

INTERSPECIFIC MATING IN THE *PIRIQUETA* *CAROLINIANA* (TURNERACEAE) COMPLEX: EFFECTS OF POLLEN LOAD SIZE AND COMPOSITION¹

JIONGQIAN WANG³ AND MITCHELL B. CRUZAN^{2,3,4}

²Department of Ecology and Evolutionary Biology; and

³Department of Botany, University of Tennessee, Knoxville, Tennessee 37996

Two taxa of *Piriqueta* (*P. caroliniana* and *P. viridis*) form a broad hybrid zone that extends over much of the central-Florida peninsula. We used genetic markers to examine the strength of the isolation barriers between these taxa and the patterns of mating at the initial stages of hybridization. Regression models were employed to analyze the effects of pollen load size and the proportions of intra- and interspecific pollen on the frequency of first-generation (F_1) hybrid formation. Overall, the postpollination mating barriers between these two taxa were relatively weak. However, there were significant effects of pollen load composition and size on the patterns of hybridization in both taxa with frequency-dependent responses to composition in both taxa. The frequency of F_1 hybrid formation was generally lower than expected based on the frequency of each pollen type on the stigma for *P. caroliniana* recipients. The lower frequencies of F_1 seeds in this taxon were apparently due to a greater competitive ability for intraspecific pollen, since hybrid seed formation decreased with increasing pollen load size. Pollen from *P. caroliniana* donors was also competitively superior on *P. viridis* recipients, leading to higher than expected frequencies of hybrid seed formation. Pollen from *P. caroliniana* did suffer higher rates of pollen-tube attrition than intraspecific pollen on *P. viridis* recipients, so the frequency of hybrid seed formation would be lower when pollen load sizes were small. In general, reproductive isolation mechanisms were stronger in *P. caroliniana*, suggesting that introgression should occur into *P. viridis* when these taxa come into close contact. Comparison of these expected patterns of mating to the distribution of hybrid genotypes in Florida provides insights into the relative roles of mating and selection in the evolution of hybrid populations of *Piriqueta*.

Key words: hybridization; interspecific mating; pollen competition; *Piriqueta*; reproductive isolation; Turneraceae.

Interspecific hybridization has been identified as an important component of the evolutionary history for a diverse set of plant and animal groups (Arnold, 1992, 1994; Rieseberg and Wendel, 1993; Masterson, 1994). Hybridization appears to be particularly common in plants. Indeed, based on levels of polyploidy it has been estimated that 70% of all flowering plant species have hybridization events in their phylogenetic histories (Stebbins, 1959; Whittman, Morrow, and Potts, 1991). In addition to ancient hybridization, studies of introgression in contemporary populations have detected numerous instances of gene transfers between subspecies, species, and even different genera of plants (Knobloch, 1972; reviewed in Arnold, 1992). Because natural hybridization is phylogenetically widespread as well as common (Stebbins, 1959; Grant, 1981; Ehrlich and Wilson, 1991; Whittman, Morrow, and Potts, 1991; Masterson, 1994), it represents a major evolutionary force for plants. Thus, to define the mechanisms that govern the frequency of natural hybridization is to define a major factor in plant evolution (Arnold, 1994). Central to the characterization of natural hybridization is an understanding of those processes of plant reproduction that may regulate interspecific mating.

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⁴ Author for correspondence.

Aspects of reproduction that might influence frequencies of interspecific hybridization include prepollination factors such as flowering time (Levin, 1978; Cruzan and Arnold, 1994), pollinator behavior (Levin, 1978; Campbell, Waser, and Melendez-Ackerman, 1997) and postpollination factors such as pollen–pistil interactions and patterns of ovule abortion (Buchholz, Williams, and Blakeslee, 1935; Levin, 1978; Carney, Cruzan, and Arnold, 1994; Rieseberg, Desrochers, and Youn, 1995). Although mating processes that limit successful crosses between different species have been recognized at least since the writings of Darwin (1859), to date there have been few analyses assessing the strength of mating barriers that exist between naturally hybridizing plant species (Smith, 1970; Kiang and Hamrick, 1978; Arnold, Hamrick, and Bennett, 1993; Carney, Cruzan, and Arnold, 1994; Rieseberg, Desrochers, and Youn, 1995; Carney, Hodges, and Arnold, 1996; Emms, Hodges, and Arnold, 1996).

In this study we test for postpollination mechanisms that affect interspecific compatibility between two hybridizing taxa of the *Piriqueta caroliniana* complex (Turneraceae); *P. caroliniana* Urban (= *P. caroliniana* var. *tomentosa*: Long and Lakela, 1971), and *P. viridis* Small (Small, 1933; = *P. caroliniana* var. *glabra*: Long and Lakela, 1971). There has been some disagreement concerning the taxonomy and delineations among forms in this group (Small, 1933; Long and Lakela, 1971; Arbo, 1995); however, for convenience we will be using Small's classification system (*P. caroliniana* and *P. viridis*) to describe the easily identifiable morphological forms that we have found in the field. Plants of *P. caroliniana* grow

in well-drained, deep sandy soils, have decumbent stems (<20 cm tall) with large elliptical leaves, and are densely pubescent with short tomentose stellate and long hirsute hairs on their leaves and stems. *P. viridis*, on the other hand, grows in the poorly drained soils of the southern pine flatwoods and is subject to periodic flooding. These plants are erect, between 20 and 60 cm tall, completely glabrous, and have narrow linear leaves. Morphologically uniform populations of each taxon can be found over broad geographic ranges with *P. caroliniana* occurring along the coastal plain of Georgia and the Panhandle region of Florida, and *P. viridis* growing in south Florida (exclusive of the Everglades) from Naples north to Lake Okeechobee. Furthermore, we have identified six diagnostic nuclear genetic markers (RAPDs: L. J. Martin and M. B. Cruzan, unpublished data) and chloroplast DNA sequence variation (S. D. Maskas and M. B. Cruzan, unpublished data) that delineate these two taxa. While it is not clear that these taxa should be considered as separate species, they do possess suites of distinguishing morphological and genetic characteristics that suggest they are more distinct than ecotypes.

Hybrids between these two taxa of *Piriqueta* have intermediate morphologies and can be found in a range of habitats throughout central Florida between Lake Okeechobee and Gainesville (Ornduff, 1970; L. J. Martin and M. B. Cruzan, unpublished data). We have found no present-day instances where populations of *P. caroliniana* and *P. viridis* are in close proximity, which suggests that the hybrid zone present in central Florida must be the result of historical interspecific mating and hybridization. Our study was initiated to understand how mating patterns that occurred at the initial stages of hybridization may have affected patterns of introgression and contemporary distributions of hybrid genotypes within and between populations.

At the initial stages of contact between two previously isolated species, we would not necessarily expect that hybridization would ensue as a result of the complete intermixing of two populations. Opportunities for interspecific mating may occur as a result of long-distance pollen or seed dispersal, particularly if the two taxa have disparate habitat affinities. As a consequence, we would expect pollen loads to contain varying amounts of intra- and interspecific pollen. To establish the strength of isolation barriers and to make predictions about patterns of mating between species it is important to understand the range of responses that might occur when flowers receive a variety of pollen load types.

Here we use mixtures of genetically marked pollen to examine the effects of different proportions of interspecific and intraspecific pollen on the siring of hybrid seeds for *P. caroliniana* and *P. viridis*. Specifically, we examine the effects of pollen load size and composition on the frequency of F₁ hybrid seed formation. The results from these experiments provide information on the potential for pre- and postzygotic processes to affect patterns of hybridization and introgression between these taxa in central Florida.

MATERIALS AND METHODS

Piriqueta taxa in North America are perennial herbs with apricot-colored flowers. The taxa studied here flower from June to August in

TABLE 1. Average pollen viability and production per anther for the long- and short-styled morphs of *P. caroliniana* and *P. viridis*. Means are followed by standard errors (in parentheses).

Morph	Species	N	Pollen number	N	Viability (%)
Long	<i>P. viridis</i>	6	2138.9 (149.1)	12	83.6 (3.3)
	<i>P. caroliniana</i>	6	1733.3 (108.2)	11	76.5 (5.7)
Short	<i>P. viridis</i>	3	1388.9 (72.2)	7	82.7 (3.5)
	<i>P. caroliniana</i>	6	1000.0 (87.1)	8	75.4 (4.1)

the field but will flower year-round in the greenhouse. In North America, plants of *Piriqueta* 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 (L morph) or flowers with short styles and long stamens (S morph). Plants used in this experiment were grown from seeds collected from allopatric populations in Effingham, Long, Wayne, and Lanier counties, Georgia in 1992 (for *P. caroliniana*), and Dade county, Florida in 1994 (for *P. viridis*).

We used controlled pollinations with genetically marked pollen in mixtures to assess the siring ability of different pollen types. Crosses were conducted using plants that were homozygous for one of two *Pgi* alleles. The 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 each morph of *P. caroliniana* and *P. viridis* 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 four randomly chosen plants of each morph from each taxon 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 using the Fluorescein Diacetate procedure (Heslop-Harrison and Heslop-Harrison, 1970; Kearns and Inouye, 1993). For each anther, the viability of 200 pollen grains was recorded by making transects across slides and counting the number of viable and dead pollen. The data were analyzed for difference in pollen viability among individual plants and taxa with ANOVA using the GLM procedure of SAS (SAS, 1985). In these analyses, the identification code for individual plants was entered as a random variable and was nested within taxon, which was entered as a fixed variable. We tested for differences among taxa using the variation among plants as the error term.

Pollen production—Before making pollen mixtures the average number of grains contained in anthers of plants from each style morph of each taxon was determined. Twelve plants of each style morph were chosen from each taxon. One anther was collected upon dehiscence from each plant and stored in 1 mL of distilled water in a microcentrifuge tube. The tubes were vortexed to disperse the pollen and the number of pollen grains in each of three subsamples of 10 μ L each were counted under a stereo microscope. Variation in pollen number among taxa was analyzed using the ANOVA procedure from SAS. Differences in the average number of pollen grains per anther produced by each taxon were found (Table 1), so adjustments were made when preparing pollen mixtures.

Pollinations—Pollinations were performed in a randomized incomplete block design using days as the blocking factor and rotating treatments among days. To more closely approximate natural pollination conditions, pollen mixtures consisted of pollen from at least five individuals of each style morph of each taxon. Wooden toothpicks were used to mix pollen and apply it to stigmas, taking care to wipe them

clean between pollinations. Pollen mixtures were made fresh each day by collecting anthers from five or more plants from both floral morphs of each taxon and mixing it together. These mixtures were then used for pollinations by covering toothpicks with pollen and gently touching the stigmas of flowers. Tests using counts of each pollen type based on their strong size difference indicated that this technique produced relatively equal and uniform mixtures of pollen (unpublished data). The styles were collected 6 h after pollination and fixed in 70% ethanol. In both *P. caroliniana* and *P. viridis*, pollen tubes have already grown through to the ovary by 3 h after pollination, and stigma removal after 6 h has no apparent effect on fruit and seed set (unpublished data). Pollen grains were stained by adding one drop of 0.1% malachite green to the styles in ethanol. The number of pollen grains adhering to stigmas were counted under a stereo microscope. Only ungerminated grains appeared to become dislodged during fixing and staining and were assumed to be inviable or to have been present on nonreceptive portions of the style.

Fruits were collected when mature (19 d 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 genotypes of the seeds determined using starch gel electrophoresis.

Starch gel electrophoresis—Seeds were ground for 10 s in 10 μ L extraction buffer (0.025 mol/L ascorbic acid, 0.01 mol/L $MgCl_2$, 0.1 mol/L KCl, 0.001 mol/L EDTA, 0.2 mol/L sucrose, 0.005 mol/L sodium bisulphate, 0.006 mol/L diethyldithiocarbamate, trace β -mercaptoethanol, and 0.2 g PVP-40T in 0.1 mol/L Tris-HCl buffer, pH 8) in 1.5-mL microcentrifuge tubes and 2×10 mm paper wicks were used to soak up the extract. Proteins were separated on 10%, 350 mL hydrolyzed potato starch gels in a morpholine-citrate pH 6.8 buffer system for 6 h at 50 mA and 180 V. The gel was then sliced and stained for PGI (phosphoglucosomerase) for 30 min in the dark at 37°C using methods described in Acquah (1992). The gel was rinsed in water and the genotype of each seed recorded. The paternity of seeds was inferred according to the genotypes of the pollen donors and recipients.

Experiments—**Pollen load composition**—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 F_1 hybrid seed formation. Pollen was mixed in the following ratios: 90:10, 75:25, 50:50, 25:75, and 10:90 of intra- to interspecific pollen. Pollinations were performed in a randomized incomplete block design with day as the blocking variable. Two flowers of each plant were pollinated with each pollen mixture and the genotypes of the resulting seeds were determined using starch gel electrophoresis as described above. The frequency of hybrids was calculated and compared to expected ratios using G tests for goodness of fit to the frequency of intra- and interspecific pollen in mixtures. The number of pollen grains and seeds were counted as described above. Regression analyses were used to assess the effect of pollen load size on the fertilization success of intra- and interspecific pollen for each pollen mixture (see below).

Pollen load size—A light pollen dosage and a heavy pollen dosage (50:50 mixture of intra- and interspecific pollen) were applied to two different flowers of the same plant to examine the effect of pollen number on the fertilization success of each pollen type. These two pollen dosages (refers to the total number of grains; intra- and interspecific pollen) were used to obtain a more even dispersion of pollen load sizes for regression analyses (see below). After pollination, styles were collected and the number of pollen grains adhering to each stigma counted as described above. Data were analyzed to test whether the observed percentage of hybrids differed significantly from the expected frequency using G tests. The effect of pollen number on siring success was ana-

lyzed using a regression model of mating system variation as described by Cruzan and Barrett (1996).

Data analysis—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 prezygotic (pollen failure before fertilization) and postzygotic (seed abortion) male gamete attrition (δ_i), and the competitive ability of pollen (α_i , the relative growth rate of each pollen type i). To examine the deviations from the expected fertilization in response to pollen grain number, we used a model similar to one previously used by Barrett and Cruzan (1996, p. 584),

$$F'_i = \delta_i + \alpha_i(G/O) \quad (1)$$

where F'_i is the deviation from the expected siring frequency for pollen type i ($F'_i = F_i - P_i$). This regression is an approximation of one given by Cruzan and Barrett (1996, Eq. 1), which 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 ; Cruzan and Barrett, 1996). When differences in pollen competitive ability exist, the proportion of seeds sired by each pollen type will depend on the total number of pollen grains present, which will produce a nonzero slope for the above regression.

Data from the pollen replacement series and pollen dosage experiments 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 G tests for goodness of fit. Pollen load size and siring success were regressed with the general linear model (GLM) procedure of SAS (SAS, 1985), using the deviation from random fertilization as the dependent variable and the total number of grains per ovule 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,

$$S/O = S/O_{\max} - \left[\frac{G/O_{\max} - G/O}{G/O_{\max}} \right]^k \quad (2)$$

where S/O is the ratio of the number of seeds to the number of ovules, S/O_{\max} is the average maximum seed set attained after full fertilization, 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, p. 584) since it allows the relationship to asymptote to seed set values that are not equal to one (as defined by S/O_{\max}). Data from the pollen dosage experiment were analyzed using Eq. 2 in nonlinear regression models (NLIN procedure: SAS, 1985) to estimate the value of S/O_{\max} and k for each recipient genotype/pollen-mixture combination.

RESULTS

Pollen production and viability—Differences in pollen viability between the two taxa of *Piriqueta* tended to be small. For both style morphs there were significant differences in pollen viability among days ($F = 21.10$, $P < 0.001$, $df = 3, 12$), but little difference among individual plants ($F = 1.67$, $P > 0.18$, $df = 20, 12$) or between morphs ($F = 0.02$, $P > 0.86$, $df = 1, 23$). The difference in pollen viability between taxa was small (Table 1) and not significant for comparisons within each morph ($F = 3.14$, $P > 0.09$, $df = 1, 11$ for the L morph and $F = 7.56$, $P > 0.05$, $df = 1, 8$ for the S morph) or for both

morphs combined ($F = 4.75$, $P > 0.06$, $df = 1, 8$). Hence, pollen of the same compatibility type from these two taxa had similar levels of viability.

There were consistent differences in the average number of pollen grains produced by each style morph in each taxon (Table 1), and adjustments were made when making mixtures of pollen by combining different numbers of anthers from each morph to compensate for differences in pollen production. Average pollen number per anther depended both on the taxon ($F = 10.09$, $P < 0.006$, $df = 1, 17$) and on the style morph ($F = 35.16$, $P < 0.0001$, $df = 1, 17$), but not on the interaction between these two factors ($F = 0.00$). There were differences in pollen production among plants ($P < 0.01$), but the magnitude of these differences was small and should have been compensated for by the inclusion of anthers from several individuals of each genotype in pollen mixtures.

Stigma load composition—The overall frequency of intraspecific and F_1 hybrid seeds was different from the expected frequencies across treatments when *P. viridis* was acting as the maternal parent ($G = 111.13$, $P < 0.001$, Fig. 1). The number of intraspecific seeds produced on *P. viridis* was significantly above the expected number by 28.1% when intraspecific pollen was only 10% in the pollen mixture (Fig. 1), but decreased from 3.0% below the expected value with 25% intraspecific pollen to 13.8% below for the 90% intraspecific pollen mixture (Fig. 1). As the pollen load composition changed across the range of values tested, the fertilization success of intraspecific pollen in *P. viridis* tended to be high when it was in the minority but increased more slowly when intraspecific pollen dominated the pollen load, which produced a nonlinear response of fertilization success to pollen load composition (Fig. 1).

The observed number of intraspecific seeds sired in *P. caroliniana* was significantly different from the expected for all the pollen mixtures (Fig. 1). The difference between the observed and expected number of intraspecific seeds decreased from 16.8% above expected at 10% intraspecific pollen to 17.8% below expected when intraspecific pollen comprised 90% of the stigma load (Fig. 1). The number of intraspecific seeds remained above the expected over the range of pollen ratios from 10 to 75%, but then decreased below expected at 90% intraspecific pollen (Fig. 1).

Pollen load size—For all pollen mixtures on *P. viridis* recipients, the frequency of intraspecific fertilizations tended to decrease as the total number of pollen grains on the stigma increased as indicated by the negative slopes for the regressions with pollen load size (as indicated by the negative regression slopes: Table 2). All of these regression slopes were similar across pollen mixtures as indicated by the lack of a significant interaction between pollen load size and composition ($F = 0.07$, $P > 0.98$, $df = 4, 28$). However, only the slope from the 25% mixture was significantly different from zero (Table 2), and there was no overall significant effect of pollen number on the frequency of intraspecific fertilizations ($F = 1.65$, $P > 0.21$, $df = 1, 28$). These nonzero slopes indicate that intraspecific pollen's competitive ability was weak in *P. viridis*, with the frequency of hybrid

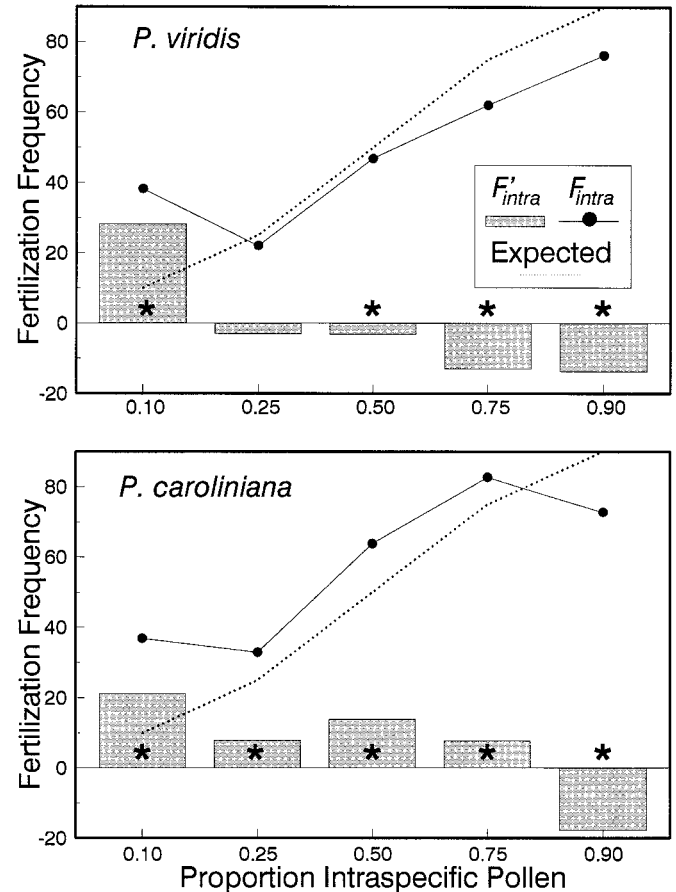


Fig. 1. The fertilization success of intraspecific pollen for pollen loads with varying proportions of intra- and interspecific pollen in *Piriqueta viridis* and *P. caroliniana*. Bars indicate deviations from the expected fertilization success of intraspecific pollen for each mixture (F'), solid lines indicate the absolute fertilization success (F), and the dotted lines represent the expected fertilization success of intraspecific pollen based on its frequency in the pollen mixture. Asterisks on the bars indicate a significant deviation from the expected fertilization frequency for that mixture.

seeds increasing when pollen load sizes were larger. There was also evidence for differences in male gamete attrition in *P. viridis*, which resulted in a relative advantage for intraspecific pollen when it was at low frequency (i.e., positive intercepts for the 10 and 25% mixtures; Table 2).

For *P. caroliniana*, the change in the frequency of intraspecific fertilization in response to pollen dosage was more complex and depended on the pollen load composition (Table 2), as indicated by a significant interaction between pollen load size and composition ($F = 8.63$, $P < 0.001$, $df = 4, 56$). As the proportion of intraspecific pollen increased in pollen loads, the relationship between load size and intraspecific fertilizations changed from a positive to a negative slope (Table 2). The fraction of seeds sired by *P. caroliniana* pollen tended to increase with pollen load size when the frequency of intraspecific pollen was between 10 and 50% (Table 2). For the 75 and 90% intraspecific pollen mixtures, on the other hand, the fraction of seeds sired by *P. caroliniana* pollen decreased as the number of grains increased (Table 2), pro-

TABLE 2. Sample sizes (N), fruit set, seed set, seed to ovule ratios (S/O), and regression analyses for different stigma load compositions of intra- and interspecific pollen in *Piriqueta caroliniana* and *P. viridis*. Boldface indicates parameters that differ significantly from zero ($P < 0.05$).

Taxon	Proportion intraspecific pollen	N	Fruit set frequency	Seeds per fruit	S/O	Intercept	Slope
<i>P. viridis</i>	0.10	10	0.16	12.2	0.48	0.268*	-0.06
	0.25	11	0.17	23.6	0.82	0.399*	-0.26*
	0.50	17	0.18	16.9	0.63	-0.028	-0.011
	0.75	7	0.44	15.6	0.73	0.257	-0.32
	0.90	5	0.31	15.2	0.84	-0.039	-0.08
<i>P. caroliniana</i>	0.10	6	0.38	7.5	0.39	-0.270	0.74**
	0.25	15	0.94	11.7	0.67	0.097	0.01
	0.50	23	0.24	11.0	0.61	-0.161*	0.39***
	0.75	18	0.28	15.1	0.71	0.088	-0.02
	0.90	18	0.28	10.1	0.49	0.021	-0.31*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ducing a significant disadvantage for intraspecific pollen when it comprised 90% of the stigma load (Fig. 1).

The superior competitive ability of intraspecific pollen when it was at lower frequencies in the stigma loads of *P. caroliniana* may have been offset by higher levels of male gamete attrition. The negative intercepts for the 10 and 50% intraspecific pollen mixtures indicate that there

were higher levels of intraspecific gamete attrition manifested either as pollen-tube attrition or abortion of ovules fertilized by intraspecific pollen (Table 2). There did not appear to be a significant effect of pollination day for either species ($P > 0.2$ for both analyses).

Patterns of fecundity—Seed numbers increased with pollen load sizes in both *P. viridis* and *P. caroliniana* (Fig. 2). By transforming seed production into relative fecundity and using the model described above (2), we can determine the level of pre- and postfertilization gamete attrition (i.e., pollen-tube attrition and seed abortion). The relative fecundity increased rapidly when pollen grain numbers were small and remained fairly constant over a range of larger pollen loads (Fig. 2). The exponential parameter describing the relationship between pollen load size and seed set (k) was larger for *P. caroliniana* ($k = 33.72$) than for *P. viridis* ($k = 10.14$; 95% confidence intervals do not overlap the means), indicating that overall rates of prefertilization gamete attrition were lower in the former taxon (i.e., a larger pollen load was required to achieve full seed set in *P. viridis* because of higher levels of pollen-tube attrition in this taxon: Fig. 2).

Levels of postfertilization gamete attrition (seed abortion) can be inferred from the asymptotic value of the relationship described above (Eq. 2: S/O_{\max}). The value of S/O_{\max} for *P. caroliniana* tended to be lower than that for *P. viridis* ($S/O_{\max} = 0.65$ for *P. caroliniana* and 0.77 for *P. viridis*; 90% confidence intervals do not overlap the means), indicating higher levels of postfertilization ovule abortion probably occurred in the former taxon.

Patterns of fecundity also changed with pollen load composition in these two taxa of *Piriqueta* (Table 2). In *P. viridis* the seed to ovule ratio (S/O) was relatively low with only 10% intraspecific pollen, but was substantially higher for pollen loads that contained lower proportions of interspecific pollen (Table 2). In *P. caroliniana* recipients, S/O values were highest for intermediate pollen mixtures and were lower when either pollen type dominated stigma loads (i.e., 10 and 90%: Table 2).

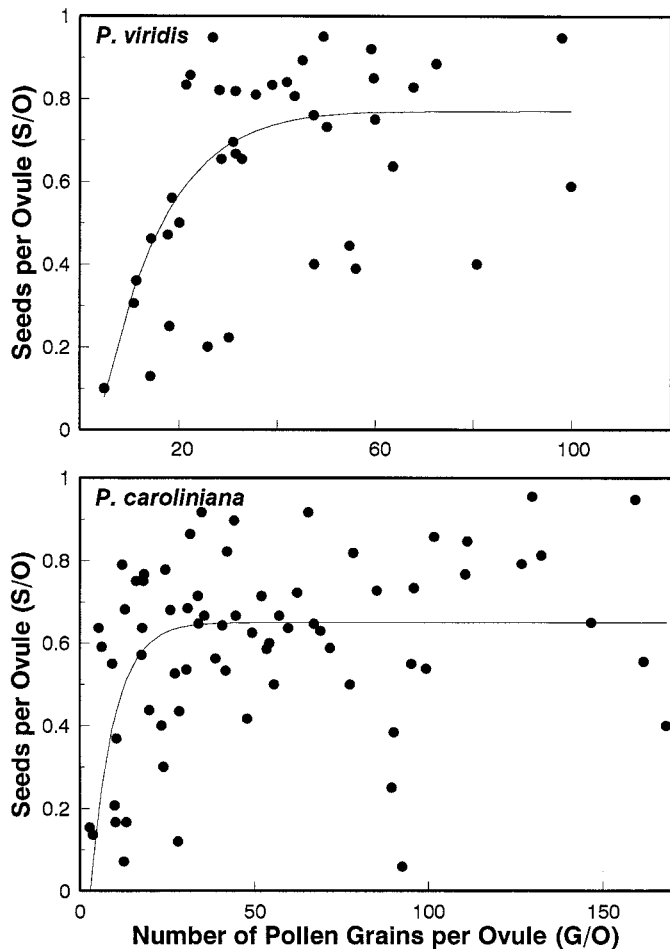


Fig. 2. Changes in fecundity in response to pollen load size in *Piriqueta viridis* and *P. caroliniana*. Predicted relationships (solid lines) are from least-squares estimates using Eq. 2 (see text for details).

DISCUSSION

Patterns of siring success for different combinations of pollen load composition and size in these *Piriqueta* taxa

suggest that the formation of F_1 hybrids is controlled by both pre- and postfertilization processes. Overall, the postpollination mating barriers between these taxa are relatively weak, and depending on the rates of interspecific pollen transfer, a high frequency of F_1 hybrid seed formation may be expected when their distributions overlap. However, the reproductive isolating mechanisms in *P. caroliniana* and *P. viridis* are not equally strong, which may result in asymmetrical introgression. Moreover, the contributions of pollen competition, pollen-tube attrition, and embryo abortion to the formation of F_1 hybrids appear to differ for the two taxa and to depend on the relative frequency of intra- and interspecific pollen in the stigma loads. The observed patterns of F_1 hybrid seed formation will have implications for the frequency of hybridization and the genetic structure of hybrid populations.

Pre- and postfertilization processes appear to be capable of reducing the frequency of F_1 hybrid seed formation by *P. caroliniana* plants, but are unlikely to limit the formation of hybrid embryos in *P. viridis*. In both taxa, a combination of pollen competition and differences in gamete attrition appeared to contribute to patterns of nonrandom fertilization. Several previous studies have used pollen-tube measurements to infer the frequency of the expected natural hybridization (Arnold, Hamrick, and Bennett, 1993; Carney, Cruzan, and Arnold, 1994; Rieseberg, Desrochers, and Youn, 1995; Carney, Hodges, and Arnold, 1996; Emms, Hodges, and Arnold, 1996), however observable differences in pollen-tube growth are not always predictive of the known patterns of hybridization (e.g., Rieseberg, Desrochers, and Youn, 1995; Emms, Hodges, and Arnold, 1996). In the current study we have used mixed loads of genetically marked pollen to infer the siring ability of different pollen types. By estimating the response of siring success and fecundity to pollen load size we can simultaneously assess the separate contributions of pre- and postfertilization processes to expected frequencies of hybridization under a variety of pollination environments.

A combination of pollen competition and attrition appeared to determine the siring success of intra- and interspecific pollen in *P. viridis* recipients. For all of the pollen mixtures except 10% intraspecific pollen treatment, the frequency of hybrid seed produced by plants of this taxon exceeded the expected frequency. Moreover, the proportion of F_1 seeds in fruits tended to increase as the size of the pollen load increased, indicating that pollen from *P. caroliniana* had a competitive advantage over intraspecific pollen in *P. viridis*. On the other hand, interspecific pollen appeared to suffer higher rates of prezygotic attrition. This was apparent from the significantly positive intercepts for pollen mixtures of 10 and 25% intraspecific pollen (indicating higher rates of gamete attrition). The higher rates of gamete attrition evident from positive intercepts and from the lower rate of increase in seed set with pollen load size (Fig. 2, i.e., k was lower for *P. viridis*) combined with the overall lower rate of postfertilization ovule abortion for *P. viridis* (i.e., S/O_{\max} was higher for *P. viridis*) suggest that the attrition of interspecific gametes occurred before rather than after fertilization. The lower overall fecundity when intraspecific pollen comprised only 10% of the pollen load com-

pared to the other pollen-mixture treatments is also indicative of higher rates of prezygotic gamete attrition for interspecific pollen.

Pollen competition appears to be largely responsible for limiting the formation of hybrids in *P. caroliniana* recipients, but patterns of fertilization in response to pollen dosage are dependent on pollen load composition. When intraspecific pollen comprised <75% of the pollen mixture, it appeared to have a competitive advantage over interspecific pollen. However, as the proportion of intraspecific pollen increased to 90%, interspecific fertilizations were higher than expected, apparently as a result of faster growth rates for *P. viridis* pollen. Frequency-dependent responses in the fertilization ability of pollen from different sources have not previously been reported in this or any other species. Such frequency-dependent effects on the relative success of intra- and interspecific pollen may have implications for patterns of mating and gene flow within and between species.

The observed frequency-dependent responses might be the result of pollen-pollen interactions or could be mediated by the style. Studies suggesting that pollen-tube growth and fertilization success may be modified depending upon the composition of the pollen pool are relatively rare (e.g., Thomson, Andrews, and Plowright, 1981; Shore and Barrett, 1984; Visser and Marcucci, 1986; Cruzan, 1990), however few attempts have been made to examine such responses. The present study differs from most others that have detected composition-dependent siring success because we have demonstrated that the fertilization ability of different pollen types in *Piriqueta* depends on their frequency in the pollen load, and not simply on the presence or absence of different pollen types (but see Shore and Barrett, 1984). The resulting seed-siring patterns change as a function of the frequencies of different pollen types on stigmas, making ecological and demographic factors that affect the pollen environment more important to the evolutionary processes of hybridization and introgression.

While pollen competition appears to be the primary interspecific isolating mechanism in *P. caroliniana*, selective abortion of hybrid embryos may also occur in this taxon. The average maximum seed set (S/O_{\max}) in *P. caroliniana* is significantly lower than in *P. viridis*, indicating higher rates of postfertilization ovule abortion. This observation combined with the relatively low fruit and seed set after pollination with mixtures that contained 90% interspecific pollen suggests that hybrid ovules may have suffered higher rates of abortion in *P. caroliniana* recipients. Selective ovule abortion has been found to contribute to differential siring success in a number of instances (Cooper and Brink, 1940; Busbice, 1968; Bertin and Sullivan, 1988; Marshall and Ellstrand, 1988; Rigney et al., 1993) including studies of interspecific hybridization (Smith and Clarkson, 1956; Cruzan and Arnold, 1994). While it is clear that overall rates of postfertilization gamete attrition are apparently higher in *P. caroliniana*, additional experiments would be required to confirm whether hybrid embryos were subject to higher rates of abortion.

The observed differences in pollen competitive ability, gamete attrition, and seed set patterns of these *Piriqueta* taxa would be expected to produce asymmetrical hybrid-

ization between these two taxa. An unequal strength in reproductive isolation barriers for pairs of plant species has been found in a number of other instances (Buchholz, Williams, and Blakeslee, 1935; Lewis and Crowe, 1958; de Nettancourt, 1977, 1984; Kiang and Hamrick, 1978; Levin, 1978; Williams and Rouse, 1988; Arnold, Hamrick, and Bennett, 1990; Gore et al., 1990; Carney, Cruzan, and Arnold, 1994; Cruzan and Arnold, 1994; Emms, Hodges, and Arnold, 1996) and may be relatively common. In most of these cases several characteristics have been identified that are associated with asymmetrical hybridization; these include (1) style length differences (short-styled species are less successful at fertilizing long-styled relatives: Buchholz, Williams, and Blakeslee, 1935; Smith, 1970; Kiang and Hamrick, 1978; Levin, 1978; Gore et al., 1990; Herrick, Murray, and Hammett, 1993; see Emms, Hodges, and Arnold, 1996, for an exception), (2) unilateral self-incompatibility (self-incompatible species form fewer hybrid seed: Lewis and Crowe, 1958; de Nettancourt, 1977; Levin, 1978; Harder, Cruzan, and Thomson, 1993), and (3) differences in ploidy (Levin, 1978). None of these mechanisms appear to be operating in *Piriqueta* since there are no obvious differences in style length between the same morphs of *P. viridis* and *P. caroliniana* (personal observation), both taxa are self-incompatible (Ornduff and Perry, 1964; unpublished data), and both taxa are diploid with $N = 7$ (Ornduff, 1970). However, such a difference in the strength of mechanisms reducing interspecific mating could also be a consequence of historical factors and past opportunities for selection to reinforce reproductive barriers (Butlin, 1987; Howard, 1993). Additional studies of the strength of isolating mechanisms for populations in different portions of each taxon's range may help elucidate the potential role of selection in producing the observed differences in F_1 hybrid seed formation.

The pattern of hybridization and the degree and direction of introgression that would be expected for these taxa of *Piriqueta* will depend largely on ecological circumstances. With widely separated populations we may expect interspecific pollen transfers to be rare, and hence pollen loads would be dominated by intraspecific pollen. Under these conditions F_1 hybrid formation would be facilitated by both taxa, but the potential for introgression would depend on the frequency of hybrids formed. On the other hand, if populations come into close contact, then we would expect the rapid formation of first-generation hybrids that were primarily produced by *P. viridis* maternal parents. However, the potential for subsequent introgression could be largely determined by the fitness of hybrid genotypes in the parental habitats (Howard et al., 1993; Wang et al., 1997) as well as mating patterns among hybrid and parental genotypes.

Genetic and morphological marker analyses indicate that the patterns of hybridization between these taxa of *Piriqueta* are generally consistent with the patterns of interspecific mating found in this study. In particular, as would be predicted from relatively weak mating barriers, introgression between these taxa has been extensive, resulting in a very broad zone that extends over much of central Florida (L. J. Martin and M. B. Cruzan, unpublished data). However, introgression has been apparently in the direction of *P. caroliniana*, rather than into *P. vir-*

idis. Since the present analysis would have predicted introgression in the opposite direction under conditions where pollen loads were large, it may have been the case that pollen loads were more moderate in size or that selection on parental and hybrid genotypes has contributed to patterns of introgression in the past. Additional studies of reproductive interactions among parental and advanced-generation hybrid genotypes and field studies of the distribution and fitness of parental and hybrid genotypes will help elucidate the contributions of nonrandom mating and selection to patterns of hybridization and introgression between this pair of *Piriqueta* taxa.

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