruzan, unpublished data).

Here I use mixtures of genetically-marked pollen to examine the effects of different proportions of inter-specific and intra-specific pollen on the siring of hybrid seeds in *P. caroliniana* and *P. viridis*. Specifically I infer the frequency of F_1 and advanced generation hybrid formation by examining the level of compatibility between parental and F_1 hybrid genotypes. The results from these experiments provide information on the effects of pre- and post-zygotic processes on patterns of hybridization and hybrid zone structure in the *Piriqueta caroliniana* complex.

Chapter II

Materials and Methods

Piriqueta taxa in North American are perennial herbs that have apricot-colored flowers. They flower from June to August in the field but year-round in greenhouses. These species can be easily distinguished based on both morphological and ecological characters. *P. caroliniana* has pubescent elliptic leaves and stems, and grows in deep quartz-sand soils in North Florida and South Georgia. *P. viridis* has glabrous linear leaves and stems, and grows in limestone-dominated soils of the Southern pine flatwoods of South Florida. Hybrids between these two species have intermediate morphologies and can be found in a range habitats throughout central Florida. While taxa of *Piriqueta* in North American have been grouped in several different ways (Long and Lakella 1971; Arbo 1995), I will use the classification scheme proposed by Small (1933).

Plants of *P. caroliniana* used in this experiment were grown from seeds collected from populations in Effingham, Long, Wayne, Lanier county, Georgia in 1992, and plants of *P. viridis* were grown from seeds

collected from Dade county, Florida in 1994 (Figure 1). F₁ plants were formed by making crosses between *P. caroliniana* and *P. viridis* plants in the greenhouse.

I used controlled pollinations with pollen mixtures to assess the siring ability of different pollen types. Crosses were conducted using plants that were homozygous for one of two PGI alleles. Siring ability of each pollen type was assessed by mixing pollen from plants that had alternative homozygous genotypes. Pollen mixtures were adjusted to account for the differences in pollen viability and average number of pollen grains per anther for *P. caroliniana*, *P. viridis* and F₁ plants. All the pollinations were performed in the greenhouse of the Department of Botany at the University of Tennessee.

Pollen Viability

Differences in pollen viability were examined by collecting four anthers from 4 randomly-chosen plants of each morph from each species

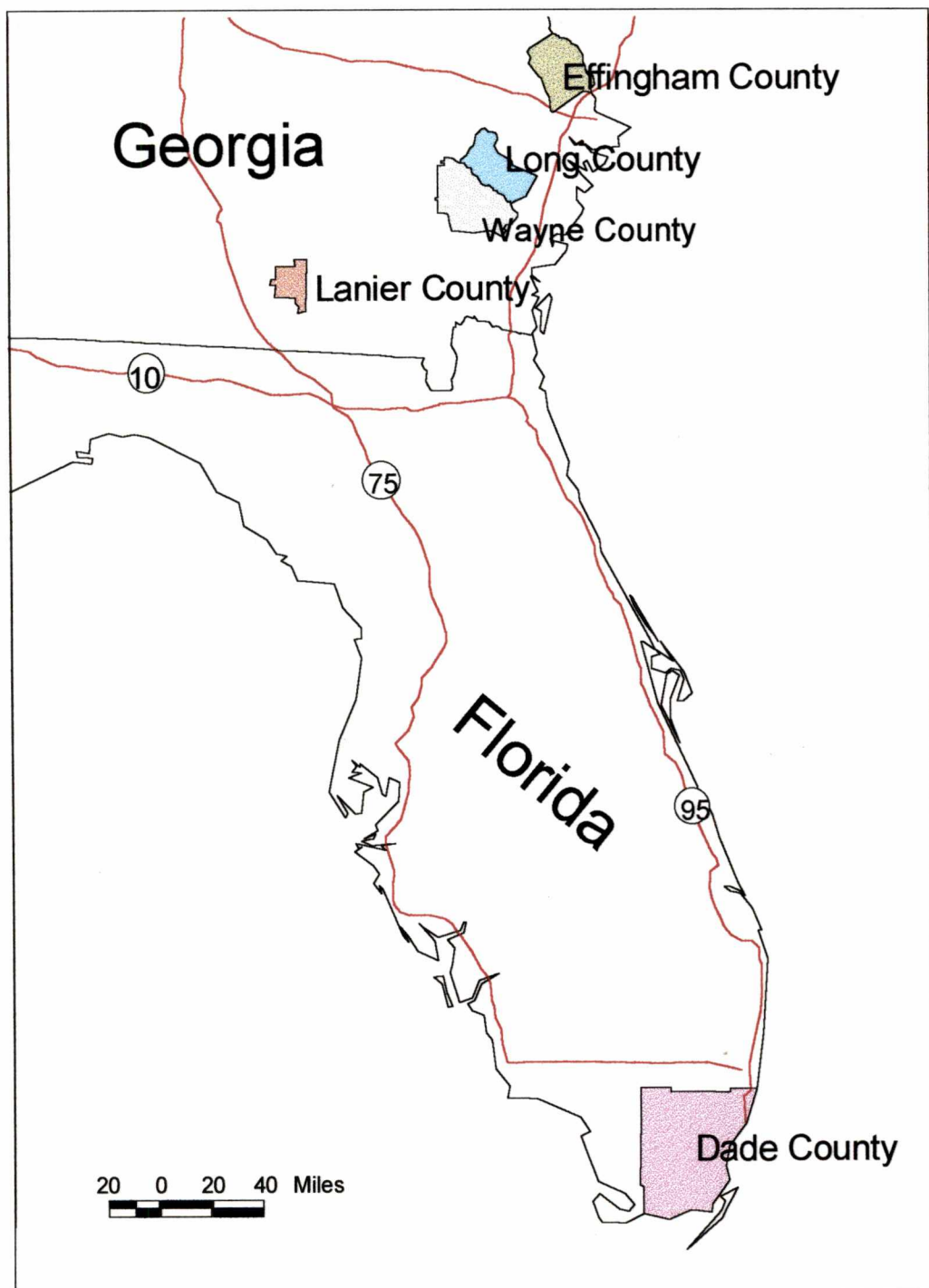


Figure 1. Natural habitats of *P. caroliniana* and *P. viridis*.

(*P. caroliniana*, *P. viridis* and F₁) shortly after dehiscence on five different days. Microcentrifuge tubes containing the collected anthers were kept in a plastic box with a moisturized paper towel on the bottom. Pollen viability was determined by a fluorochromatic procedure using fluorocene diacetate (Kearns and Inouye 1993). For each anther, the viability of 200 randomly chosen pollen grains was recorded and the percentages of viable and dead pollen were calculated. The data were analyzed for difference in pollen viability for *P. caroliniana*, *P. viridis* and F₁ plants with ANOVAs using the GLM procedure of SAS (SAS Institute, Inc. 1985). In these models, plant was entered as a random variable nested within species, which was entered as a fixed variable. I tested for differences among species using the variation among plants as the error term.

Pollen Production

Before making pollen mixtures the average number of grains contained in anthers of *P. caroliniana*, *P. viridis* and F₁ plants was determined. Twelve plants of each style morph were chosen from each species (*P. caroliniana*, *P. viridis* and F₁). One anther was collected upon dehiscence

from each plant and stored in 1 ml of distilled water in microcentrifuge tubes. The tubes were vortexed to disperse the pollen and the number of pollen grains in each of three subsamples of 10 μ l water was counted under a stereo microscope. Variation in pollen number among species was analyzed using the MEAN procedure from SAS. Differences in the average number of pollen grains produced by each species and the F₁ hybrids were found (Table 1) and adjustments were made when making mixtures of pollen from different genotypes.

Pollinations

Pollinations were performed in a randomized incomplete block design using days as the blocking factor and rotating treatments among days. Pollinations were made with a toothpick to mix pollen and apply it to stigmas. Pollen mixtures were made by collecting anthers from five or more plants from both floral morphs of each species and mixing it together. These mixtures were then used for pollinations by covering toothpicks with pollen and gently touching the stigmas of flowers. The

Table 1. The number of pollen grains per anther for *P. caroliniana*, *P. viridis* and F_1 and the adjustment of anther ratio for the 50:50 pollen mixture.

Style Morph	Species	Mean pollen number	S. D.	Pollen mixture	Anther ratio
Long	<i>P. caroliniana</i>	1733.3	343.8	<i>P. caroliniana</i> : <i>P. viridis</i>	21 : 17
	<i>P. viridis</i>	2138.9	389.8	<i>P. viridis</i> : F_1	26 : 21
	F_1	2600.0	484.5	F_1 : <i>P. caroliniana</i>	17 : 26
Short	<i>P. caroliniana</i>	1000.0	296.1	<i>P. caroliniana</i> : <i>P. viridis</i>	7 : 5
	<i>P. viridis</i>	1388.9	220.5	<i>P. viridis</i> : F_1	3 : 2
	F_1	2058.3	575.8	F_1 : <i>P. caroliniana</i>	1 : 2

styles were collected 6 hours after pollination and fixed in 70% ethanol. In *P. caroliniana*, *P. viridis* and F_1 styles, pollen tubes have already grown through the base of the styles and reached the ovules by 3 hours after pollination. Stigma removal after 6 hours has no apparent effect on fruit and seed set (unpublished data). Pollen grains were stained by adding 1 drop of 0.1% Malachite Green to the styles in ethanol. The number of pollen grains on the stigma was either counted under a stereo microscope or was estimated using video image analysis software (Optimas 5.2). For the latter method, the total area of the pollen grains on the stigma and the average area of a single pollen grain were estimated. The number of pollen

grains was then calculated as the total area divided by the average area of a single grain.

Fruits were collected when mature (19 days after pollination).

Harvested fruits were stored at room temperature for a week before the number of seeds and ovules were counted under a stereo microscope. Ten seeds from each fruit were randomly chosen and the genotype of the seeds were determined using starch gel electrophoresis.

Starch Gel Electrophoresis

Seeds were ground for 10 second in 10 μ l seed extraction buffer in 1.5 ml microcentrifuge tubes and 2mm * 10mm paper wicks were used to soak up the extract. Proteins were separated on 10%, 350 ml hydrolyzed potato starch (Sigma S-4501) gels in a Morpholine-citrate pH 6.8 buffer system for 6 hours at 50mA and 180V (Acquaah, 1992). The gel was then sliced and stained for PGI (Phosphoglucosomerase) for 30 minutes in the dark at 37 °C (stain mixture in Appendix). The gel was rinsed in water and the genotype of each seed was recorded. The paternity of seeds was inferred according to the genotypes of the pollen donors and recipients.

Experiments

1. Pollen Replacement Series. Hand pollinations were conducted with mixtures of genetically-marked pollen from *P. caroliniana* and *P. viridis* to assess the effect of stigma load composition on the probability of F₁ hybrid seed formation. Pollen was mixed in the following ratios: 100:0, 90:10, 75:25, 50:50, 25:75, 10:90 and 0:100 of intra- to inter-specific pollen. Pollinations were performed in a randomized incomplete block design with pollen mixture ratio as the blocking variable. For each pollen mixture, pollen was taken from 8 plants of each species (2 from each combination of pistil morphology and PGI genotype). Two flowers of each plant were pollinated with each pollen mixture. The genotypes of seeds were determined using starch gel electrophoresis as described above. The frequency of the hybrids was calculated and compared to expected ratios using χ^2 -tests for goodness of fit. The number of pollen grain on the stigma and seeds in each fruit were counted under a stereo microscope.

2. Pollen Dosage. A light pollen dosage and a heavy pollen dosage (50 : 50 mixture) was applied to two different flowers of the same plant to

examine the effect of pollen number on the fertilization success of each pollen type. After pollination, styles were collected and the number of pollen grains adhering to each stigma was counted under a stereo microscope. Data were analyzed to test whether the observed percentage of hybrids differed significantly from the expected frequency using χ^2 -tests. The effect of pollen number on siring success was analyzed using a regression model of mating system variation (see below; Cruzan and Barrett 1996).

3. Advanced-generation hybrid formation. F₁ hybrids were produced using controlled hand pollinations between *P. caroliniana* and *P. viridis* and their genotypes were determined using starch gel electrophoresis. Only hybrids that were homozygous at the PGI locus were chosen and used in this experiment as either pollen donors or recipients. Three types of pollen mixture, each containing equal amounts of two pollen types, were applied to flowers of F₁ hybrid, *P. caroliniana* and *P. viridis* plants: (a) pollen from *P. caroliniana* and *P. viridis* (c/v mixture), (b) pollen from *P. caroliniana* and F₁ (c/F₁ mixture), (c) pollen from *P. viridis* and F₁ (v/F₁ mixture). The

number of anthers used to make pollen mixtures was adjusted according to ratios in table 1 to produce 50:50 mixtures. Pollinations were performed as described above with plants as the blocking variable. For each pollen mixture, 48 flowers on four plants of each species and F_1 hybrids were pollinated.

Styles and seeds were collected and analyzed as described above. Data were analyzed by testing whether the observed percentage of the F_1 and advanced generation hybrids differed significantly from the expected. Differences in the percent of different hybrid genotypes produced among maternal plants and taxa (F_1 , *P. caroliniana*, and *P. viridis*) was tested using ANOVA.

4. Data analysis based on the decomposition model of mating system.

The model proposed by Cruzan and Barrett (1996) attempts to decompose pollen siring ability into the effects of pollen competition and attrition. For each pollen type i present on the stigma of a flower, the proportion of seeds sired (F_i) could be affected by four factors: the proportion of pollen i present on the stigma (P_i), the ratio of the total number of grains present on

the stigma to the number of ovules (G/O), the level of gamete attrition (δ_i), and the competitive ability of pollen (α_i , the relative growth rate of each pollen type i). Cruzan and Barrett (1996) suggested that when there are not enough pollen grains to fertilize all of the ovules (e.g. G/O ratio < 1), the proportion of seeds sired depends only on a pollen type's frequency and its level of attrition; thus,

$$F_i = P_i + \delta_i \quad (1)$$

Once the number of grains on the stigma exceeds the number of ovules ($G/O > 1$), there is potential for competition among pollen types and the number of seeds sired by a pollen type is given by the regression

$$F_i = (P_i + \delta_i) + \alpha_i P_i [(G/O) - 1] \quad (2)$$

To examine the deviations from the expected fertilization in response to pollen grain number, I used a modification of equation (2),

$$F'_i = \delta_i + \alpha_i G \quad (3)$$

where $F'_i = F_i - P_i$. This transformation allows the direct estimation of the level of gamete attrition as the deviation from a zero intercept (δ_i) and pollen competitive ability as the slope (α_i). When differences in pollen

competitive ability exist, the proportion of seeds sired by different pollen types will depend on the number of pollen grains present.

Data from the pollen replacement series and pollen dosage experiments, and crosses with F_1 plants were analyzed as the proportion of seeds fertilized by pollen from different genotypes for each pollination. The deviation of each pollen type's siring success from a random expectation in the pollen replacement series was tested using χ^2 . Based on the decomposition model (3) above, the stigma load size and siring success in the pollen dosage experiment were regressed with the general linear model (GLM) procedure of SAS (SAS Institute, Inc. 1985), using the deviation from random fertilization (F_i) as the dependent variable and the total number of grains as the independent variable.

The fecundity of plants could also be affected by the mechanism of discrimination among pollen types. An exponential regression model was used to examine the relationship between pollen-load size and seed set,

$$S/O = S/O_{max} - \left\{ \frac{G/O_{max} - G/O}{G/O_{max}} \right\}^k \quad (4)$$

where S/O is the ratio of the number of seeds to the number of ovules, S/O_{max} is the maximum seed set attained, G/O_{max} is the maximum pollen

load size observed, and k is an exponent describing the shape of the relationship. This model differs from the one proposed by Cruzan and Barrett (1996) since it allows the relationship to asymptote to seed-set values that are not equal to one (defined by S/O_{max}). Data from the pollen dosage experiment were analyzed using linear regression (GLM procedure, SAS Institute 1985) and equation (4) in nonlinear regression (NLIN procedure, SAS Institute, Inc. 1985) to estimate the value of S/O_{max} and k for each recipient genotype / pollen mixture combination.

Chapter III

Results

Pollen Viability

There were no significant differences in pollen viability among L-morph plants of *Piriqueta caroliniana*, *Piriqueta viridis* and F₁ genotypes, but the pollen viability in this morph did differ among days (Table 2). There were also no among-plant differences in pollen viability for the L-morph plants (Table 2). For S-morph plants, no significant variation was found in pollen viability among *P. caroliniana*, *P. viridis* and F₁ genotypes (Table 2). As in the L-morph, there was among day variation in pollen viability, but there was significant variation among individual plants within genotypes (Table 2).

Pollen Production

Differences in the average of number of pollen grains produced by each species and the F₁ hybrids were found and adjustments were made

Table 2. Analysis of variance in pollen viability in *P. caroliniana*, *P. viridis* and F₁ plants.

Source		df	Sum of squares	F	P<F
L-morph	species	2	0.031695	2.07	0.1538
	plant	19	0.1454571	1.96	0.1514
	day	2	0.2532849	32.37	0.0001
	error	9	0.0352151		
S-morph	species	2	0.0040443	0.18	0.839
	plant	14	0.1592133	22.37	0.0014
	day	1	0.0052083	10.25	0.024
	error	5	0.0025417		

when making mixtures of pollen from different genotypes (Table 1). The analysis of variance indicated that there were significant difference in the pollen production for *P. caroliniana*, *P. viridis* and F₁ plants (Table 3). Differences were also found between the two types of flowers within a species and different plants (Table 3).

Pollen Replacement Series

The overall frequency of intra-specific and F₁ hybrid seeds was different from the expected frequency across treatment for *P. viridis*

Table 3. Analysis of variance in pollen production per anther of *P. caroliniana*, *P. viridis* and F1 plants.

Source	df	Sum of squares	F	P<F
Species	2	16708276.5	83.02	<0.0001
Morph	1	10633359.4	105.67	<0.0001
Plant	29	9678515.6	3.32	<0.0001
Error	66	6641666.7		

($\chi^2 = 111.13$, $p < 0.001$, Table 4). The number of intra-specific seeds produced on *P. viridis* was significantly above the expected number by 28.14% when intra-specific pollen was only 10% in the pollen mixture (Table 4, Figure 2), but decreased below the expected by 2.98% as the pollen from *P. viridis* was 25% in the pollen mixture. The frequency of the intra-specific seeds sired by *P. viridis* was 13.81% below the expected when intra-specific pollen was increased to 90% in the pollen mixture (Table 4, Figure 2).

Further analysis indicated significant effects of both pollen load size and composition on the frequency of hybrid seed formation (Table 5).

However, there was no significant interaction between pollen load size and composition in *P. viridis* (Table 5).

Table 4. The results of pollen replacement experiment. The observed and expected number of seeds are given for each pollination treatment.

Species	Intra-specific pollen percentage	N	Percent fruit set	Seeds per fruit	S/O	Number of intra specific seed	Number of hybrids	Chi-square
<i>P. viridis</i>	0.10	10	0.16	12.2	0.48	37 (9.70)	60 (87.3)	85.37*
	0.25	11	0.17	23.6	0.82	27 (29.95)	72 (69.05)	0.42
	0.50	17	0.18	16.9	0.63	55 (70.50)	86 (70.5)	6.82*
	0.75	7	0.44	15.6	0.73	36 (43.50)	22 (14.5)	5.17*
	0.90	5	0.31	15.2	0.84	48 (56.70)	15 (6.3)	13.35*
<i>P. caroliniana</i>	0.10	6	0.38	7.5	0.39	11 (4.10)	30 (36.9)	12.90*
	0.25	15	0.94	11.7	0.67	46 (35.00)	94 (105)	4.61*
	0.50	23	0.24	11.0	0.61	134 (105.00)	76 (105)	16.02*
	0.75	18	0.28	15.1	0.71	134 (121.50)	28 (40.5)	5.14*
	0.90	18	0.28	10.1	0.49	96 (119.70)	37 (13.3)	46.92*

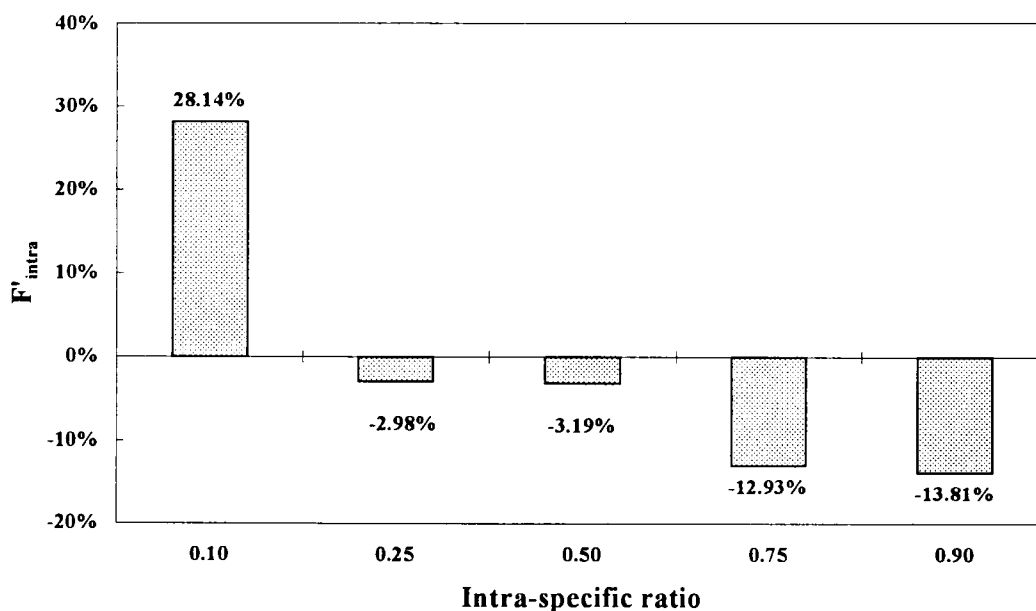


Figure 2. The deviation of the frequency of intra-specific fertilization from the expected for *P. viridis*. F'_{intra} is the deviation of intra-specific fertilization from the expected frequency.

Table 5. Analysis of variance in the deviation of the frequency of intra-specific seeds from the expected (F_{intra}) for *Piriqueta viridis*.

Source	df	Sum of Square	Mean Square	F	P < F
Plant	8	0.8409	0.1051	2.34	0.045
Ratio	4	0.7935	0.1984	4.41	0.007
Pollen	1	0.1956	0.1956	4.35	0.046
Pollen*ratio	4	0.0182	0.0045	0.1	0.981
Error	29	1.3045			

The observed number of intra-specific seeds sired in *P. caroliniana* was significantly different from the expected for all the pollen mixtures (Table 4, Figure 3). The percent difference between the observed and expected number of intra-specific seeds decreased from 16.8% above expected to 17.8% below expected as the amount of intra-specific pollen increased from 10% to 90% (Fig. 3). The number of intra-specific seeds remained above the expected as the percent intra-specific pollen increased from 10% to 75%, but then decreased below expected at 90% intra-specific pollen (Table 4, Fig. 3).

Further analysis indicated that there was not a significant overall effect of pollen mixture treatment, but hybrid formation depended on an

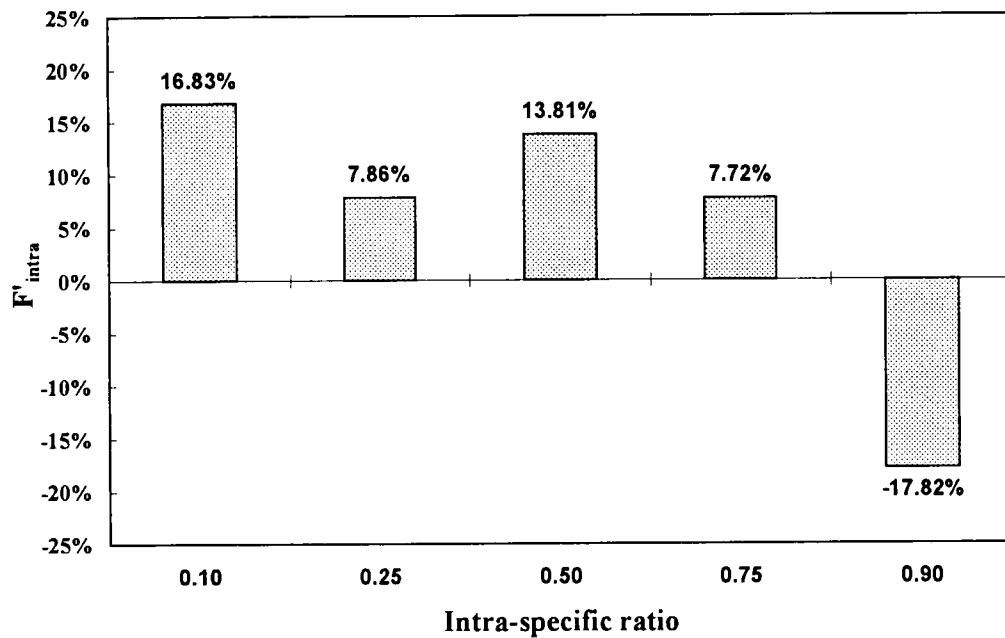


Figure 3. The deviation of intra-specific fertilization from the expected frequency of *P. caroliniana*. F'_{intra} is the deviation of intra-specific fertilization from the expected frequency.

interaction between the pollen load size and composition in *P. caroliniana* (Table 6).

Pollen Dosage

For all pollen mixtures combined, the frequency of intra-specific fertilizations tended to decrease (slope = - 0.00010, $t = 2.21$, $P < 0.05$) as the number of pollen grains on the stigma increased for *P. viridis* plants (Table 5). The Non-zero slope indicates that pollen competitive ability

Table 6. Analysis of variance in the deviation of the frequency of the intra-specific seeds from the expected (F_{intra}) for *Piriqueta caroliniana*

Source	df	Sum of Square	Mean Square	F	P < F
Plant	7	0.3254	0.0465	0.87	0.5325
Ratio	4	0.3704	0.0926	1.74	0.1537
Pollen	1	0.2457	0.2457	4.62	0.0359
Pollen*Ratio	4	1.8352	0.4588	8.63	0.0001
Error	56	2.9774			

was largely responsible for determining the siring success of pollen from *P. caroliniana* and *P. viridis* on *P. viridis* recipients. There was also a high level of gamete attrition for *P. viridis* pollen, which resulted in the negative intercepts for pollen mixtures which consisted of 10% and 25% intra-specific pollen (Table 7).

For *P. caroliniana*, the change in the frequency of intra-specific fertilization in response to pollen dosage was more complex and depended on the pollen load composition (Table 6). The fraction of seeds sired by *P. caroliniana* tended to increase for the pollen ratio of 10% and 50% as the number of grains on the stigma increased, while for the pollen mixture of ratio 90%, the fraction of seed sired by *P. caroliniana* tended

Table 7. Regression analysis of pollen load size on fertilization success of five pollen mixtures on *P. carolinia* and *P. viridis*.

Recipient	Intra-specific pollen rati	Fruits	Intercept	Probability	Slope	Probability
<i>P. viridis</i>	0.10	8	0.26827	<0.05	-0.00006	0.390
	0.25	10	0.39947	<0.05	-0.00026	<0.05
	0.50	14	-0.02775	0.432	-0.00011	0.262
	0.75	6	0.25733	0.153	-0.00032	0.091
	0.90	5	-0.03905	0.351	-0.00008	0.146
<i>P. caroliniana</i>	0.10	6	-0.26982	<0.10	0.00074	<0.01
	0.25	15	0.09655	0.189	0.00001	0.461
	0.50	18	-0.16097	<0.05	0.00039	<0.001
	0.75	18	0.08853	0.178	-0.00002	0.330
	0.90	18	0.02070	0.436	-0.00031	<0.05

to decrease as the number of grains increased (Table 7). The non-zero slopes indicate that pollen competitive ability is partly responsible for the siring patterns of hybrids in *P. caroliniana*. Fertilization success in *P. caroliniana* was also determined by the level of gamete attrition, as indicated by non-zero intercept for the 10% and 50% intra-specific pollen mixtures. The negative intercepts in both pollen mixtures indicates that there might be stronger gamete attrition against intra-specific pollen in *P. caroliniana*.

Patterns of Fecundity

Seed number increased with pollen load sizes in both *P. viridis* (Figure 4) and *P. caroliniana* (Figure 5). By transforming seed production into relative fecundity and using the model proposed by Cruzan and Barrett (1996), we can determine the level of pre- and post-fertilization gamete attrition in *P. viridis* and *P. caroliniana*. The relative fecundity increased rapidly when pollen grain numbers were small and remained fairly constant over a range of pollen loads (Figures 4-5). The

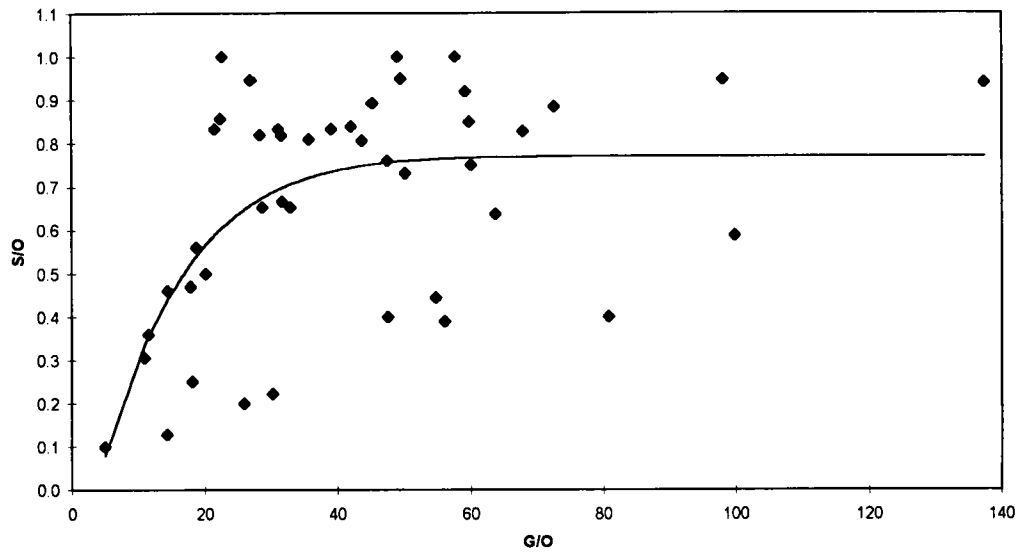


Figure 4. Patterns of fecundity of *P. viridis*. G/O is the ratio of the number of pollen grain to the number of ovule, S/O is the number of seed to the number of ovule.

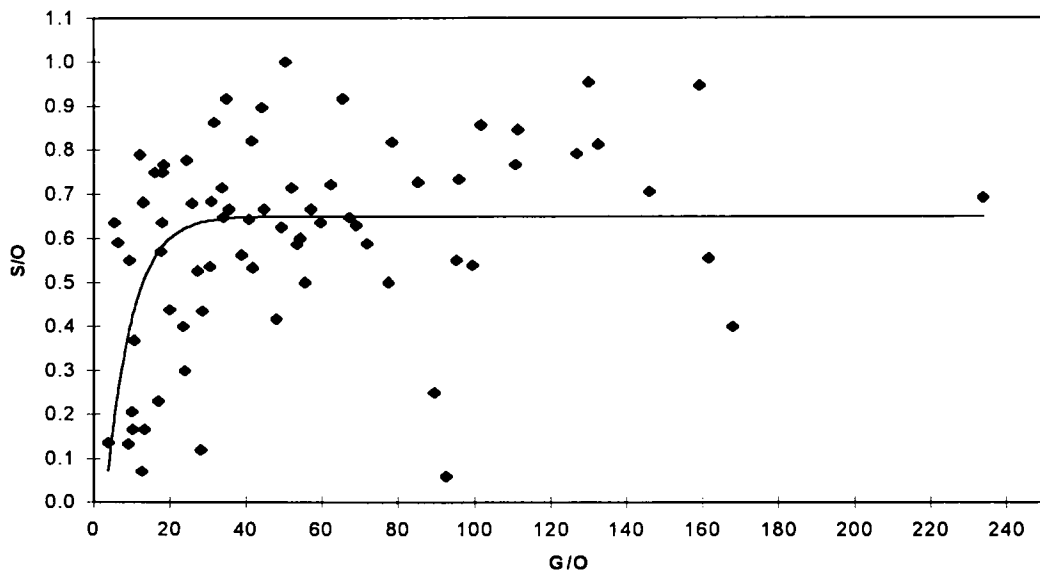


Figure 5. Patterns of fecundity of *P. caroliniana*. G/O is the ratio of the number of pollen grain to the number of ovule, S/O is the number of seed to the number of ovule.

exponential parameter describing the relationship between pollen load size and seed set (k) was significantly larger for *P. caroliniana* ($k = 33.72$) than for *P. viridis* ($k = 10.14$) as indicated by the non-overlapping 95% confidence interval.

Levels of post-fertilization gamete attrition can be inferred from the asymptotic value of the relationship described above (equation 4: $S/O_{\max}$). Since the value of $S/O_{\max}$ for *P. caroliniana* was significantly lower than that for *P. viridis* (0.65 for *P. caroliniana*, 0.77 for *P. viridis*, $P < 0.10$ for confidence interval), higher level of post-fertilization gamete attrition probably occurred in *P. caroliniana*.

Advanced-Generation Hybrid Formation

The frequency of formation of parental genotypes, F_1 hybrid, and advanced-generation hybrid seeds depended on the composition of the pollen load and the recipient genotype used. On *P. caroliniana* maternal plants, the proportion of seeds sired by mixtures of c/v and v/ F_1 pollen are significantly different from the expected (Table 8, Figure 6). The

Table 8. Summary of the fertilization results from the experiments of formation of advanced-generation hybrids.

Maternal plant	Pollen mixture	Fruits	Type of seed	Number of seed	Type of seed	Number of see	Chi-square
<i>P. caroliniana</i>	<i>c/v</i>	23	Intra	116	F_1	63	15.69*
	<i>c/F_1</i>	27	Intra	135	$B(F_1*c)$	107	3.240
	<i>v/F_1</i>	24	F_1	117	$B(F_1*v)$	86	4.73*
<i>P. viridis</i>	<i>c/v</i>	18	Intra	56	F_1	63	0.410
	<i>c/F_1</i>	24	F_1	100	$B(F_1*v)$	72	4.56*
	<i>v/F_1</i>	16	Intra	66	$B(F_1*v)$	62	0.130
<i>F1</i>	<i>c/v</i>	26	$B(c*F_1)$	122	$B(v*F_1)$	80	8.73*
	<i>c/F_1</i>	24	$B(c*F_1)$	91	F_2	54	9.44*
	<i>v/F_1</i>	21	$B(v*F_1)$	100	F_2	90	0.530

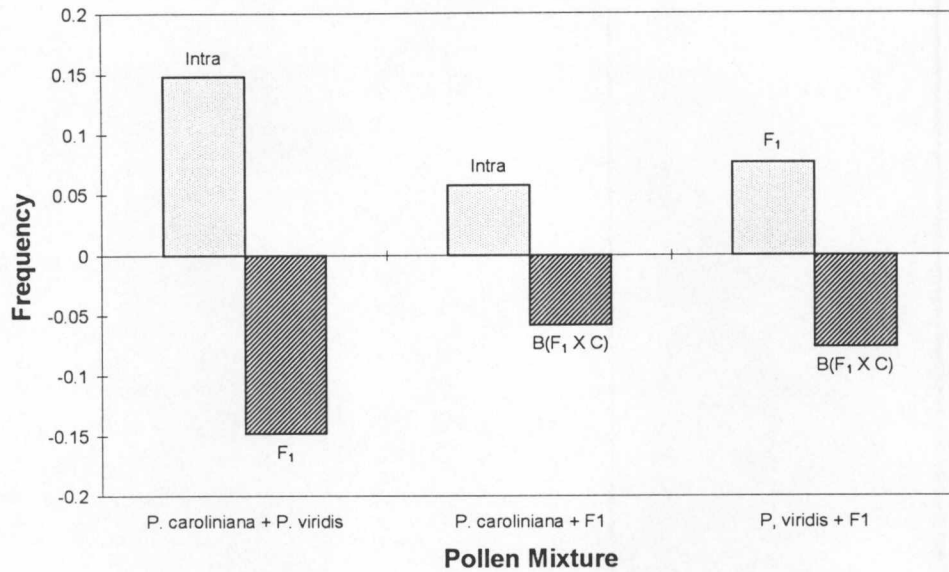


Figure 6. Formation of parental genotypes, F₁ hybrid, and advanced-generation hybrid seeds on *P. caroliniana*.

number of backcross seeds fertilized by F₁ pollen is below the expected for both pollen mixtures containing F₁ pollen (c/F₁ and v/F₁: Table 8, Figure 6). When *P. caroliniana* pollen was included in the mixture, intra-specific fertilization always exceeded the expected frequency.

On *P. viridis*, the proportion of seeds sired by the c/F₁ pollen mixture is significantly different from the expected ratio (Table 8, Figure 7). The number of backcross seeds sired by F₁ pollen is below the expected number in both pollen mixtures of c/F₁ and v/F₁. Pollen from *P.*

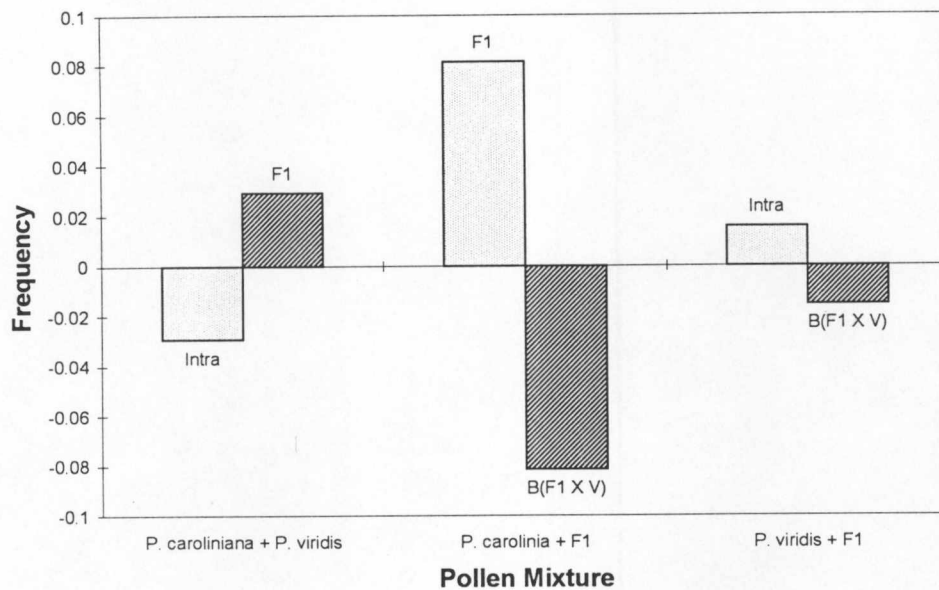


Figure 7. Formation of parental genotypes, F₁ hybrid, and advanced-generation hybrid seeds on *P. viridis*.

caroliniana appears to have a stronger siring ability than *P. viridis* on *P. viridis* (Table 8, Figure 7). On F₁ maternal plants, the number of backcross seeds sired by the pollen from *P. caroliniana* is much greater than the number sired by *P. viridis* pollen (Table 8, Figure 8). The number of F₂ hybrids produced on F₁ plants is below the expected number for both pollen mixtures containing F₁ pollen (c/F₁ and v/F₁). For these two pollen mixtures, more backcross seeds are produced than F₂ hybrids (Table 8, Figure 8).

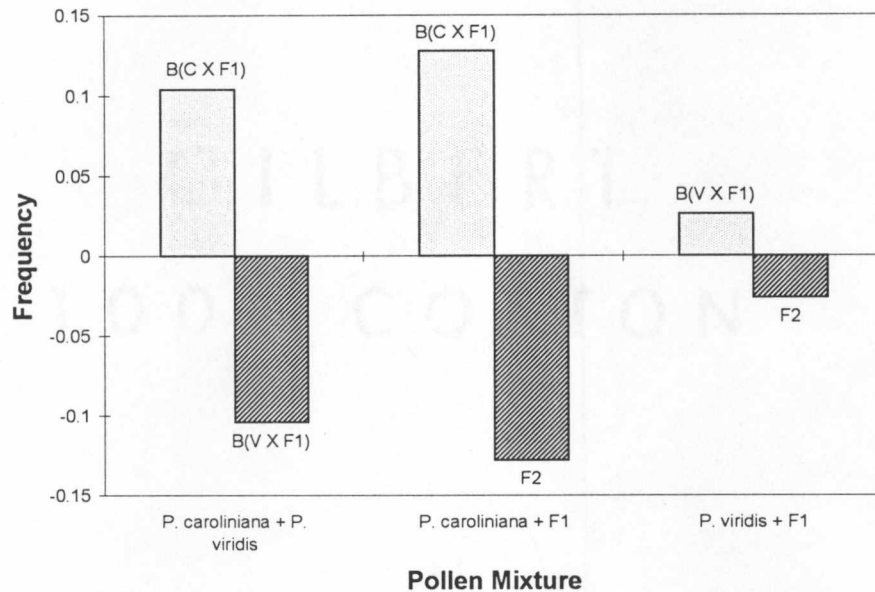


Figure 8. Formation of parental genotypes, F₁ hybrid, and advanced-generation hybrid seeds on F₁ plants.

(a). Results of pollen dosage and seed set on *P. caroliniana*. When the c/v pollen mixture was applied on flowers of *P. caroliniana*, the result of the pollen dosage analysis indicates that as the pollen dosage increases, the fraction of seeds sired by *P. caroliniana* didn't increase significantly, but the intercept was significantly above zero (Table 9, Figure 9). The average seed set was lower (Table 10, Figure 10) indicating that post-fertilization gamete attrition might be higher for the c/v pollen mixture than that for the other two pollen mixtures (c/F₁, v/F₁).

Table 9. Regression analysis of pollen load size on fertilization success of *P. caroliniana* in the experiment of advanced-generation hybrid formation.

Pollen mixture	Intercept	Probability	Slope	robability
<i>P. caroliniana</i> / <i>P. viridis</i>	0.126	0.1178	0.00139	0.256
<i>P. caroliniana</i> / F_1	-0.188	<0.01	0.005	<0.001
<i>P. viridis</i> / F_1	0.124	<0.0657	-0.0008	0.586

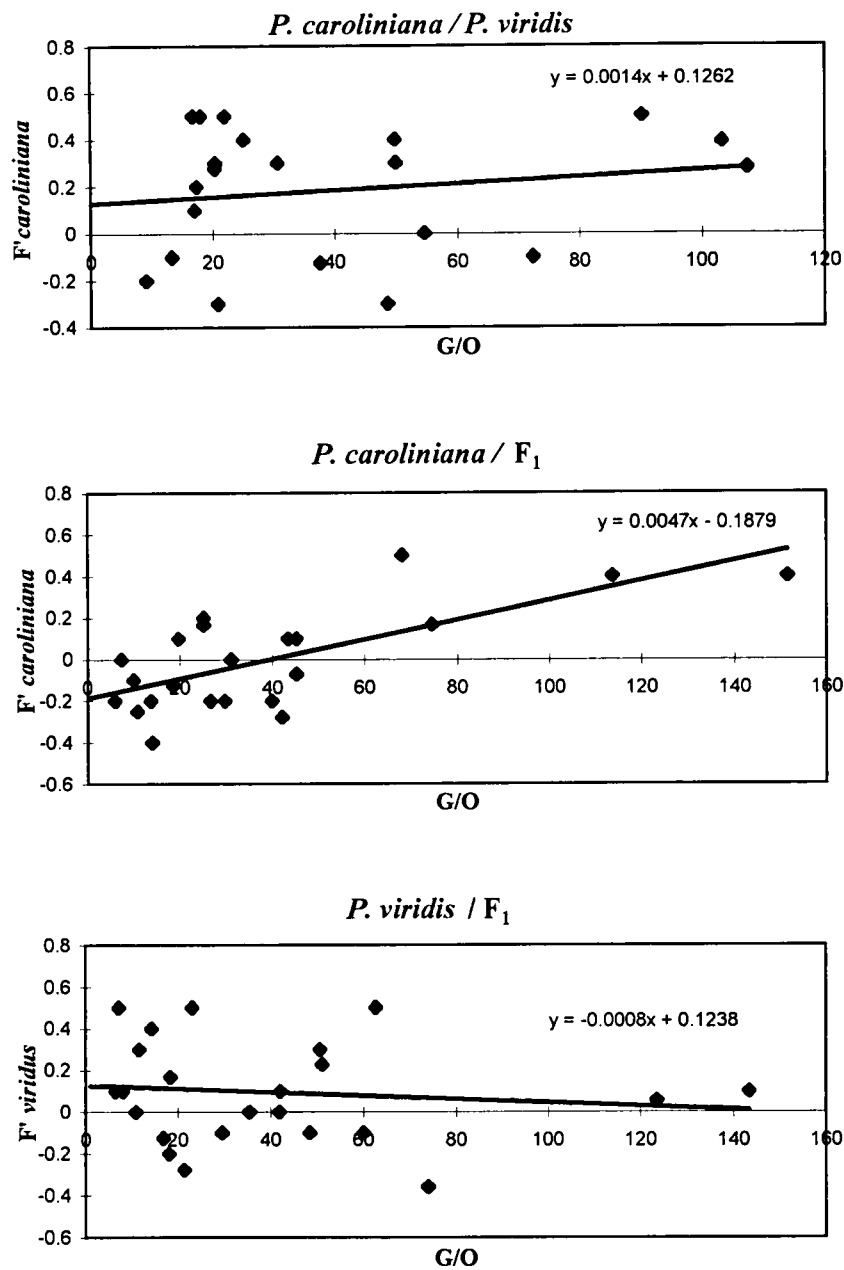


Figure 9. Fertilization success of *P. caroliniana* in the experiments of advanced-generation hybrid formation.

Table 10. Analysis of the seed set data in the experiment of advanced-generation hybrid formation.

Recipient	Pollen mixture	k	Average maximum seed set
<i>P. caroliniana</i>	<i>c/v</i>	-	0.73
	<i>c/F₁</i>	21.89	0.73
	<i>v/F₁</i>	47.5	0.729
<i>P. viridis</i>	<i>c/v</i>	18.08	0.802
	<i>c/F₁</i>	-	0.651
	<i>v/F₁</i>	27.02	0.71
<i>F₁</i>	<i>c/v</i>	51	0.716
	<i>c/F₁</i>	-	0.736
	<i>v/F₁</i>	-	0.829

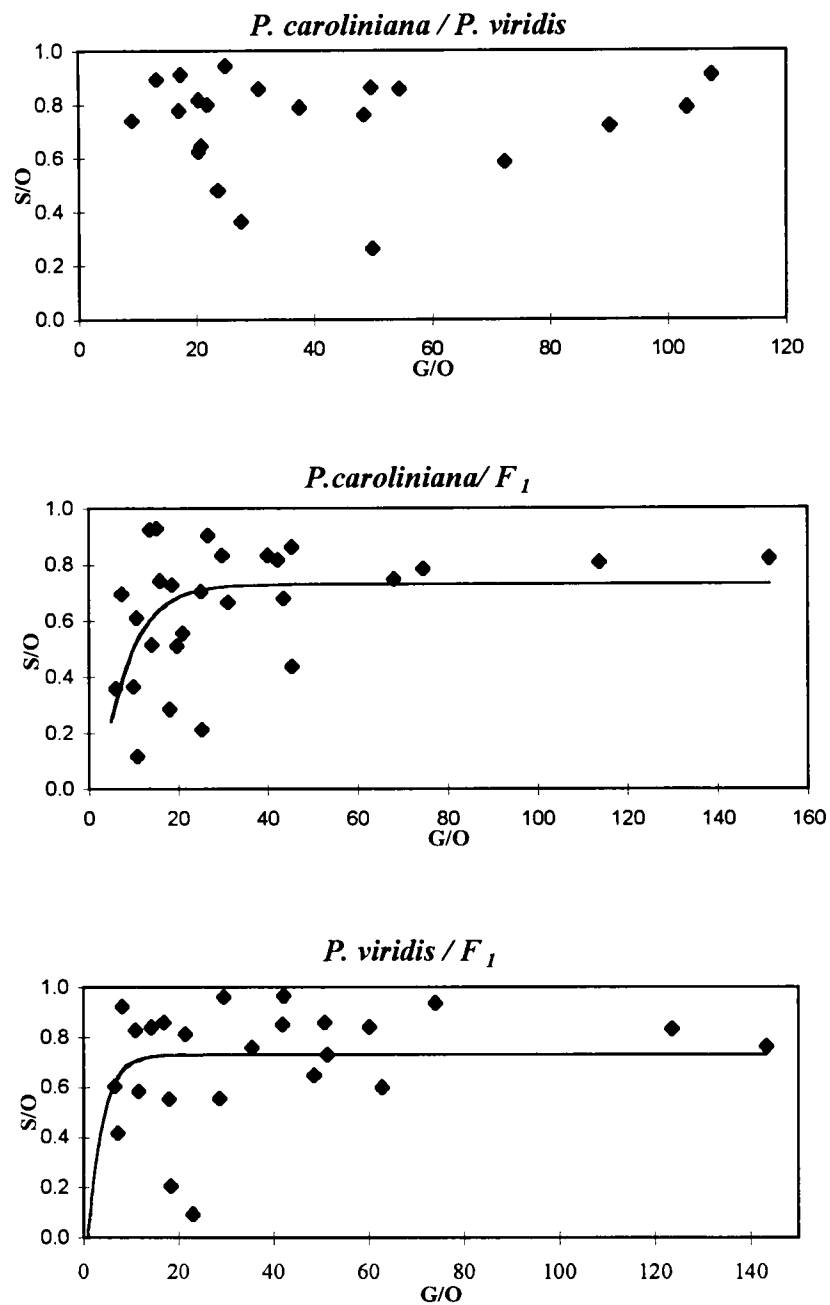


Figure 10. Fertilization success on *P. caroliniana* in the experiments of advanced-generation hybrid formation.

As the number of pollen grains increases for the c/F_1 pollen mixture, the proportion of seeds sired by *P. caroliniana* increased significantly (Table 9, Figure 11). The non-zero slope indicates that the competitive ability of pollen is largely responsible for determining siring success. There was also high level of gamete attrition against *P. caroliniana* pollen, which resulted in the negative intercept (Table 9, Figure 10). The exponential parameter for the seed set is 21.9 (Table 10), which is lower than that of pollen mixture v/F_1 . The average seed set is the same for both the c/F_1 and v/F_1 pollen mixtures (Table 10).

The proportion of seed sired by pollen from *P. viridis* doesn't increase as the pollen dosage increased for v/F_1 pollen mixture (Table 9, Figure 9), but there might be pollen attrition against F_1 pollen as indicated by the positive intercept (Table 9). The exponential parameter k is larger for v/F_1 pollen mixture than that for c/F_1 pollen mixture (Table 10, Figure 10), but the seed set is the same which indicates that stronger pre-fertilization gamete attrition might have happened in the v/F_1 pollen mixture.

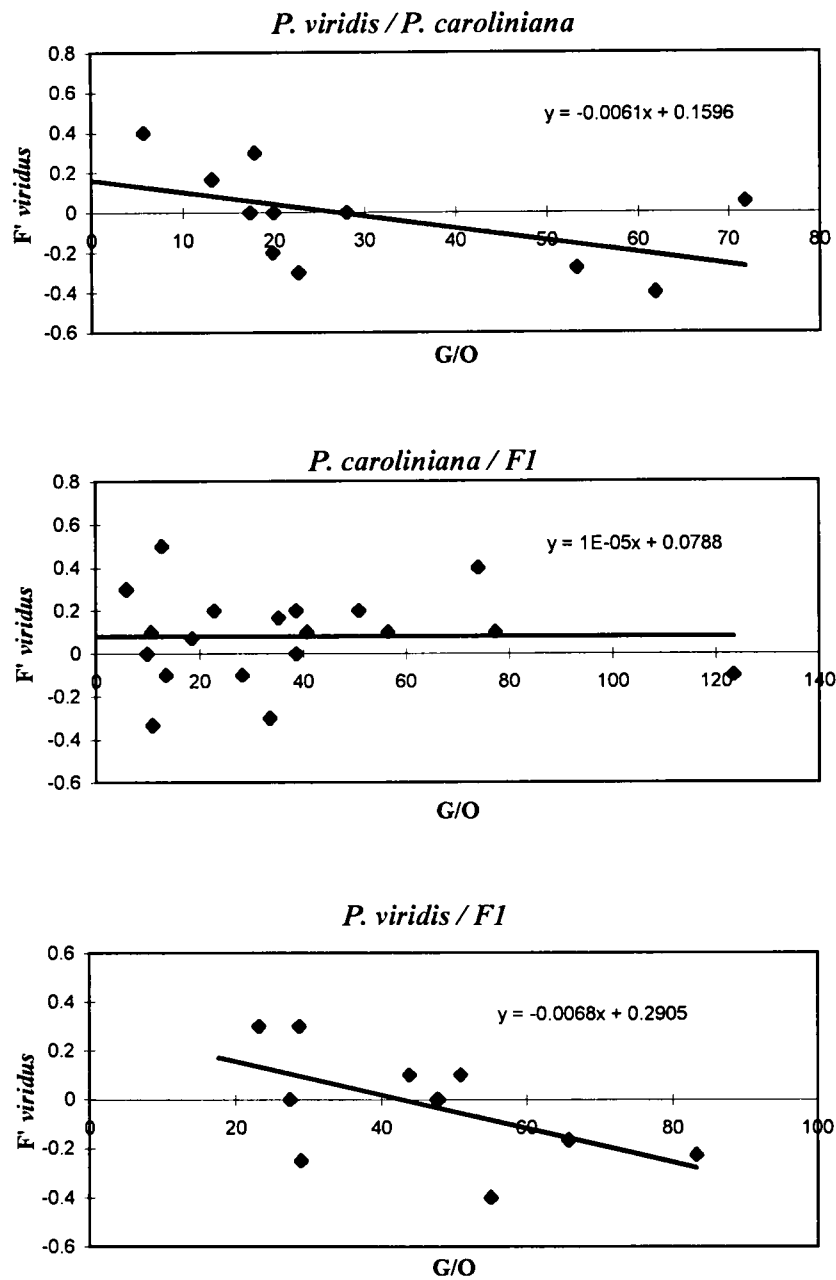


Figure 11. Fertilization success of *P. viridis* in the experiments of advanced-generation hybrid formation.

(b). Pollen dosage and seed set on *P. viridis*. There was full seed set for all three pollen mixtures in *P. viridis*, which is an obvious contrast to *P. caroliniana*. This suggests that lower levels of post-fertilization gamete attrition probably occurred in *P. viridis* than in *P. caroliniana*.

In *P. viridis*, the fraction of seeds sired by *P. viridis* tended to decrease as the number of pollen grains on the stigma increased (Table 11, Figure 11), indicating that *P. viridis* pollen has a lower competitive ability. The average maximum seed set was 0.80 (Table 10, Figure 12), which was higher than that of the other two pollen mixtures (c/F₁ and v/F₁).

The fraction of seeds sired by *P. caroliniana* after pollination with the c/F₁ pollen mixture doesn't change as the pollen dosage increased, as indicated by the insignificant slope in the linear regression (Table 11, Figure 11). However, the average maximum seed set was very low (Table 10, Figure 12), which indicates that post-fertilization ovule abortion might be responsible for the fecundity pattern for this pollen mixture.

When the v/F₁ pollen mixture was applied to the stigma of *P. viridis*, the proportion of seeds sired by *P. viridis* decreased as the pollen dosage

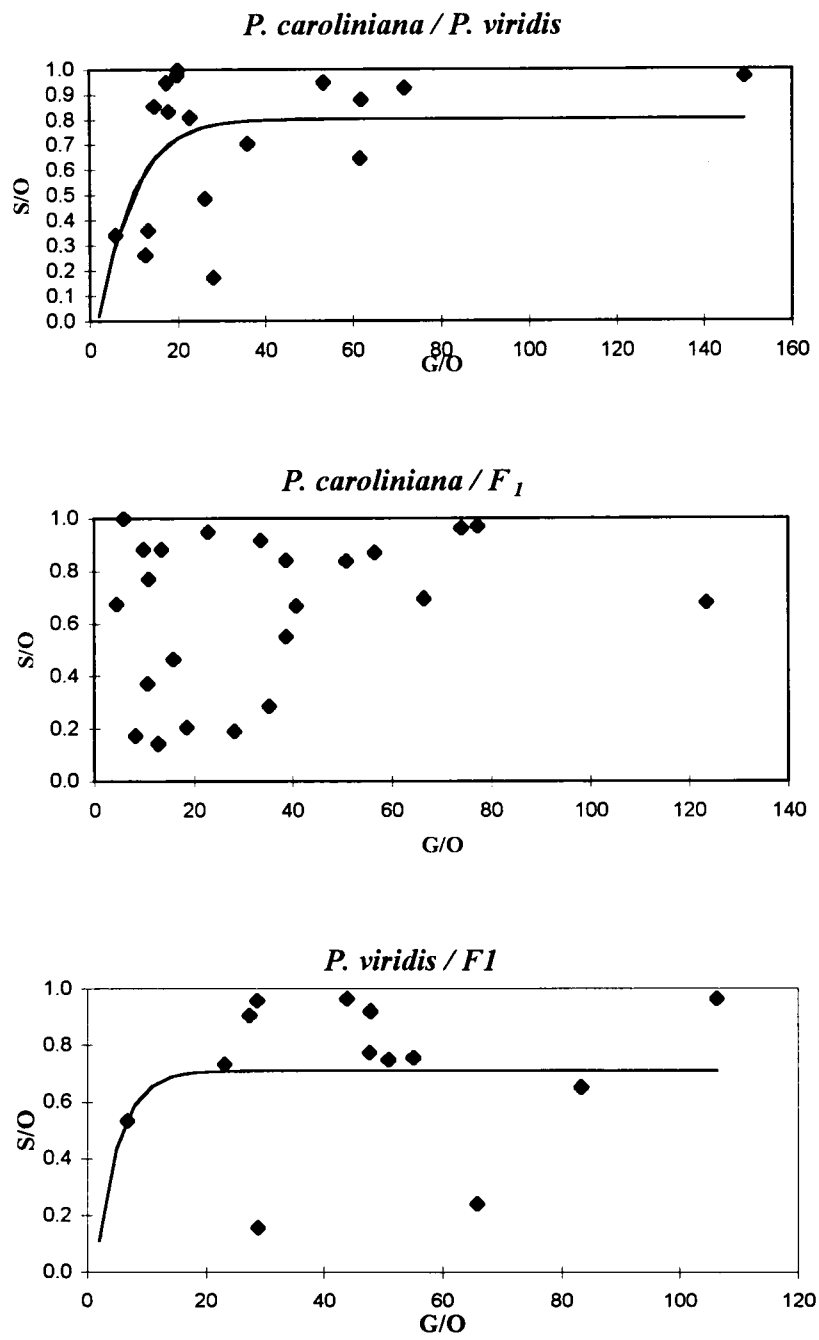


Figure 12. Fertilization success on *P. viridis* in the experiments of advanced-generation hybrid formation.

Table 11. Regression analysis of pollen load size on fertilization success of *P. viridis* in the experiment of advanced-generation hybrid formation.

Pollen mixture	Intercept	Probability	Slope	robability
<i>P. caroliniana</i> / <i>P. viridis</i>	0.160	0.220	-0.006	<0.05
<i>P. caroliniana</i> / F_1	0.079	0.340	0.000	0.990
<i>P. viridis</i> / F_1	0.291	<0.05	-0.007	<0.05

increased (Table 11, Figure 11, $P < 0.05$) indicating that pollen from F_1 genotypes has a better competitive ability than intra-specific pollen on *P. viridis*. The positive intercept (Table 11, Figure 11, $P < 0.05$) indicates that gamete attrition is also responsible for determining the siring success of the two different pollen types. There may be high level of gamete attrition against pollen from F_1 plants. Further analysis of the seed set data showed that the exponential parameter k is higher than that in pollen mixture of c/v and the average maximum seed set was lower than that of the c/v pollen mixture (Table 10, Figure 12), suggesting that higher post-fertilization gamete attrition might be occurring against F_1 pollen.

(c). Pollen dosage and seed set on F₁ plants. The proportion of seeds sired by *P. caroliniana* pollen increased as the pollen dosage increases for the pollen mixture of *P. caroliniana* and *P. viridis* (Table 12, Figure 13, $P < 0.05$). The positive slope indicates that pollen from *P. caroliniana* is at advantage in term of pollen competitive ability. No obvious differential pollen attrition is found since the intercept is not significantly different from zero (Table 12, Figure 13, $P = 0.62$).

When pollen mixture of *P. caroliniana* and F₁ was applied on *P. viridis* stigmas, the fraction of seeds sired by *P. caroliniana* didn't change as the pollen dosage increased (Table 12, Figure 13, $P = 0.885$). No gamete attrition is evident as indicated by the zero intercept (Table 12, Figure 13).

The proportion of seeds sired by *P. viridis* pollen decreased as the pollen dosage increased for the pollen mixture from *P. viridis* and F₁ plants. The negative slope indicates that pollen from F₁ plants has a stronger competitive ability than that from *P. viridis* in the pollen mixture (Table 12, Figure 13, $P < 0.1$). The significant positive intercept indicates

Table 12. Regression analysis of the pollen load size on fertilization success of F_1 in the experiment of advanced-generation hybrid formation.

Pollen mixture	Intercept	Probability	Slope	robability
<i>P. caroliniana</i> / <i>P. viridis</i>	0.0318	0.621	0.0034	<0.05
<i>P. caroliniana</i> / F_1	0.1005	0.350	0.0004	0.885
<i>P. viridis</i> / F_1	0.1183	<0.05	-0.0019	0.083

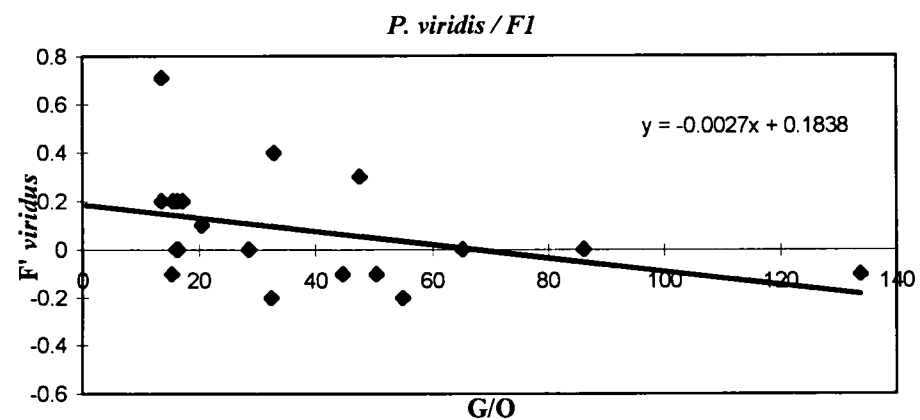
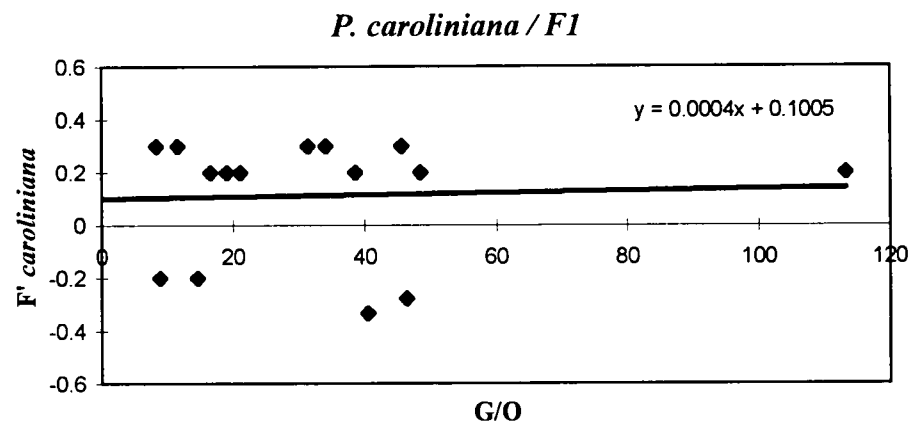
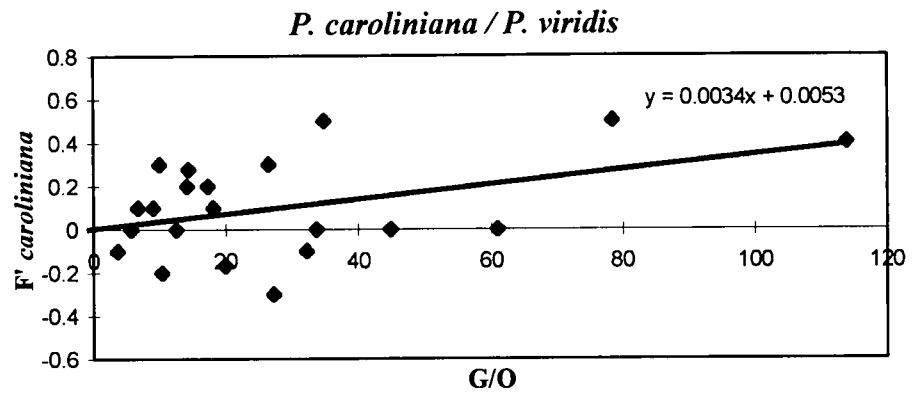


Figure 13. Fertilization success of F₁ in the experiments of advanced-generation hybrid formation.

that selective gamete attrition may be acting against pollen from F_1 plants (Table 12, Figure 13, $P < 0.05$). The average seed set for the v/F_1 pollen mixture was the highest of the three mixtures used (Table 10, Figure 14), which indicates that the observed gamete attrition is due to pollen tube attrition rather than ovule abortion.

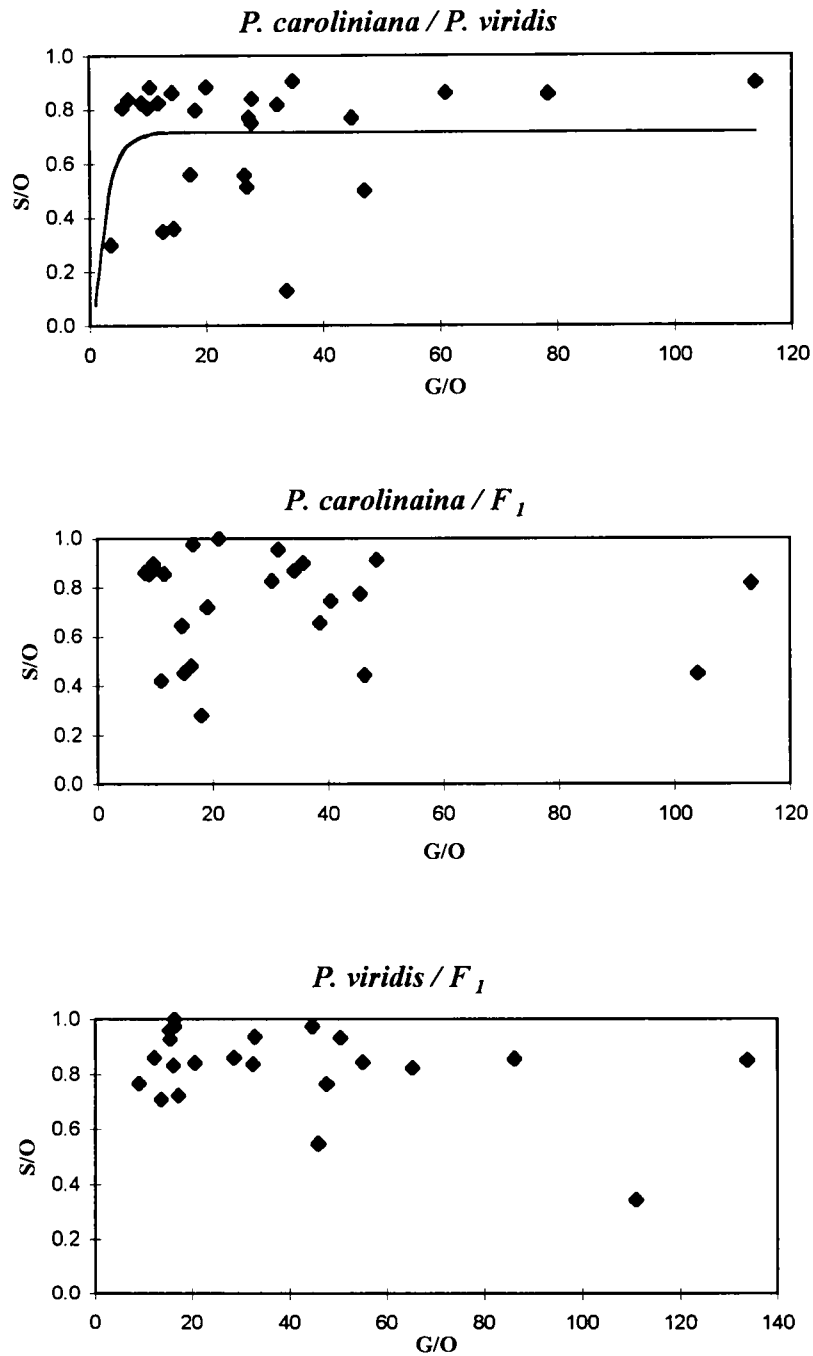


Figure 14. Fertilization success on F_1 in the experiments of advanced-generation hybrid formation.

Chapter IV

Discussion

Patterns of siring success for the different combinations of stigma load composition and size in these *Piriqueta* species suggest that the formation of hybrids is controlled by a combination of pre- and post-fertilization processes. Hybrid seeds were produced at frequencies that were significantly different from the expected for both *P. viridis* and *P. caroliniana*. However, the patterns and contributions of pollen competition, pollen-tube attrition, and embryo abortion to the formation of hybrids appear to differ for the two species.

F₁ hybrid formation

In *P. viridis*, pollen replacement and pollen dosage data indicate that pre-fertilization processes are unlikely to limit the formation of F₁ hybrid embryos. For all the pollen composition ratios except the 10% intraspecific pollen mixture, the frequency of hybrid seed exceeded the expected

frequency, and tended to increase as the proportion of intraspecific pollen increased. Pollen from *P. caroliniana* appears to have a competitive advantage in *P. viridis* recipients, as indicated by the negative relationship between pollen load size and fertilization for intra-specific pollen. Several studies have reported the apparent effects of pollen competition (Carney et al. 1994, 1996; Emms et al. 1996) and conclude that it could be a major factor in determining the frequency of hybrids produced between two species. However, gamete attrition was not examined in most of these studies and could be another factor in determining the frequency of hybrids in natural populations.

Differences in pollen competitive ability may not limit the formation of hybrids in *P. viridis*, however, gamete attrition apparently limits the frequency of hybrids produced by this species when pollen load sizes are small. This is apparent from the significant positive intercept for the 0.1 and 0.25 pollen mixtures. Furthermore, gamete attrition in *P. viridis* appears to act pre-zygotically rather than occurring after ovule fertilization since the average maximum seed set is higher in *P. viridis* than that in *P. caroliniana*. Pollen of *P. caroliniana* may be subject to higher levels of

attrition during pollen-tube growth providing an advantage to conspecific pollen in *P. viridis* when pollen loads are small.

In *P. caroliniana*, the frequency of hybrids seed is lower than expected in all the pollen treatments except the 90% intraspecific pollen mixture. Pollen competition is largely responsible for the limitation of the formation of the hybrids in this species, but the response of fertilization pattern to pollen dosage depends on the load composition. When intra-specific pollen is no more than 75% in the pollen mixture, pollen from *P. caroliniana* has a competitive advantage. However, as the proportion of intra-specific pollen increased to 90%, inter-specific fertilizations occur at higher than expected frequencies apparently because of faster inter-specific pollen-tube growth rates.

The different pattern of fertilization in response to pollen load composition might be the result of pollen-pollen and/or pollen-style interaction during pollen-tube growth in *P. caroliniana*. Previous studies have suggested that pollen tube growth rate and the fertilization success of one donor's pollen may be modified depending upon the composition of the pollen pool (Nettancourt 1977; Visser 1983; Cruzan 1990).

Post fertilization gamete attrition may have contributed to the reduced frequency of F₁ hybrid seeds formation in *P. caroliniana*. The average maximum seed set in *P. caroliniana* is significantly lower than that of *P. viridis*, indicating that after fertilization there might be a selective abortion of ovules. It's very likely that intra-specific ovules are subject to the ovule abortion as indicated by the negative intercept in regression analysis of pollen dosage on fertilization success for this species. This reduction in seed production coincided with my observation of shrunken pink ovules in some of the fruits in this species (0.48 and 0.31 pink ovules per fruit in *P. viridis* and *P. caroliniana*, respectively.) I infer the shrunken pink ovules and are aborted ovules because they're larger than unfertilized white-small ovules, but smaller than fully-developed seeds. Since the rate of pink-aborted seeds is very low and selected abortion may be high in these species, I infer that some of the white-small ovules might be aborted ovules in the early stage of seed formation. Selective ovule abortion has been reported in a number of studies (Cooper and Brink 1940; Busbice, 1968; Bertin and Sullivan 1988; Marshall and Ellstrand 1988; Rigney et al. 1993)

including studies of inter-specific hybridization (Smith Clarkson 1956; Cruzan and Arnold 1994).

An important implication of the pollen competitive ability, gamete attrition, and seed set patterns of the *Piriqueta* species is that there is a directional bias in the formation of hybrid progeny. The findings suggest that the majority of F₁ hybrids will be produced by *P. viridis* plants when these species interact in nature. Asymmetric hybridization has been reported in many studies and has been suggested to be common in both plants (Buchholz et al. 1935; Lewis and Crowe 1958; de Nettancourt 1977, 1984; Kiang and Hamrick 1978; Levin 1978; Williams and Rouse 1988; Gore et al. 1990; Arnold et al. 1990; Carney et al. 1994; Emms et al. 1996) and animals (Coyne and Orr 1989; Kaneshiro 1990; Howard and Gregory 1993; Palumbi 1994; Wade et al. 1994). To further confirm the asymmetric hybridization in *Piriqueta*, more work is needed to check the patterns of natural hybridization.

The asymmetric pattern of fertilization of *Piriqueta* species is not expected based on results from other species. In some case short-styled taxa are less successful at fertilizing long-styled relatives (Buchholz et al.

1935; Smith 1970; Kiang and Hamrick 1978; Levin 1978; Gore et al. 1990; Herrick et al. 1993) with the exceptions of Emms et al.'s study of Louisiana irises (1996), in which they found short-styled *I. fulva* is more successful in siring hybrid seeds. In *Piriqueta*, however, there was no obvious difference in style length between *P. viridis* and *P. caroliniana*. *P. caroliniana* is more successful in siring seeds than *P. viridis*, so differences in style length is not responsible for the asymmetric pattern for these species. Pollen from *P. caroliniana* has a stronger competitive ability than pollen from *P. viridis*, therefore differences in pollen-tube growth rates contribute to this pattern.

Differential abortion might also cause the asymmetric pattern in *Piriqueta* species. Emms et al. (1996) suggested that selective abortion could be advantageous if hybrid embryos or hybrid seeds were less viable or fit than intraspecific seeds, given that resource were to be saved for other offspring. However, some evidence in the direction and severeness of differential ovule abortion in *Piriqueta* species needs to be further studied using molecular markers like RAPDs or RFLPs. While differences in self-compatibility and in ploidy level (Lewis and Crowe 1958; de Nettancourt

1977, 1984; Levin 1978) have been reported to cause asymmetric hybridization in other species pairs, both *P. caroliniana* and *P. viridis* are self-incompatible and diploid (Orrnduff, 1970), indicating that these factors are not responsible for the asymmetric hybridization patterns in *Piriqueta*.

Advanced generation hybrid formation.

The directionality to F₁ hybrid formation was also confirmed in the advanced-generation hybrid experiment. As in the previous experiment, *P. viridis* plants produced higher proportions of F₁ hybrid seed than *P. caroliniana*. The formation of F₁ hybrids can be a bridge and catalyst for higher rates of advanced generation hybrid production (Hodges et al. 1996).

In *P. caroliniana*, backcross seeds were produced at the same frequency as the intra-specific seeds, but the backcross frequency decreased significantly when interspecific pollen was present, where F₁ hybrid formation was favored. In *P. viridis*, backcross seed formation is much lower than F₁ hybrid formation, but is the same as intra-specific seed formation. On F₁ plants, both backcross and F₂ hybrids are produced.

Given that backcross and F_2 hybrid formation is frequent in these species, the production of F_1 hybrid would be expected to lead to the formation of a hybrid swarm.

Among both animal and plant taxa the F_1 hybrids are often less fit than the parental genotypes (Arnold and Hodges 1995). For example, F_1 progeny of *Helianthus annuus* and *H. petiolaris* have extremely low pollen fertility and seed production (means of 14% and 2% respectively (Heiser et al. 1969). But in *Piriqueta*, the F_1 hybrids has no obvious lower pollen fertility or lower seed viability. The high fitness of these *Piriqueta* hybrids may have to do with recent divergence between these species (Grun, 1976).

Based on the crosses conducted, we would expect that if F_1 hybrids were in close proximity to *P. caroliniana* plants, predominantly intra-specific and F_1 hybrid progeny would be produced on *P. caroliniana* plants and backcross seeds would be formed at a high frequency by F_1 hybrids. If F_1 hybrids were near *P. viridis* plants, then backcross seeds will probably be formed in equal proportions with intra-specific seeds by *P. viridis* plants, and backcross seeds plus F_2 hybrids would be produced at the same

frequency by F_1 plants. So when initial F_1 establishment occurs, introgression is likely to occur into the *P. viridis* genome since more backcross seeds would be produced by *P. viridis* plants. For F_1 plants occurring near *P. caroliniana*, backcross plants would be much less common and introgression would be restricted.

Given the patterns of F_1 and advanced-generation hybrid formation, in *Piriqueta* species, the formation of a hybrid swarm would be expected with introgression towards the genome of *P. viridis*. F_1 hybrids would be produced more by *P. viridis* than by *P. caroliniana*. After the establishment of initial hybrids, backcross seeds would be largely formed by *P. viridis*. Backcrossing would also occur with *P. caroliniana*, but at much less frequency especially when competing with the formation of F_1 hybrids. More complex backcross individuals would be expected to be formed after the formation of F_2 hybrids by F_1 plants. With the establishment of F_1 hybrids between *P. caroliniana* and *P. viridis*, introgression would occur into *P. viridis* with F_1 individuals acting as the catalyst.

Chapter V

Conclusion

1. There were no significant differences in pollen viability among *P. caroliniana*, *P. viridis* and F₁ plants. However, there was day-to-day variation in pollen viability.
2. There were significant differences in pollen production per anther among *P. caroliniana*, *P. viridis* and F₁ plants. Differences were also found between the two floral morphs within each species and among different plants.
3. F₁ hybrid seeds were produced at frequencies that were significantly different from the expected values for both *P. caroliniana* and *P. viridis*. For *P. viridis*, the frequencies of F₁ hybrids were higher than the expected for all the pollen mixtures except for 10% intra-specific pollen mixture. For *P. caroliniana*, the F₁ hybrids were formed at lower frequencies than the expected for all the pollen mixtures except for 90% intra-specific pollen mixture.

4. As the number of pollen grains on the stigma increased for *P. viridis*, the frequency of intra-specific fertilization tended to decrease indicating that the siring success of pollen was largely determined by the competitive ability of pollen. There was a high level of gamete attrition for *P. viridis* pollen which resulted in the negative intercepts for pollen mixtures consisting of 10% and 25% intra-specific pollen.
5. For *P. caroliniana*, the change in the frequency of intra-specific fertilization in response to pollen dosage was more complex and depended on the pollen load composition. The competitive ability of pollen was partially responsible for the reduced number of hybrids. There may also be stronger gamete attrition against intra-specific pollen in *P. caroliniana*.
6. The experimental parameters describing the relationship between pollen load size and seed set were $k = 33.72$ and $S/O_{\max} = 0.65$ for *P. caroliniana* and $k = 10.14$ and $S/O_{\max} = 0.77$ for *P. viridis*, respectively. Higher levels of post-fertilization gamete attrition probably occurred in *P. caroliniana*.

7. Pre-fertilization processes are unlikely to limit the formation of hybrid embryos in *P. viridis*. However, pollen from *P. caroliniana* was apparently subjected to high levels of pre-zygotic gamete attrition and may have provided an advantage to intra-specific pollen in *P. viridis*.
8. Pollen competition was largely responsible for the limitation of the formation of hybrids in *P. caroliniana*. Post-fertilization ovule abortion may have attributed to the hybridization patterns in *P. caroliniana*.
9. There was a directional bias in the formation of hybrid seeds. The majority of hybrids were produced by *P. viridis* plants, whereas *P. caroliniana* was more successful at siring seeds in both species than *P. viridis*.
10. Backcross seeds were produced at the same frequency as the intra-specific seeds, but decreased significantly when competed with F_1 hybrids. In *P. viridis* backcross seed formation was much lower than F_1 hybrid formation, but was the same as the intra-specific seed formation. On F_1 plants both backcross seeds and F_2 hybrids were produced with higher frequencies for backcross seeds.

11. Based on these results, I would expect that introgression would be more likely to occur to *P. viridis* populations when these species interact in nature.

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