

## Reproductive assurance for a rewardless epiphytic orchid in Puerto Rico: *Pleurothallis ruscifolia* (Orchidaceae, Pleurothallidinae)

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**ABSTRACT.**—Most angiosperms sustain pollinator visits by offering a reward, such as nectar or pollen, yet there are plants that do not offer rewards and instead depend on deception for successful pollen transfer. Unless rewardless plants have an extremely efficient means of deceit or are autogamous, they tend to experience less fruit set than those that offer pollinator rewards. In Puerto Rico, we studied the reproductive biology of a rewardless epiphytic orchid, *Pleurothallis ruscifolia* (Pleurothallidinae), a widespread Neotropical species. The Pleurothallidinae are primarily myophilous, self-incompatible, and pollinator dependent for fruit set and seed production. However, because our preliminary observations indicated that populations of *P. ruscifolia* on the island of Puerto Rico exhibited an unusually high fruit set, we hypothesized that our population is autogamous and that reproductive effort and fruiting success are resource limited. We monitored 168 plants in a single population during the 2009 reproductive season to determine the mating system of *P. ruscifolia* while looking for evidence of reproductive constraints. Fruit set in our population was high (66%) and no floral visitors were seen. Fruit set for our pollinator exclusion experiment was no different than fruit set for open pollinated plants. Most plants produced both chasmogamous and cleistogamous flowers, the majority of fruits produced by the latter. Furthermore, reproductive effort and fruiting success were limited by plant size, which is likely the result of resource constraints. We expect that autogamy is prevalent in *P. ruscifolia* of the West Indies, but outcrossing should be dominant in continental populations. Selection for reproductive assurance in the absence or rarity of an effective pollinator is the likely process leading to this autogamous system.

**KEYWORDS.**—autogamy, cleistogamy, fruit production, plant size, deception pollination, reproductive constraints, Pleurothallidinae, Orchidaceae

### INTRODUCTION

The majority of flowering plants sustain pollinator visitation by offering rewards such as nectar or pollen. Although this is an effective system to attract and maintain pollinator interest, there are nevertheless plants that do not offer rewards and instead rely on deception for successful pollen transfer. Unless rewardless plants have an extremely efficient means of deceit or are autogamous, they tend to experience less fruit set than plants that do provide pollinator rewards (Tremblay et al. 2005). In most cases, this low fruiting success can be attributed to infrequent pollinator visitation rates, which

typically results from pollinators learning to avoid rewardless plants (Heinrich 1975; Ackerman 1981, 1983; Firmage and Cole 1988; Ayasse et al. 2000; Tremblay et al. 2005).

Most food deceptive plants are found within the Orchidaceae (Ackerman 1986). Comprising 1/15 of all flowering plants, orchids may be the largest family of flowering plants with over 28,000 accepted species (Govaerts et al. 2010), at least 1/3 of which are food deceptive (van der Pijl and Dodson 1966). Despite the fact that these plants do not offer pollinator rewards, deceptive species appear to be rather successful and are clearly well represented within the Orchidaceae. Nonetheless, the evolution of

deceptive pollination has remained an enigma, although substantial progress has been made in our understanding of the patterns and processes involved (Nilsson 1992; Jersáková et al. 2006).

Whether a plant relies on deception to attract pollinators or instead offers rewards, the function of any animal-mediated pollination system requires all players involved to co-exist. Furthermore, pollinator service should outweigh the costs of maintaining an animal pollinated system. Where pollinator service is rare or non-existent, many species, including orchids, have either a mixed mating system or are entirely autogamous as a means of reproductive assurance (Catling 1990; Jacquemyn et al. 2005; Goodwillie et al. 2005; Micheneau et al. 2008). Such conditions are rather common in regions of highly variable and severe weather and on islands where the pollinator fauna is often depauperate and effective pollinators rare (Hagerup 1952; Carlquist 1974; Barrett 1996; Pansarine and Amaral 2006; Wheelwright et al. 2006). Indeed, the frequency of autogamy in orchids is substantially higher on relatively small islands such as Puerto Rico and Réunion than it is for orchid floras of Panama, North America, or Madagascar (Ackerman 1985; Jacquemyn et al. 2005). Furthermore, autogamy is likely to be more common for island species with deceptive pollination systems since they already tend to experience infrequent pollinator visitation.

Herein we report on the reproductive biology of *Pleurothallis ruscifolia* (Jacq.) R. Br., a rewardless epiphytic orchid with a widespread Neotropical distribution (Ackerman 1995). We determined its mating system and looked for correlates of reproductive effort and success in a Puerto Rican population. Although there are approximately 4,100 species within the subtribe Pleurothallidinae (Pridgeon 2005), the reproductive biology of only a few species is known. Most authors have regarded the group to be primarily myophilous and self-incompatible, completely requiring animal pollinators for fruit set and seed production (e.g. Borba and Semir 2001; Borba et al. 2001; Borba et al. 2002; Barbosa et al. 2009; Gontijo et al. 2010). Additionally, this myophilous mat-

ing system is considered a basal characteristic of the subtribe. However, there is at least one account of self-compatibility within the Pleurothallidinae: Kirchner (1922) reported that a cultivated plant of *P. ruscifolia* was autogamous (though he did not report the provenance of the specimen). Thus, self-compatibility and autogamy may exist in the subtribe despite the spectrum of deleterious effects associated with inbreeding. Because our preliminary observations indicated that populations of *P. ruscifolia* on the island of Puerto Rico exhibited an unusually high fruit set for a rewardless orchid, we hypothesized that our population is autogamous and that reproductive effort (flower production) and fruiting success are resource limited.

## MATERIALS AND METHODS

### *Study Site*

Twenty-nine patches (268 plants) of *Pleurothallis ruscifolia* were located along the Quebrada Sonadora in the Luquillo Experimental Forest (El Yunque National Forest) of northeastern Puerto Rico (18° 19' N, 65° 49' W). Our study site covered an elevational gradient from approximately 300 to 550 m and was located within a wet, subtropical forest dominated by tabonuco, *Dacryodes excelsa* (Burseraceae) (Holdridge life zone system, Ewel and Whitmore 1973). Furthermore, Puerto Rico is the smallest (8876 km<sup>2</sup>) of the principal islands of the Greater Antilles some 640 km from Venezuela, the closest mainland source.

### *Study Plant*

*Pleurothallis ruscifolia* in Puerto Rico grows epiphytically on trees or upon boulders at 300 to 1100 m elevation. The caespitose, sympodial plants consist of one to 60 shoots or more (mean = 13.7 shoots per plant, SD = 11.8). For 5-6 weeks from May to July, flowers appear on short, fasciculate inflorescences (1-22 flowers per inflorescence; mean = 4.7, SD = 3.8), remaining open for approximately one to three weeks. The pale yellow to yellow-green flowers are scentless, about 7 mm long, with some flowers fully opening (chasmogamous) while

others remain closed (cleistogamous). The perianth parts of chasmogamous flowers remain open and separated during fruit maturation whereas the perianth parts in suspected cleistogamous flowers remain completely fused and tightly closed. Because of this discrepancy, we were able to determine whether fruits were produced by chasmogamous or cleistogamous flowers.

### *Mating System*

To determine whether *Pleurothallis ruscifolia* is autogamous and self-compatible we covered 15 randomly selected shoots from different plants with net-bag enclosures to exclude potential pollinators. Bagging occurred early in the reproductive season before flower anthesis. Bag enclosures were made of a fine-mesh bridal veil with a wire frame for support. The total number of flower buds was noted for each bagged plant at the beginning of the reproductive season and the total number of fruits was noted at the end of the reproductive season. Fruit set for our bagged treatments was compared to natural fruit set levels for open pollinated plants in the rest of the population. We also assessed the degree to which fruit set in *P. ruscifolia* was a result of cleistogamous flowers by determining whether fruits were formed from open (chasmogamous) or closed (cleistogamous) flowers. To determine whether *P. ruscifolia* attracts pollinators we made 22 half-hour observations over a 10-day period at the peak of flowering (15-25 June 2009). Observation periods were scattered throughout the day from 0700 to 2100 hours, however, the majority of our observations were carried out from 0700 to 1400 hours. Pollinator observations were not conducted during periods of rain.

### *Reproductive Effort and Success*

To assess the relationship between flower production (reproductive effort) and fruit production (reproductive success) with plant size, we randomly selected and monitored 168 plants from all 29 patches during the 2009 reproductive season. Three census periods were carried out at regular inter-

vals from 8 June to 29 July 2009. During each census plants were examined for their total number of flowers (including buds, unopened and open flowers), pollinaria removals, and fruits. To determine an effective measure of plant size the number of shoots per plant, stem lengths, and leaf lengths were recorded (after Montalvo and Ackerman 1987); however, because stem and leaf lengths remained fairly constant throughout size classes (Table 1), we chose only to use the number of shoots per plant as our measure of plant size. We grouped plants into 16 size classes based on the number of shoots per plant. Total flower and fruit production per plant were estimated by taking the average number of flowers or fruits produced per shoot multiplied by the total number of shoots for each plant. Reproductive measures were then averaged for each size class and analyzed using linear regressions to assess the relationship between plant size and reproductive measures.

We also compared seed viability with plant size. Three fruits per plant were selected from 29 plants of different size classes. Approximately 200 seeds per fruit were examined under a compound microscope at low power (40 $\times$ ). All seeds with an embryo were considered viable (Montalvo and Ackerman 1987). The average percent of seeds with embryos was calculated for plants in each size class and analyzed using a linear regression. Because the minimum number of pollinia is often sufficient to fertilize all ovules in at least some orchids (Montalvo and Ackerman 1987), we assumed a low percent seed viability was not a consequence of pollen limitation, but rather an indication of either partial self-incompatibility, selective seed abortion, inbreeding depression, or resource constraints (e.g., Stort and de Lima Galdino 1984; Peter and Johnson 2009).

## RESULTS

### *Mating System*

Our net-bag pollinator exclusion experiment, mating system observations, and pollinator observations supported our prediction that *Pleurothallis ruscifolia* is pri-

TABLE 1. Average reproductive and vegetative measurements for *Pleurothallis ruscifolia* in Puerto Rico. Dashes indicate size classes with no data available for the particular measurement category.

Measurement:	Number of shoots per plant															Overall plant averages	
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-25	26-30	31-35	36-40	41-45		46+
Tot. flower production	2.9	15.3	20.2	26.2	45.9	69.8	96.7	102.1	106.4	73.6	177.3	158.6	148.6	224.6	349.6	298.8	119.8
Flowers per shoot	2.4	3.6	3.4	3.8	4.0	5.2	6.7	4.5	5.7	3.1	7.3	6.1	3.1	5.8	11.5	5.9	5.1
Tot. fruit production	1.4	8.6	13.7	17.5	34.4	31.4	72.5	57.1	70.3	62.4	112.3	147.5	105.8	178.1	279.6	289.3	92.6
Fruit set (%)	47.3	56.0	67.9	66.7	75.0	45.0	75.0	55.9	66.1	84.8	63.3	93.0	71.2	79.3	80.0	96.8	70.2
Missing pollinaria (%)	11.1	17.0	23.7	35.5	56.9	87.5	41.2	50.0	66.7	1.00	81.3	22.2	54.3	29.5	50.0	81.8	50.5
CL flowers (%)	100	76.6	79.9	74.4	77.2	69.9	80.6	60.4	78.5	72.7	69.6	0.88	82.4	79.2	91.5	67.1	78.0
Viable seeds (%)	-	77.4	31.4	90.9	90.0	89.1	90.7	66.0	62.4	66.4	49.6	93.8	81.3	89.9	73.6	-	75.2
Stem length (cm)	6.6	8.2	8.9	10.2	11.3	10.6	13.2	11.5	12.6	10.4	10.8	13.2	14.2	13.9	16.7	11.9	11.5
Leaf length (cm)	7.8	8.0	8.4	8.6	9.1	8.2	8.9	9.8	9.5	8.9	9.2	9.7	9.8	9.2	10.5	9.6	9.1

CL = cleistogamous.

marily autogamous. Fruit set for plants in insect-exclusion bags was not significantly different from natural fruit set levels for open pollinated plants (Mann-Whitney U-test:  $Z = -0.68, P = 0.49$ ). Fruit set for bagged plants was 62.3% ( $n = 15, SE = 7.5$ ); natural fruit set for open pollinated plants was 66.6% ( $n = 150, SE = 2.3$ ). Both chasmogamous and cleistogamous flowers produced fruit but not equally; fruit set for cleistogamous flowers was 100% (i.e. all set fruit:  $n = 1413$ ), where as only 47% of chasmogamous flowers set fruit (i.e. 429 fruits from 912 flowers). Cleistogamous flowers accounted for more than 60.7% of all flowers produced ( $n = 2,325$  total flowers) and more than 75% of all fruits produced ( $n = 1842$  total fruits). Eighty-one percent of observed plants had both cleistogamous and chasmogamous flowers; the remaining 19% had only cleistogamous flowers ( $n = 104$  plants). None of the plants observed had only chasmogamous flowers. The proportion of cleistogamous flowers did not change with plant size (Table 1;  $R^2 = 0.002; d.f. = 15; P = 0.88$ ). Furthermore, no floral visitors were observed visiting flowers during our 22 observation periods nor were any visitors seen during any other time spent in the field near our study plants.

*Reproductive Effort and Success*

Both measures of reproductive effort were dependent upon plant size: total flower production increased with plant size (Table 1; Fig. 1A:  $R^2 = 0.90, d.f. = 15, P < 0.0001$ ) and so did the number of flowers produced per shoot (Table 1; Fig. 1B:  $R^2 = 0.33, d.f. = 15, P = 0.02$ ). Similarly, female reproductive success was also dependent upon plant size: total fruit production and fruit set increased with the number of shoots per plant (Table 1; total fruit production, Fig 1C:  $R^2 = 0.94, d.f. = 15, P < 0.0001$ ; percent fruit set, Fig. 1D:  $R^2 = 0.50, d.f. = 15, P = 0.002$ ). Conversely, pollinia were absent from approximately 60% of chasmogamous flowers ( $n = 569$  observed chasmogamous flowers), the frequency of which was not dependent upon plant size (Table 1;  $R^2 = 0.02; d.f. = 14; P = 0.58$ ). Average percent of seeds with embryos per fruit was 75.2%

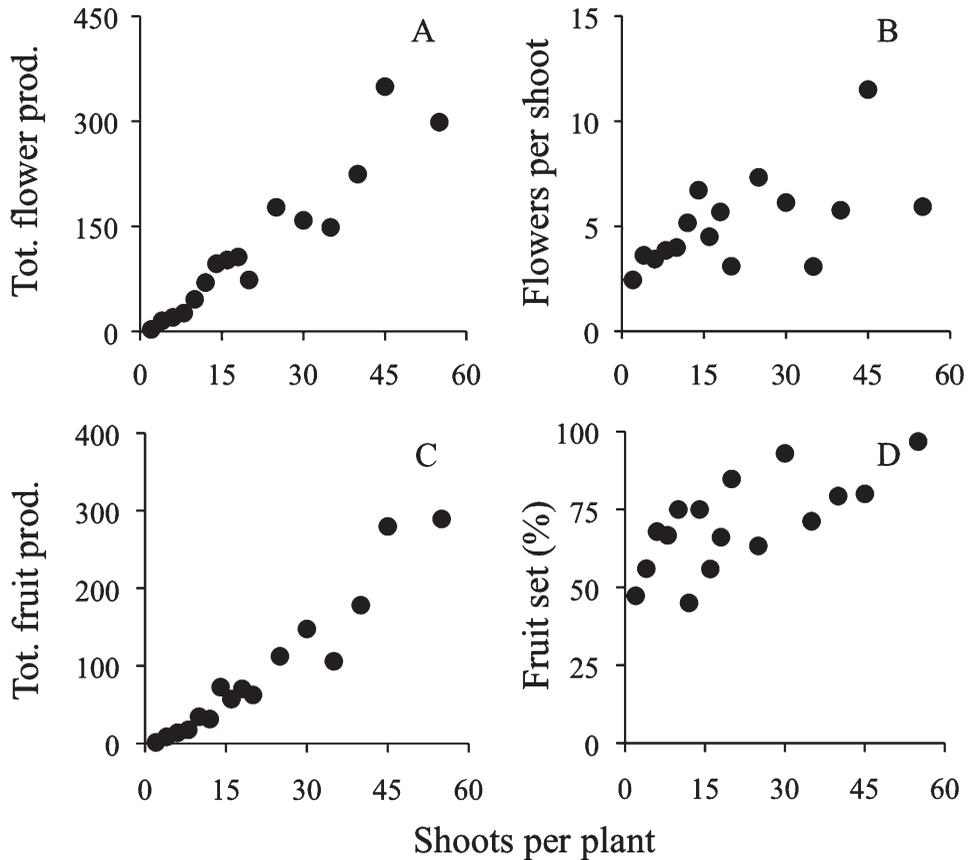


FIG. 1. Relationships between plant size (number of shoots per plant) and measures of reproductive effort and fruiting success: A. total flower production per plant, B. number of flowers produced per shoot, C. total fruit production per plant, and D. percent fruit set per plant.

( $n = 87$  fruits,  $SE = 1.9$ ), which was not correlated with plant size (Table 1;  $R^2 = 0.03$ ;  $d.f. = 13$ ;  $P = 0.58$ ).

#### DISCUSSION

Average fruit set for our population of *Pleurothallis ruscifolia* was approximately 70%, which far exceeds the 20% average for deceptive orchids (Tremblay et al. 2005). We attribute this high fruit set to autogamy based on several lines of evidence. First, our pollinator exclusion experiment resulted in fruit set that was no different from natural fruit set levels for open pollinated plants. Second, we found that the majority of fruits were produced autogamously from cleistogamous flowers. Finally, we did not observe any pollinator activity during our 22 obser-

vation periods nor during any other time spent near our study plants. Although infrequent pollinator visits are a hallmark of deceptive orchids (Ackerman 1986; Neiland and Wilcock 1998; Tremblay et al. 2005), visits to *P. ruscifolia* in Puerto Rico are likely rare or do not occur at all in our population. Instead it appears that in our population *P. ruscifolia* does not depend on pollinators for reproduction, but rather, is completely reliant upon a self-compatible, autogamous mating system.

Because fruit set levels did not increase for open pollinated plants compared to that of bagged plants, the majority of fruit production for *P. ruscifolia* can be attributed to self-pollination. According to Kirchner (1922), *P. ruscifolia* self-pollinates when the whole anther comes in contact with the

stigma. This method of self-pollination likely accounts for the numerous missing pollinaria in chasmogamous flowers that we had recorded as "pollinarium removals". Similar selfing mechanisms occur in other orchids and can be facilitated by agitation from wind and rain (Catling 1980); this form of wind and rain assisted self-pollination has been known to occur in our general study area in Puerto Rico (González-Díaz and Ackerman 1988). However, because we did not directly examine stigmas, pollen tube growth, or ovule fertilization, a more thorough investigation is necessary to determine the exact selfing mechanism of *P. ruscifolia*; with our current findings, the occurrence of other forms of autogamy, such as agamospermy, cannot be ruled out.

In normally outcrossing plants, self-pollination (autogamous or geitonogamous) typically results in inbreeding depression often expressed by decreased seed viability, reduced seed production, slower growth, or abnormal development (e.g., Darwin 1877; Catling 1990; Tremblay et al. 2005). Outcrossing orchids often show differences in seed production or viability between fruits resulting from self- and cross-pollinations (Stort and Lima Galdino 1984; Meléndez-Ackerman and Ackerman 2001; Peter and Johnson 2009). Some isolated autogamous populations may purge their deleterious alleles over multiple generations, reducing the effects of inbreeding (Byers and Wallers 1999). Genetic purging may have already occurred in *P. ruscifolia* based on the fact that both fruit production and seed viability were relatively high. Still, because plants in our population are capable of producing two flower types (chasmogamous and cleistogamous) the potential for a mixed mating system exists (xenogamy and autogamy), and if activated, could resurrect lost genetic diversity (McNamara and Quinn 1977; Lloyd 1984).

Although cleistogamous flowers were dominant in our population, most plants (80%) produced both cleistogamous and chasmogamous flowers, a condition not unknown in orchids (Catling 1990; Pansarin and Amaral 2006). Either this population is in a transitional stage between chasmogamy and cleistogamy, or fitness contributions of

the occasional pollinator visit helps maintain the current ratio of cleistogamous to chasmogamous flowers. All of the plants in our population had cleistogamous flowers and more than 60% of all flowers produced were cleistogamous. Not only are cleistogamous flowers less costly to develop and maintain than chasmogamous flowers (Schemske 1978; Oakley et al. 2007), they also provide a much more efficient means of self-pollination (Schoen and Lloyd 1984). Therefore, in the absence of an outcrossing advantage, one would expect selection to favor cleistogamous flower production (Culley and Klooster 2007). In our population, selfing efficiency of chasmogamous flowers (47% fruit set) is clearly imperfect and was more than two times less than the fruit set of cleistogamous flowers (100% fruit set). Furthermore, in some plant species, including orchids, the production of cleistogamous flowers is plastic depending on plant size or prevailing ecological conditions (e.g. pollinator availability), presumably allowing a plant to track ideal conditions for cross-pollination (Schoen and Lloyd 1984; Catling 1990; Oakley et al. 2007). For example, in the mesophytic forests of Serra do Japi, Brazil, Pansarin and Amaral (2006) found that the majority of flowers produced by *Polystachia estrellensis* were cleistogamous, which they attributed to the scarcity of pollinators in that area. Nevertheless, we found no relationship with plant size or pollinator abundance, so if such plasticity occurs in the production of cleistogamous flowers, the underlying conditions by which it occurs remain unknown.

Fruit production in outcrossing orchids is often very low, which is attributed to pollination limitation (Tremblay et al. 2005); however, the severity of pollination constraints is affected by resource availability (Montalvo and Ackerman 1987; Calvo and Horvitz 1990). In contrast, fruit production in selfing orchids is high but not always 100%, generally as a result of floral herbivory, fruit predation, an imperfect self-pollination mechanism, or resource constraints. While we did not see any significant florivory or fruit predation, self-pollination for both types of flowers resulted in a high percentage fruit set. Still, resource

availability appears to account for some variation in fruit set as is apparent in other autogamous orchids (Catling 1990; González-Díaz and Ackerman 1988). After all, the patterns of both reproductive effort and fruiting success in our population were limited by plant size (Fig. 1), which is likely the result of resource constraints. Larger plants (those with more shoots) may produce more flowers and fruits because they have greater access to available resources or are better able to translocate resources throughout the plant. Furthermore, this relationship between reproductive measures and plant size would be expected for plants with an efficient autogamous mating system based on the fact that pollinator limitation is no longer a factor.

Departures from an animal-mediated outcrossing strategy generally occur where pollinator service is either lacking or unreliable (Hagerup 1952; Barrett 1996). For example, continental populations of *Cochleanthes flabelliformis* are primarily chasmogamous and outcrossing, whereas those of the Antilles are autogamous (Ackerman 1995). Its sister species, *C. aromatica*, is pollinated by large euglossine bees, and we expect the same for *C. flabelliformis* (Dodson 1965 cited in Williams 1982). However, only one euglossine species is native to the Antilles, a Jamaican endemic, and it is likely too small to be an effective pollinator. Similarly, mating system variation may occur even within an island population. Ortiz-Barney and Ackerman (1999) found that most plants in eastern Puerto Rican populations of *Prosthechea* (as *Encyclia*) *cochleata* were autogamous whereas those in the western parts of the island were dependent upon pollinators for successful pollen transfer. Nevertheless, fruit set was so rare and the effects of inbreeding depression so minimal that if an efficient selfing mutation occurred in western populations, then it should spread rapidly. We do not yet know whether the mating system of *P. ruscifolia* varies in a like manner across the island, the Antilles, or over its entire Neotropical range.

In conclusion, *Pleurothallis ruscifolia* in Puerto Rico appears to be primarily autogamous. Fruit set for bagged plants was no

different than fruit set levels for open pollinated plants indicating that self-pollination is the primary means of fruit production. Self-pollination via cleistogamy accounts for the majority of fruits produced, however, chasmogamous flowers also set fruit, but to a lesser degree. Both reproductive effort and fruiting success are not pollinator limited, but instead limited by plant size. Selection for reproductive assurance in the absence or rarity of an effective pollinator is the likely process leading to this autogamous system. Although we expect the mating system of *P. ruscifolia* to depend on animal-mediated outcrossing on the continent, comparative studies across its wide distribution are needed to determine whether autogamy in Puerto Rico is an island phenomenon, typical throughout its range, or phenotypically plastic.

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