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## POLLEN COMPETITION IN *TURNERA ULMIFOLIA* (TURNERACEAE)<sup>1</sup>

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We performed mixed pollinations, involving self vs. within- or between-population pollen, and used starch gel electrophoresis to assay 2,210 progeny to detect pollen competition in *Turnera ulmifolia*. Within populations there was no evidence for a competitive advantage to outcross pollen. Between populations a marked advantage to self pollen occurred for most populations, and the extent of this competitive advantage was correlated with increasing morphological divergence of the outcross pollen donor population. A comparison of rates of ovule abortion revealed that the biased paternity ratios cannot be the result of ovule abortion alone, and that pollen competition must be the major contributing factor. We suggest that reproductive divergence among the populations, perhaps through adaptation to different pollinators or through the evolution of increased selfing, has resulted in the evolution of reproductive isolation through pollen competition.

In many self-compatible hermaphroditic plants, stigmas capture a mixture of self and outcross pollen. The proportion of self to outcross pollen, however, will not necessarily reflect the proportion of self to outcross seeds (Jones, 1922; Johnston, 1993; Rigney et al., 1993; Niovi Jones, 1994), as a number of mechanisms may lead to differential fertilization success (Marshall and Ellstrand, 1986; Lyons et al., 1989). The processes of pollen germination (Thomson, 1989) and growth of pollen tubes down the style provide the opportunity for competition among male gametophytes for access to ovules. Additionally, it may be possible for the maternal plant to influence the probability that a particular embryo will mature to seed and hence exhibit choice among potential mates.

For pollen competition to occur it is necessary that a plant receive excess pollen in relation to the amount required for full seed set (Mulcahy, Curtis, and Snow, 1983; Marshall and Ellstrand, 1986; Ramstetter and Mulcahy, 1986; Snow, 1986a, b; Mulcahy and Bergamini Mulcahy, 1987; Snow and Mazer, 1988; Snow and Spira, 1991b; Spira et al., 1992), and this often seems to be the case (Snow, 1986a, b; Spira et al., 1992). Several studies have suggested that genes determining the vigor of a diploid sporophyte also influence pollen tube growth rates and may mediate pollen competition (Mulcahy, 1971; Mulcahy and Bergamini Mulcahy, 1975; Johnson and Mulcahy, 1978; Mulcahy and Kaplan, 1979; Mulcahy and Bergamini Mulcahy, 1987; Charlesworth and Charlesworth, 1992; Hormaza and Herrero, 1992).

The effects of pollen competition have been investigated both within species, and to a lesser extent, between species. There are a number of consequences that nonrandom fertilization, due to pollen competition, may have within a plant population. Pollen competition favoring outcross pollen may result in a decrease in the production of selfed

progeny and hence both reduce the extent of inbreeding depression (although the effect might be small, see Charlesworth and Charlesworth, 1992) and alter the genetic structure of the population. Pollen competition can also affect the number of seeds that a plant sires (Jones, 1922; Marshall and Ellstrand, 1986; Marshall, 1991). Self-discrimination mechanisms involving specific reactions between the maternal parent and self pollen, pollen tubes, or embryos may determine the outcome of the competition (e.g., Aizen, Searcy, and Mulcahy, 1990; Montalvo, 1992; Rigney et al., 1993; but see Johnson and Mulcahy, 1978; Snow and Spira, 1991a). Few studies of interspecific pollen competition have been carried out, but it may be important in determining the degree of hybridization and gene exchange among taxa (Arnold, Hamrick, and Bennett, 1993; Carney, Cruzan, and Arnold, 1994).

Female plants may also influence the postpollination success of mates (e.g., Janzen, 1977; Marshall and Ellstrand, 1986, 1988; Marshall, 1988; Lyons et al., 1989). Mechanisms by which female plants choose mates are important since by influencing the paternity of their seeds or by adjusting the seed size and number based on paternity, females may be able to improve offspring fitness. Possible mechanisms of maternal choice include maternal control of pollen germination, pollen tube growth rate, fertilization, seed abortion, fruit abortion, and seed filling (Marshall and Ellstrand, 1988). Selective seed abortion within a population has regularly been implicated as a source of maternal influence although it was not documented until recently (Marshall and Ellstrand, 1988; although see Lyons et al., 1989).

Much of the work on postpollination selective mechanisms has concentrated on pollen competition with particular focus on the differential success of self vs. outcross pollen (Jones, 1922; Johnson and Mulcahy, 1978; Mulcahy, Curtis, and Snow, 1983; Johnston, 1993; Rigney et al., 1993; Snow and Spira, 1993). Here we conduct experiments to detect within- as well as between-population pollen competition in *Turnera ulmifolia* L., a species known to exhibit a mixed mating system (Belaoussoff and Shore, 1995).

Since inbreeding depression occurs within populations of *T. ulmifolia* (Belaoussoff and Shore, 1995), we might expect that there would be a competitive advantage to outcross pollen within populations. However, populations are often small and may be highly inbred (Belaous-

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TABLE 1. Localities of populations and numbers of plants used in pollination (as male and female parents) and morphological studies.

Population	Locality	Number of plants		
		Female	Male	Morphology
Pelican Lake (BAH)	Grand Bahama Island	—	3	17
Bermuda (BER)	Bermuda	—	4	3
Jamaica				
Falmouth (FAL)	Falmouth, Trelawny	8	6	20
Irish Town (IRT)	Irish Town, St. Andrew	6	6	20
Mandeville (MAN)	Mandeville, Manchester	3	6	8
Paradise (PAR)	Paradise, Hanover	10	17	20
Quaco Rock (QUA)	Quaco Rock, Trelawny	—	8	14

soff and Shore, 1995) and genetically depauperate. As a result, we also carry out between-population competitive pollinations. We expected that for populations that are not too genetically divergent there would be a competitive advantage to outcross pollen. For very divergent populations, we might expect a competitive advantage favoring self (or within population) pollen, perhaps due to reproductive divergence among populations. We address specifically the following questions: 1) Is there competition between self and outcross pollen within populations of *T. ulmifolia*? 2) Is there competition between self and outcross pollen for between-population crosses, and is it associated with morphological divergence among populations? 3) Is there evidence for maternal choice via selective abortion?

## MATERIALS AND METHODS

**The study organism**—*Turnera ulmifolia* L. (Turneraceae) is an insect-pollinated, perennial weed native to the Neotropics (Barrett and Shore, 1987). The hexaploid populations used in this study show varying degrees of herkogamy (stigma-anther separation) as well as differences in other morphological characteristics (see Results). The plants are self-compatible, and a negative correlation between the amount of selfing and degree of herkogamy occurs (Barrett and Shore, 1987; Belaoussoff and Shore, 1995). Outcrossing rates vary from 0 to 69% among Jamaican populations, and therefore a considerable number of selfed seeds are set in natural populations (Belaoussoff and Shore, 1995). There is no evidence for inbreeding depression at early portions of the life history, but multiplicative fitness functions provide evidence for inbreeding depression across life history stages (Belaoussoff and Shore, 1995).

**Population samples**—Populations used in this study included five populations from Jamaica, one from the Bahamas, and one from Bermuda (Table 1). The Jamaican populations belong to one of two intergrading taxonomic varieties—*T. ulmifolia* var. *angustifolia* Willd. or var. *ulmifolia*—and considerable morphological variation occurs among populations on Jamaica (du Quesnay, 1971; Barrett and Shore, 1987). The plants from Grand Bahama Island and Bermuda are *T. ulmifolia* var. *acuta*. Hybrids have been made among all populations (with the exception of the plants from Bermuda, which were only recently

collected), and they are always highly pollen fertile (J. Shore, unpublished data). The Jamaican population from Quaco Rock was propagated from selfed seeds and cuttings sampled from the population in 1977 (Barrett #1337). The Bahamian plants were propagated from seeds taken from a herbarium specimen (Correll #40638), and the Bermuda sample was propagated from a bulk seed collection. Relatively few plants were available for these three populations and so they were used only as outcross pollen donors (males). All the remaining populations were sampled from a number of open-pollinated maternal plants in Jamaica in 1991 and 1992. The Paradise and Falmouth populations were chosen to be the primary focus of the study involving between-population crosses. These samples contained the greatest number of plants and also show a considerable degree of herkogamy (Belaoussoff and Shore, 1995), reducing the opportunity for contamination due to autogamy.

**Morphological comparisons**—Seventeen morphological characters were measured for all populations to determine the extent of morphological divergence among the populations. The characters measured included style length, stamen length, hypanthium length, flower diameter, flower depth (the distance from the base of the ovary to the top of the open petals), petal length and width, sepal length, bracteole length, width, and number of teeth, leaf length, width, and number of teeth, petiole length, and pollen length and width. Continuous characters were measured to the nearest 0.1 mm using calipers, and pollen length and width were measured using a calibrated ocular micrometer on a compound microscope. We used canonical discriminant analysis to construct a set of Mahalanobis distances among populations (Klecka, 1980; SAS, 1988) and later used this information as a possible predictor of pollen competitive ability.

**Pollinations**—Two types of competitive pollinations, as well as pure self and outcross control pollinations, were made in a pollinator-free glasshouse. The experimental treatment used most frequently involved a pollination in which one stigma of a flower was pollinated with two anthers of self pollen and a second stigma of the same flower was pollinated with two anthers from a genetically marked outcross pollen donor. This type of cross will be referred to as a “separate” pollination. Fine-tipped forceps were used to pollinate the stigmas. The remaining unpollinated stigma and anthers were removed from the flowers. Our rationale for using this type of competitive pollination was that since populations may differ in pollen production, it would be best to saturate each stigma with one pollen source to circumvent difficulties in attempting to mix equal numbers of pollen grains from two populations. A smaller number of “simultaneous” pollinations were also performed in which anthers from both self and outcross donors were applied simultaneously to all stigmas. Control crosses for both types of competitive pollinations involved pollinating stigmas of different flowers on the same maternal plant with only outcross pollen from the pollen donor or self pollen.

Seed capsules from competitive and control pollinations were collected when ripe, approximately 16–21 d after the pollinations were made. Seeds from each cross

were stored in separate envelopes until sown. The number of aborted or unfertilized ovules and the number of seeds were counted. Small white ovules were scored as unfertilized/aborted since we can't distinguish between these two possibilities (henceforth, we will refer to them as aborted). Seeds from each cross were sown into separate pots, following the methods of Shore and Barrett (1985). For each pot, percent germination was recorded, and seedlings were assayed using isozyme electrophoresis.

Within-population competitive pollinations were performed for the Paradise, Falmouth, and Mandeville populations. Between-population competitive pollinations were made using plants from Paradise, Falmouth, Mandeville, and Irish Town populations as maternal parents. Individuals from all populations served as outcross pollen donors.

**Isozyme markers**—The parental plants were screened for variation at six enzymes. The assays for esterase (EST), aconitase (ACO), phosphoglucosomerase (PGI), phosphoglucosomutase (PGM), malate dehydrogenase (MDH), and 6-phosphoglucosomate dehydrogenase (6-PGD) followed the methods of Shore and Barrett (1987) and Wendel and Weeden (1989). Ovaries from mature flowers were used since they yield high activity. Of the enzymes screened for the parental plants, PGM, PGI, and MDH showed the highest levels of activity in seedlings so these provided the genetic markers used to determine the paternity of offspring from competitive pollinations. Seedlings were generally run at the cotyledon stage and were destructively sampled. For the majority of crosses, all seedlings were assayed.

**Statistical analyses**—Paternity data were analyzed using goodness of fit and heterogeneity  $G$ -tests (Sokal and Rohlf, 1981).  $G$ -statistics were calculated as follows: 1) the progeny of each cross were tested against the null hypothesis of equal paternity (1:1 ratio); 2) all of the progeny for every cross in an experimental class were pooled (i.e., all progeny for a particular set of within- or between-population crosses) and tested against the null hypothesis of equal paternity ( $G_{\text{pooled}}$ ); and 3) a heterogeneity statistic,  $G_{\text{het}}$ , was calculated to determine if the paternity ratios differed among the individual crosses in each set.

To determine if maternal choice via selective embryo abortion might contribute to the nonrandom paternity ratios, the mean proportion of aborted ovules for all treatment and control pollinations was compared using analysis of variance. The proportion of aborted ovules was angular-transformed prior to analysis. If selective ovule abortion accounts for the deviant paternity ratios, then we might expect to see higher levels of ovule abortion in competitive pollinations compared to the controls. Control pollinations included both self and outcross pollinations. Separate analyses were conducted for crosses involving populations from Falmouth, Mandeville, and Paradise.

## RESULTS

The populations used in this study differ in a number of attributes including both vegetative and floral char-

acteristics. Some of the characters showing marked and significant differences among the populations are listed in Table 2. Significant differences in style length and pollen size (Table 2) might be important in determining siring success when competitive pollinations between populations are carried out. The population from Quaco Rock is noteworthy as it possesses the largest pollen and longest styles. Its flowers are unique among populations as the petals do not reflex, and therefore, at anthesis, the corolla is tubular rather than dish-shaped. Its long styles protrude well beyond the corolla tube. Crossing studies (J. Shore, unpublished data) reveal that it sets few seeds when used as a female parent in crosses with other populations, although hybrids made in reciprocal crosses are fertile.

We carried out a discriminant analysis to compare the morphology of populations and construct a set of Mahalanobis distances among them (Table 3). The population centroids are plotted in a space defined by the first two discriminant functions, which possess 87% of the discriminating power (Fig. 1). The plants from Quaco Rock appear to be the most morphologically divergent. This population occurs in the Cockpit Country of Jamaica, an area having high rates of endemism. The plants from Bermuda and Grand Bahama Island are morphologically divergent from the Jamaican populations.

A total of 2,210 progeny from competitive pollinations was assayed electrophoretically to determine their paternity. The parental plants were chosen for crosses such that they were homozygous for alternative alleles at one or more isozyme loci. The paternity of individual progeny could be easily determined, as outcrossed progeny were heterozygotes while selfed progeny were homozygotes.

**Within-population pollinations**—A total of 765 progeny from within-population crosses was scored. These included within-population crosses for the Falmouth, Mandeville, and Paradise populations (Tables 4–6). There is no evidence for a competitive advantage favoring outcross pollen for these populations (Tables 4–6). The Falmouth crosses and the single cross from Mandeville show no significant deviation from a 1:1 paternity ratio (Tables 5, 6), nor is there significant heterogeneity among crosses (Table 5). For the population from Paradise, the pooled progeny ratios actually show a significant excess of selfed progeny (Table 4). Considerable heterogeneity, however, occurs among replicate crosses, with some individual crosses showing a statistically significant excess of selfed progeny while one shows the reverse (Table 4). The simultaneous pollinations appear to give more variable results. Pooling the within-population crosses for the three populations, the ratio of selfed to outcross progeny is 412:353 ( $G_{\text{pooled}} = 4.6$ ,  $P < 0.05$ ;  $G_{\text{het}} = 9.2$ ,  $P < 0.01$ ). Overall, there appears to be a bias favoring self pollen over outcross pollen.

**Between-population pollinations**—The remaining 1,445 progeny assayed resulted from between-population competitive pollinations (Tables 4–7). For pollinations using plants from Paradise as maternal parents, only pollinations involving the Mandeville population (as paternal parents) did not show a pooled deviation from a 1:1 ratio (Table 4). The results for all remaining crosses showed a large and significant excess of selfed progeny. While there

TABLE 2. Means  $\pm$  SD for seven morphological characters that distinguish populations. Pollen length is given in  $\mu\text{m}$ , all other measurements are in mm.

Character	Population							F
	PAR	IRT	FAL	MAN	BER	BAH	QUA	
Style length	25.6 $\pm$ 1.6a	25.9 $\pm$ 2.1a	23.9 $\pm$ 2.0a	20.4 $\pm$ 1.7b	15.7 $\pm$ 0.6b	18.9 $\pm$ 1.1b	40.7 $\pm$ 3.1c	191.7*
Pollen length	90.1 $\pm$ 3.4a	86.8 $\pm$ 3.1abcd	85.4 $\pm$ 3.9bd	82.3 $\pm$ 3.8c	81.3 $\pm$ 3.6cde	80.9 $\pm$ 2.3ce	104.2 $\pm$ 2.3f	83.4*
Flower depth	18.1 $\pm$ 3.8acde	18.2 $\pm$ 2.8ade	21.0 $\pm$ 2.7a	14.0 $\pm$ 2.5cde	12.8 $\pm$ 2.4de	16.6 $\pm$ 2.0e	37.8 $\pm$ 2.0f	113.6*
Flower diameter	48.1 $\pm$ 6.0a	45.1 $\pm$ 6.6a	46.8 $\pm$ 5.8a	41.9 $\pm$ 4.9acd	28.4 $\pm$ 1.7b	35.6 $\pm$ 2.2bce	36.4 $\pm$ 3.3bde	19.1*
Bracteole width	7.0 $\pm$ 1.7a	3.8 $\pm$ 2.2b	6.5 $\pm$ 2.4ac	5.1 $\pm$ 1.6abd	2.8 $\pm$ 0.4bc	2.3 $\pm$ 0.4b	4.4 $\pm$ 0.9bcd	16.0*
Leaf length	66.0 $\pm$ 10.9a	61.8 $\pm$ 10.1ac	58.6 $\pm$ 9.9ab	52.4 $\pm$ 7.5ab	41.9 $\pm$ 5.2bc	48.5 $\pm$ 7.3b	57.3 $\pm$ 7.4ab	8.1*
Leaf width	25.1 $\pm$ 4.7ab	21.9 $\pm$ 3.8bc	20.3 $\pm$ 3.0cd	22.6 $\pm$ 3.3abc	12.9 $\pm$ 3.9de	8.0 $\pm$ 1.7e	26.8 $\pm$ 3.8a	50.9*

<sup>a</sup> F-values from a one-way ANOVA are provided. Means with the same lowercase letter do not differ significantly based on Scheffe's test. \* P < 0.001.

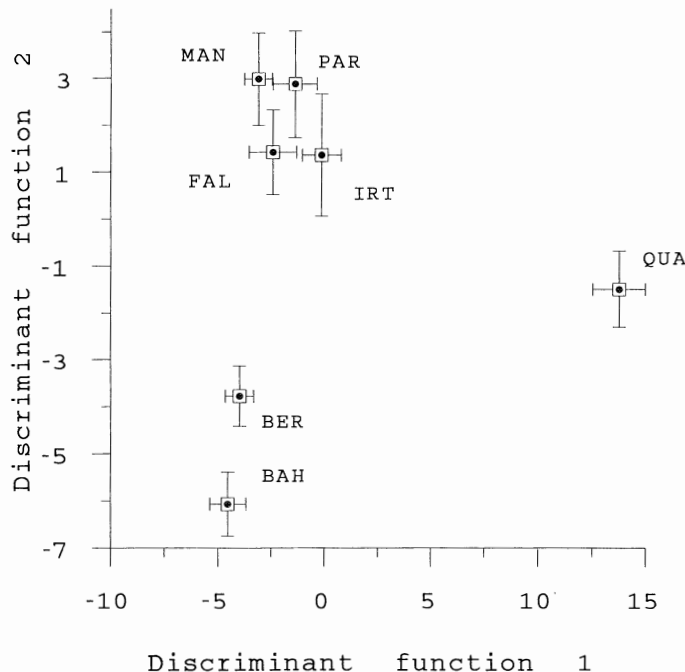


Fig. 1. Ordination of centroids of seven populations of *T. ulmifolia* defined by the first two functions from a discriminant analysis based on 17 characters. Error bars represent 1 SD on either side of the population centroid.

is statistically significant heterogeneity among these crosses, 19 of the 20 crosses show a greater number of selfed than outcrossed progeny (Table 4). The pooled results from this experiment are plotted in Fig. 2A. Populations are ranked along the X-axis according to their increasing Mahalanobis distance from the maternal Paradise population. There appears to be an increasing bias favoring selfed progeny production when morphologically more divergent populations are used as paternal parents in competitive pollinations. Spearman's nonparametric correlation was calculated between the mean percent of selfed progeny and the rank of morphological distance from the maternal population and it is positive and statistically significant (Fig. 2A).

A similar set of results emerge when the Falmouth population is used as a source of maternal plants for between-population pollinations (Table 5). An increasing excess of selfed progeny result when the outcross pollen-donating population is more phenotypically divergent (Fig. 2B). There is significant heterogeneity among individual

TABLE 3. Matrix of pairwise Mahalanobis distances from a discriminant functions analysis based on 17 morphological characters for seven populations of *T. ulmifolia*.

	BAH	BER	FAL	IRT	MAN	PAR
BER	34.3					
FAL	69.1	66.9				
IRT	84.1	79.0	34.6			
MAN	101.8	57.2	22.6	41.4		
PAR	92.9	83.7	19.7	19.6	31.3	
QUA	357.2	342.6	276.3	215.3	316.6	254.5

TABLE 4. Number of offspring sired by self vs. outcross pollen for within- and between-population competitive pollinations where the population from Paradise provided the source of maternal plants. *G*-tests for goodness of fit to 1:1 ratios are provided for each cross as well as the pooled data. The heterogeneity statistic tests the hypothesis that paternity ratios are homogeneous.<sup>a</sup>

Cross ♀ × ♂	Self	Outcross	df	<i>G</i>
<b>Paradise × Paradise</b>				
1 × 17	19	23	1	0.3
4 × 1	9	10	1	0.1
4 × 12	15	4	1	6.8**
4 × 13	12	12	1	0.0
6 × 3	31	24	1	0.9
6 × 3	38	16	1	9.2**
7 × 3	23	32	1	1.5
7 × 11	7	3	1	1.7
14 × 10	19	13	1	1.1
17 × 10	12	11	1	0.0
2 × 5 <sup>s</sup>	16	1	1	16.0***
4 × 1 <sup>s</sup>	2	16	1	12.4***
14 × 3 <sup>s</sup>	29	0	1	40.2***
Pooled	232	165	1	11.4***
Heterogeneity	—	—	12	78.8***
<b>Paradise × Irish Town</b>				
2 × 4	23	20	1	0.2
4 × 1	10	9	1	0.5
18 × 1	29	7	1	14.5***
18 × 1	35	1	1	40.8***
14 × 5 <sup>s</sup>	69	0	1	95.0***
Pooled	166	37	1	88.6***
Heterogeneity	—	—	4	62.5***
<b>Paradise × Mandeville</b>				
6 × 5	4	1	1	1.8
14 × 2	16	16	1	0.0
14 × 2	6	12	1	2.0
15 × 1	18	27	1	1.8
14 × 1 <sup>s</sup>	16	14	1	0.1
Pooled	60	70	1	0.8
Heterogeneity	—	—	4	5.1
<b>Paradise × Bermuda</b>				
4 × 2	15	1	1	14.7***
14 × 2	27	1	1	29.7***
15 × 2	14	4	1	5.9*
4 × 2 <sup>s</sup>	18	1	1	18.5***
7 × 2 <sup>s</sup>	1	2	1	0.3
Pooled	75	9	1	59.3***
Heterogeneity	—	—	4	10.4*
<b>Paradise × Bahamas</b>				
4 × 2	24	0	1	33.3***
7 × 1	15	4	1	6.8**
14 × 2	12	1	1	11.0***
15 × 2	35	3	1	31.7***
18 × 2 <sup>s</sup>	50	0	1	69.3***
Pooled	136	8	1	137.8***
Heterogeneity	—	—	4	14.2**
<b>Paradise × Quaco Rock</b>				
4 × 6	8	1	1	6.2*
6 × 2	35	1	1	40.8***
9 × 2	15	0	1	20.8***
19 × 4	33	4	1	26.0***
6 × 7 <sup>s</sup>	69	0	1	95.7***
Pooled	160	6	1	178.5***
Heterogeneity	—	—	4	10.9*

<sup>a</sup> \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; <sup>s</sup> = simultaneous pollinations.

TABLE 5. Number of offspring sired by self vs. outcross pollen for within- and between-population competitive pollinations where the population from Falmouth provided the source of maternal plants. *G*-tests for goodness of fit to 1:1 ratios are provided for each cross as well as the pooled data. The heterogeneity statistic tests the hypothesis that paternity ratios are homogeneous.<sup>a</sup>

Cross ♀ × ♂	Self	Outcross	df	<i>G</i>
<b>Falmouth × Falmouth</b>				
1 × 4	12	25	1	4.7*
6 × 9	36	40	1	0.2
6 × 4	56	46	1	1.0
7 × 9	30	42	1	2.0
7 × 5	22	19	1	0.2
Pooled	156	172	1	0.8
Heterogeneity	—	—	4	7.3
<b>Falmouth × Paradise</b>				
1 × 8	2	9	1	4.8*
6 × 1	47	43	1	0.2
6 × 5	33	34	1	0.0
2 × 5 <sup>s</sup>	58	21	1	18.0***
3 × 1 <sup>s</sup>	16	5	1	6.0*
Pooled	156	112	1	7.3**
Heterogeneity	—	—	4	21.8***
<b>Falmouth × Irish Town</b>				
6 × 5 <sup>s</sup>	66	12	1	41.1***
<b>Falmouth × Quaco Rock</b>				
1 × 3	26	0	1	36.0***
1 × 5	15	2	1	11.3***
7 × 3	30	0	1	40.9***
Pooled	71	2	1	82.9***
Heterogeneity	—	—	2	6.0*

<sup>a</sup> \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; <sup>s</sup> = simultaneous pollinations.

crosses, but seven of nine crosses show an excess of selfed progeny.

Fewer crosses were undertaken when the Mandeville plants were used as maternal parents. No significant deviation from a 1:1 ratio occurred when Paradise plants were the outcross pollen donors, as was the case for the reciprocal pollinations (Tables 4, 6). All other crosses showed a significant excess of selfed progeny, which was correlated with increasing morphological distance from the maternal population (Table 6; Fig. 2C). A few additional crosses were undertaken using Irish Town plants as maternal parents (Table 7). An excess of selfed progeny occurred for both sets of pooled results. Overall, there appears to be a bias favoring selfed progeny production for most between-population pollinations.

We also explored whether pollen size and style length alone might be predictors of self pollen success. We calculated the absolute value of the difference in style length between the maternal and paternal population (and did similar calculations for pollen length) and then calculated Spearman's rank correlation over all between-population crosses. There is a significant positive correlation between the proportion of selfed progeny and the difference in style length ( $r_s = 0.64$ ,  $P < 0.01$ ) but not for pollen length ( $r_s = 0.15$ ,  $P = 0.56$ ).

We carried out competitive pollinations involving the separate or simultaneous application of competing pollen to stigmas. The results of simultaneous pollinations showed

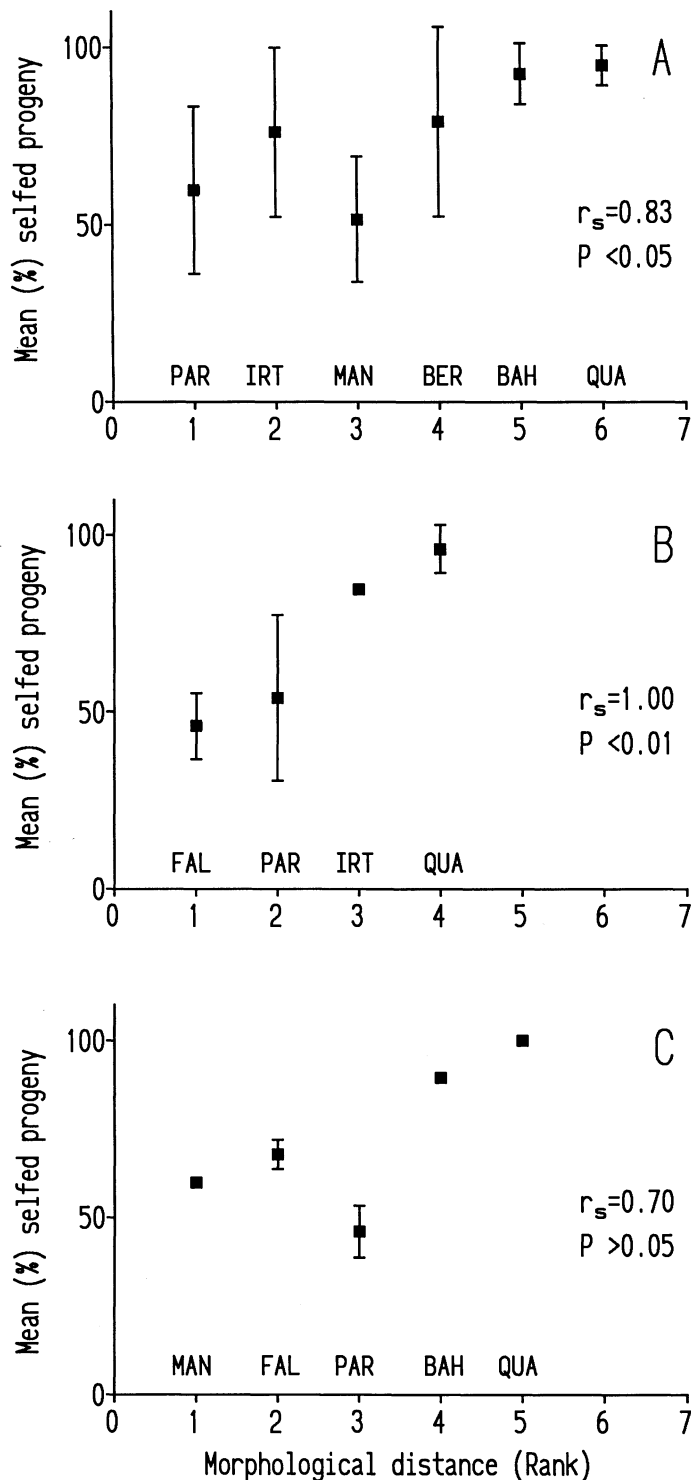


Fig. 2. Plots of the mean percent of selfed progeny vs. increasing rank of morphological divergence (based on Mahalanobis distances) from the maternal population. Spearman's rank correlation coefficient is also provided. A. Maternal population was Paradise. Pollen donors were PAR, IRT, MAN, BER, BAH, and QUA. B. Maternal population was Falmouth. Pollen donors were FAL, PAR, IRT, and QUA. C. Maternal population was Mandeville. Pollen donors were MAN, FAL, PAR, BAH, and QUA.

TABLE 6. Number of offspring sired by self vs. outcross pollen for within- and between-population competitive pollinations where the population from Mandeville provided the source of maternal plants. *G*-tests for goodness of fit to 1:1 ratios are provided for each cross as well as the pooled data. The heterogeneity statistic tests the hypothesis that paternity ratios are homogeneous.<sup>a</sup>

Cross ♀ × ♂	Self	Outcross	df	<i>G</i>
<b>Mandeville × Mandeville</b>				
4 × 3	24	16	1	1.6
<b>Mandeville × Falmouth</b>				
4 × 5	17	7	1	4.3*
4 × 5 <sup>s</sup>	13	7	1	1.8
Pooled	30	14	1	14.7***
Heterogeneity	—	—	1	4.0*
<b>Mandeville × Paradise</b>				
4 × 14 <sup>s</sup>	9	13	1	0.7
4 × 16 <sup>s</sup>	22	21	1	0.0
Pooled	27	34	1	0.1
Heterogeneity	—	—	1	0.6
<b>Mandeville × Bahamas</b>				
4 × 1	34	4	1	27.1***
<b>Mandeville × Quaco Rock</b>				
3 × 1	13	0	1	18.0***
4 × 7	27	0	1	37.4***
1 × 6 <sup>s</sup>	11	0	1	15.3***
Pooled	51	0	1	70.7***
Heterogeneity	—	—	2	0.0

<sup>a</sup> \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; <sup>s</sup> = simultaneous pollinations.

comparable ratios or, on occasion, a greater bias in selfed progeny production than did the separate pollinations (e.g., Table 4, Paradise × Irish Town, Paradise × Mandeville). Given the differences in pollen size among populations and the strong possibility that this would also be associated with differences in pollen production (Table 2), we carried out a majority of separate pollinations for reasons stated in the methods.

TABLE 7. Number of offspring sired by self vs. outcross pollen for between-population competitive pollinations where the population from Irish Town provided the source of maternal plants. *G*-tests for goodness of fit to 1:1 ratios are provided for each cross as well as the pooled data. The heterogeneity statistic tests the hypothesis that paternity ratios are homogeneous.<sup>a</sup>

Cross ♀ × ♂	Self	Outcross	df	<i>G</i>
<b>Irish Town × Falmouth</b>				
5 × 5	11	29	1	8.4**
3 × 8 <sup>s</sup>	33	1	1	38.1***
Pooled	44	30	1	2.7
Heterogeneity	—	—	1	43.9***
<b>Irish Town × Bahamas</b>				
5 × 2	12	0	1	16.6***
1 × 3 <sup>s</sup>	6	2	1	2.1
Pooled	18	2	1	14.7***
Heterogeneity	—	—	1	4.0*

<sup>a</sup> \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; <sup>s</sup> = simultaneous pollinations.

TABLE 8. Mean percent  $\pm$  SD (*N*) of aborted ovules for pooled within- and between-population, separate and simultaneous competitive pollinations, and their respective outcross controls, and selfs. *F*-values from a one-way ANOVA comparing treatments for each population are provided.

Pollination treatment	Maternal population		
	Falmouth	Mandeville	Paradise
Separate	21.3 $\pm$ 22.9 (17)	9.2 $\pm$ 7.7 (6)	26.1 $\pm$ 19.6 (36)
Sep. control	26.3 $\pm$ 26.9 (10)	44.7 $\pm$ 37.0 (3)	40.5 $\pm$ 28.3 (17)
Simultaneous	15.8 $\pm$ 18.6 (10)	30.3 $\pm$ 20.5 (10)	31.3 $\pm$ 20.7 (18)
Sim. control	23.5 $\pm$ 17.5 (10)	29.6 $\pm$ 28.0 (12)	37.8 $\pm$ 34.2 (16)
Self	17.1 $\pm$ 7.0 (4)	40.0 — (1)	12.4 $\pm$ 9.3 (5)
<i>F</i> -value	0.4 ns <sup>a</sup>	1.4 ns	2.0 ns

<sup>a</sup> ns = not statistically significant.

**Ovule abortion and maternal choice**—The mean percent of aborted ovules was calculated and compared for treatment, controls, and selfs for the Falmouth, Mandeville, and Paradise populations (Table 8). The mean percent of aborted ovules was quite variable and ranged from 9.2 to 44.7 (Table 8). There were no significant differences in mean percent of aborted ovules among the treatments, controls, or self-pollinations for the three maternal populations (Table 8).

Table 9 shows the detailed results from 11 between-population competitive pollinations in which the number of aborted ovules is low and the percent germination and proportion of progeny screened electrophoretically are both high. Using these particular crosses it is possible to determine whether deviations from 1:1 paternity ratios can be accounted for solely by maternal choice via selective abortion or pollen competition. Table 9 also lists the potential number of outcrossed progeny that would have been produced assuming that all aborted ovules were the result of outcrossing (and subsequent abortion) and that all ungerminated seeds and progeny that were not screened were also outcrosses. For all 11 crosses, there is a marked deviation from a 1:1 paternity ratio even given these assumptions, indicating that pollen competition must be occurring.

## DISCUSSION

We conducted competitive pollinations and used genetic markers to test the hypothesis that outcross pollen would sire more offspring than self pollen. Using seven populations of *T. ulmifolia*, a species known to experience considerable self-fertilization in the field (Belaoussoff and Shore, 1995), we found no evidence for a competitive advantage to outcross pollen. While inbreeding depression appears to manifest itself later in the life history of this species (Belaoussoff and Shore, 1995), there appears to be no advantage to outcrossed pollen at the level of pollen tube competition or embryo abortion.

For one population (Paradise), the pooled paternity ratio showed a significantly greater proportion of selfed progeny (Table 4). This is an unexpected result since a number of studies have shown that self pollen tube growth rates are slower than those of outcross pollen (Aizen, Searcy, and Mulcahy, 1990; Cruzan and Barrett, 1993; Rigney et al., 1993; but see Johnson and Mulcahy, 1978; Mulcahy, Curtis, and Snow, 1983). Paternity ratios have been examined in a number of species capable of both selfing and outcrossing, and generally, in competition, a

greater number of progeny are the result of outcrossing (e.g., *Erythronium grandiflorum*, Rigney et al., 1993; *Clarkia gracilis*, Niovi Jones, 1994). In some species, however, there may be a great deal of variation among individuals such that self pollen grows faster in some individuals but slower in others (e.g., *Lobelia cardinalis*, Johnston, 1993; *Hibiscus moscheutos*, Snow and Spira, 1991a, b, 1993). The significant heterogeneity among replicate pollinations for the Paradise population (Table 4) could indicate that this phenomenon is occurring, and the excess of selfed progeny in the pooled data may be a result of this heterogeneity.

An additional possibility for the bias in selfed progeny production has been proposed by Johnson and Mulcahy (1978) for maize. They suggest that genetic factors may play a role in differences in pollen competitive ability. Repeated selfing of maize lines over many generations has led to intensive selection for growth of self pollen in a particular stylar environment and to small but significant increases in the competitive ability of self relative to outcross pollen. It is perhaps unlikely that this explanation can be applied to our within-population crosses, but it may provide an explanation for the marked advantage of selfed pollen for between-population crosses of *T. ulmifolia*.

TABLE 9. Detailed results for 11 between-population competitive pollinations. Numbers of seeds and aborted ovules, as well as the number of selfed and outcrossed progeny assayed, are provided. Potential indicates the number of outcrossed progeny that would have been produced assuming, that in addition to the observed outcrosses, all aborted ovules, ungerminated seeds, and seedlings not assayed were the result of outcrossing. Percentage of seeds assayed and percent germinated are also provided.<sup>a</sup>

Cross	Seeds	Aborted ovules	Number of progeny			Percent	
			Self	Outcross	Potential	Assayed	Germinated
PAR $\times$ QUA	43	7	35	1	15	84	86
PAR $\times$ QUA	44	3	33	4	14	84	84
PAR $\times$ QUA <sup>s</sup>	73	1	69	0	5	95	96
PAR $\times$ IRT	47	5	35	1	17	77	77
PAR $\times$ IRT <sup>s</sup>	80	7	69	0	18	86	86
PAR $\times$ BAH <sup>s</sup>	53	11	50	0	14	94	96
FAL $\times$ IRT <sup>s</sup>	78	5	66	12	17	100	100
MAN $\times$ QUA	13	3	13	0	3	100	100
MAN $\times$ QUA	27	6	27	0	6	100	100
MAN $\times$ BAH	56	4	34	4	26	68	68
IRT $\times$ FAL <sup>s</sup>	52	0	33	1	19	65	65

<sup>a</sup> s = simultaneous pollination.

Our results support the hypothesis that pollen tube competition or possibly different rates of pollen germination (although limited observations we have made of stained, pollinated stigmas showed that pollen does germinate in among-population crosses) explain the deviant ratios for between-population crosses rather than ovule abortion. There were no significant differences among ovule abortion rates for treatment, control, or self pollinations. If ovule abortion were an important contributor to deviant paternity ratios we might expect to see increased ovule abortion when competitive pollinations are carried out. In addition, in noncompetitive pollinations among populations of *T. ulmifolia*, comparable levels of seed set are achieved for most crosses, and viable and highly fertile progeny are obtained (J. Shore, unpublished data).

Studies examining the importance of ovule abortion in influencing paternity ratios have yielded variable results. For example, in *Hibiscus moscheutos* ovule abortion is not an important factor contributing to a deviant paternity ratio (Snow and Spira, 1991a), whereas ovule abortion plays an important role in influencing paternity ratios in *Raphanus sativus* (Marshall and Ellstrand, 1988). Arnold, Hamrick, and Bennett (1993) used interspecific competitive pollinations among *Iris fulva* and *I. hexagona* and found no hybrid progeny among the 201 seeds analyzed, although the species are known to hybridize at low rates in the field. They argue that pollen competition is the cause of the lack of hybrid production. In later work, using genetic markers and measures of pollen tube growth, Carney, Cruzan, and Arnold (1994) concluded that both pre- and postfertilization processes contribute to low levels of hybrid seed formation in *Iris*. It is perhaps more likely to expect postfertilization effects, such as embryo abortion, to occur for interspecific crosses rather than the intraspecific crosses used here.

An examination of particular crosses showing low rates of ovule abortion indicate more directly that pollen competition is likely the major contributor to excess selfed progeny production for between-population competitive pollinations. For a specific cross, if the paternity ratio of the progeny differs significantly from a 1:1 ratio, and all ovules become seeds and all progeny are screened, pollen competition, rather than maternal choice (via selective abortion), must be the cause of nonrandom paternal success. While we found only a single instance where all ovules produced seeds, a number of pollinations showed low rates of ovule abortion (Table 9). The biased ratios for these crosses must result wholly, or in part, from pollen competition. While it is not possible to rule out ovule abortion as a contributor to the biased ratios with certainty, pollen competition appears to be the major contributing factor.

A number of the populations show marked differences in morphological traits (Table 2; Fig. 1), and the more divergent the population, the less competitive is its pollen (Fig. 2). Plants from the Bahamas and Bermuda have the smallest flowers, short styles, small pollen, and have stigmas and anthers in close contact. Both are highly autogamous. While outcrossing rates have not been measured in these populations, data from other populations of *T. ulmifolia* demonstrate that there is a correlation between degree of herkogamy and outcrossing rate (Barrett and Shore, 1987; Belaussoff and Shore, 1995), and therefore,

these two populations are probably highly selfing. The plants from Quaco Rock are herkogamous, and have the longest styles, largest pollen, and an unusual floral architecture. We have not had the opportunity to investigate pollination in this population, but suspect that it may be adapted to a different pollinator(s) based on its unusual floral morphology, presence of a purple petal spot, and copious nectar production (J. Shore, unpublished data). The evolution of these different floral forms may be responsible for the poor competitive ability of pollen in between-population crosses, as pollen may have been selected to grow optimally in a native stylar environment. We did observe a positive correlation between the proportion of selfed progeny and the absolute value of the difference in style length between the maternal and the pollen-donating population, which might support this hypothesis, although we found no significant correlation with the deviation in pollen size.

Our results for between-population crosses were, on the whole, very striking. While we compared only self vs. outcross pollinations, we expect that within-population outcross pollen would have a similar competitive advantage over pollen from a different population. Our results reveal a mechanism that might form the basis of reproductive isolation among diverging natural populations. Any outcross pollen from rare pollen flow events would likely be outcompeted by self or within-population pollen. We believe that divergence in the reproductive systems of these populations, perhaps in response to selection by different pollinators or through the evolution of increased self-fertilization, is the cause of reduced interpopulation pollen competitive ability. It is unclear whether structural and/or physiological aspects of the reproductive systems are responsible for the poor competitive ability of outcross pollen. Pollen tube competition might be a very important prezygotic isolating mechanism, and our results may show how plant speciation might be driven by local adaptation to different pollinators.

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