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Fruit production frequency as an indication of self-pollination in *Lepanthes eximia* (Orchidaceae: Pleurothallidinae) in Monteverde, Costa Rica

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ABSTRACT

Most orchids are capable of self-pollination, but outcrossing promotes the variation in flower structure and the specificity of pollination mechanisms prevalent in orchids. For this reason there are many elaborate pollination mechanisms involving specific pollinators. Highly specific mechanisms ensure efficient pollination; yet low fruit production is common in orchids and in the genus *Lepanthes*. An exception to this trend is *Lepanthes eximia*, which has a relatively high fruit set. This may indicate that *L. eximia* does not depend on rare pollination events, but self-pollinates, accepting low genetic variability in order to increase its number of offspring. Two groups of *L. eximia*, one exposed to potential pollinators and one covered with mesh bonnets, were observed for differences in fruit production in the orchid garden of Dr. Karen Masters in Monteverde, Costa Rica to determine if *L. eximia* self-pollinates. Comparisons in morphological variation of *L. eximia* and *L. pygmaea*, both exposed to potential pollinators, were made to examine the differences in variance between inbred and outbred species. *Lepanthes eximia* was found to be capable of self-pollination, but no significant difference in the variability of morphological traits of inbred and outbred species of *Lepanthes* exists.

RESUMEN

La mayoría de las orquídeas son capaces de polinizarse a sí mismas, pero la estructura floral y los mecanismos específicos de la polinización tienden a la polinización cruzada. Por eso, hay muchos mecanismos complicados de polinización con polinizadores específicos. Los mecanismos muy específicos aseguran la polinización eficiente, sin embargo la baja producción de frutas es común en las orquídeas y en el género *Lepanthes*. Una excepción a esta tendencia es *Lepanthes eximia*, que tiene un grupo alto de frutas. Esto indica que *L. eximia* no depende de los eventos raros de polinización, pero se poliniza a sí misma, aceptando baja variabilidad genética para aumentar el número de descendencias. Dos grupos de *L. eximia*, uno expuesto a polinizadores potenciales y otro cubierto con gorras de malla, estuvieron siendo observados para ver las diferencias en la producción de frutas en el jardín de Karen Masters en Monteverde, Costa Rica para determinar si *L. eximia* se poliniza a sí misma. Las comparaciones entre la variación morfológica de *L. eximia* y *L. pygmaea*, ambos expuestos a polinizadores potenciales, han hecho para examinar las diferencias en variación entre especies innatas y especies no innatas. Se encontró que *L. eximia* es capaz de polinizarse a sí mismo, pero no hay diferencia significativa en la variabilidad de características morfológicas de especies innatas y no innatas de *Lepanthes*.

INTRODUCTION

Orchidaceae is the largest family of angiosperms with recent estimates exceeding 20,000 species and 700 genera (Christenson 2004). Among the flowering plants, orchids claim the most species richness, with its peak in the American tropical cloud forest (Walter 1983; Dressler 1990). One of the most fascinating characteristics of orchids is the great variety of pollination mechanisms and the specificity of the relationship between an

orchid species and its pollinator (Christenson 2004). The variation in floral shapes, sizes and colors reflects the variety and specificity of orchid pollinators (Dressler 1990). The diversity and complexity of orchid pollination mechanisms and relationships have been the subjects of many studies that attempt to understand the evolution of the most diverse flowering plant family on Earth.

Pollination is a key mechanism in plant reproduction and may underpin the high levels of speciation in orchids (Esquilín and Tremblay 1999). There are two basic types of pollination. The first type, cross-pollination, increases heterozygosity and fitness by reducing inbreeding depression in offspring (Esquilín and Tremblay 1999; Jersáková and Johnson 2006). The second type, self-pollination, increases homozygosity, causing the loss of genetic variability. However, it has the short-term advantage of producing huge numbers of seeds without depending on the often rare and erratic pollinator visitation that is characteristic for orchids (Dressler 1990; Esquilín and Tremblay 1999; Walter 1983). Most orchids are capable of self-pollination, but outcrossing is more prevalent, increasing variation in flower structure and specificity of pollination mechanisms in future populations (Dressler 1990; Walter 1983). Selective pressures encouraging pollination with a conspecific has promoted the evolution of highly specific pollination relationships (Dressler 1990).

Orchids are adapted for rare and specific pollination events (Christenson 2004). Pollination mechanisms may be simple with orchids offering rewards to their pollinators such as nectar, oil, or wax in exchange for pollen dispersal (Walter 1983). Other mechanisms are more elaborate than others. One mechanism is “buzz” pollination, where the pollinator has to buzz at a specific frequency in order to have access to the reward. Another pollination mechanism may require the elongated proboscis of a sphingid moth. Not all orchid species offer rewards. Instead, they use deception mechanisms that include floral structures mimicking male intruders or female mates of the pollinator species to provoke attack or copulating behavior in a potential pollinator. Orchids also mimic nectar-producing flowers and give off fragrances that attract pollinators but do not actually reward the pollinator with nectar (Walter 1983). The pollination of orchids is highly efficient due to these pollinator specific mechanisms and they ensure that pollinia will be transferred to an individual of the same species.

Even though orchids have various species-specific pollination mechanisms, fruiting failure and low fruit production are common in orchids (Blanco and Barboza 2005; Calvo 1990; Janzen 1998; Tremblay 1997), indicating low pollination success and pollinator-limitation (Janzen 1998). Although outcrossing is favored promoting specialized relationships with pollinators, autogamy (self-pollination within one flower on an individual plant) and cleistogamy (self-pollination because the flower never opens) are known among some orchids, possibly to maintain populations that would otherwise never be pollinated (Endress 1994).

As with most orchids, many species of the epiphytic genus *Lepanthes* (Pleurothallidinae) experience infrequent pollinator visitation and commonly have low fruit sets (Blanco and Barboza 2005). Pollinators for *Lepanthes* are still largely unknown, but a few studies indicate that sexual deception to induce pseudocopulatory pollination by small Diptera is a possibility (Blanco and Barboza 2005; Tremblay 1997; Tremblay et al. 1998). Although *Lepanthes* species lack any detectable floral rewards for potential

pollinators, the majority of the species share the same basic floral structures that may attract male Diptera (Blanco and Barboza 2005).

One exception to the low fruit production trend in *Lepanthes* orchids is the species *L. eximia*. No pollinator has been observed for this species and contrary to the trend for congeners, *L. eximia* has a very high fruit set (K.L. Masters, pers. comm. 2006). The flowers and flowering patterns of this species have been described in the cloud forest by Hammel et al. (2003). The purpose of this study is to investigate how *L. eximia* is able to produce a high fruit set despite pollinator-limitation in the genus. I hypothesize that *L. eximia* is self-pollinated and is therefore able to produce a high fruit set because it does not depend on rare pollination events. I predict that fruit production of *L. eximia* will not differ significantly between groups where outbreeding is prevented and groups where it is allowed. I further predict that *L. eximia* will vary little morphologically because of low genetic variation and inbreeding in comparison to an outbred congener, *L. pygmaea* (K.L. Masters, pers. comm. 2006).

MATERIALS AND METHODS

This study was conducted in November of 2006 in the orchid garden of Dr. Karen Masters located southwest of La Estación Biológica de Monteverde in Monteverde, Costa Rica. The orchid garden consists of various orchid species living on frames covered in moss. The site is surrounded by Lower Montane Wet Forest at 1530 m. I first observed *Lepanthes eximia* in order to define its developmental stages for my study. The stages included the following succession: a new flower bud develops into a mature bud, the mature bud opens to a flower, the flower closes, then the pedicel may swell and produce a fruit. New and mature flower buds were distinguished from each other in this study by their size. A new flower bud measures a few millimeters in length with a width equal to or smaller than the width of the pedicel and has a red tip. The mature bud is larger than the pedicel and is ready to open as a flower. The pedicel is located just below the flower bud and as soon as it showed any swelling or a capsule-like appearance I called it a fruit.

Two groups of *L. eximia* individuals were previously separated in the garden. These two groups represented the experimental and control groups when testing for self-pollination. The experimental group consisted of 26 individuals enclosed in green mesh bonnets shaped with malleable wire to prevent access by potential pollinators. The control group consisted of 23 individuals without mesh bonnets and was easily accessible to potential pollinators. Before beginning my observations to test for self-pollination, I counted the number of mature buds and fruits that were produced previous to my observations (Figure 1). The previously produced mature buds and fruits remained on the plants for the duration of the study.

On the first day of the study and on ten days (not always consecutive) thereafter, I counted the number of leaves, new and mature flower buds, new fruits, and open flowers on each individual of the exposed and capped *L. eximia* (Figure 1). Leaves were not counted if they had not completely unfurled. New and mature flower buds and new fruits were identified by the characteristics previously described. Open flowers were noted as remaining open for a period of 24 hours.

Not all new mature buds were observed to open and close, and the exact developmental stage of the “mature buds” observed previous to the experiment was not

known. Before the pedicel swells, which occurs between five and six days after a flower closes in the observed *L. eximia*, a flower that has opened and closed looks like a mature bud. Therefore a closed flower may have been counted as a mature bud even though it had already passed this developmental stage. It is unlikely that double counting of mature buds occurred because of close observation, but that possibility exists. There was also the possibility that some previously produced mature buds were not identified as such and were actually closed flowers.

To test the variation in morphological traits between an inbred and an outbred species, I compared 23 individuals of *L. eximia* (inbred) and 17 individuals of *L. pygmaea* (outbred). *L. pygmaea* is similarly a tiny, epiphytic orchid and as in *L. eximia*, these individuals had been collected in 2005 and positioned along frames in the garden. The lengths of 94 leaves of *L. eximia* and 117 leaves of *L. pygmaea* were measured (in millimeters) using a caliper. The Equality of Variances F-test was used to compare the variance of leaf length between *L. eximia* population and the *L. pygmaea* population. I then tested the variation in average leaf lengths between individuals in each population. Bartlett's test was used to compare the variation of average leaf lengths of 17 individuals within the *L. pygmaea* population and within subsets of 23 *L. eximia* individuals.

RESULTS

Over the course of two weeks, four new mature buds, zero open flowers, and three new fruits were observed in 23 individuals of the *L. eximia* population exposed to pollinators (Figure 1). Although there were three new fruits, only one fruit was produced by a new mature bud observed in all stages of development. Nine new mature buds, four open flowers, and twelve new fruits were observed in the 26 capped individuals of *L. eximia* (Figure 1). Although there were twelve new fruits, only three fruits were produced by new mature buds observed in all stages of development. Four flowers were observed open in the capped *L. eximia* and remained open for about a 24-hour period. Three of these flowers produced fruits within the time frame of the study. No pollinators were observed at any time during the study.

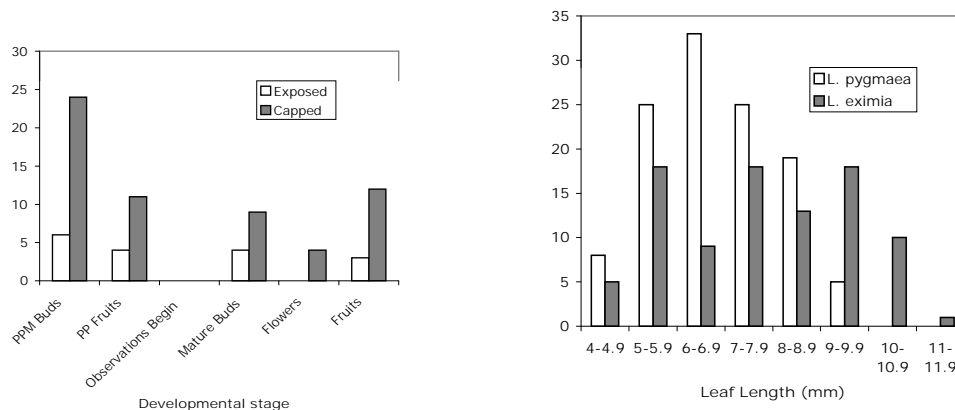


FIGURE 1. Observed number of previously Produced mature buds (PPM Buds) and fruits (PP Fruits), mature buds, open flowers, and fruits in the exposed and capped group of *Lepanthes eximia* for the duration of 2 weeks in Monteverde, Costa Rica.

FIGURE 2. The frequency of leaf lengths (mm) in 94 leaves of *L. eximia*, an inbred species, and 117 leaves of *L. pygmaea*, an outbred species.

There was significant difference in the amount of variability between the inbred population of *L. eximia* and the outbred population of *L. pygmaea* with *L. eximia* demonstrating greater variability in morphological traits (F-test, $F = 1.908$, $P = 0.0012$, $df = 93$, $n = 94$, $n = 117$) (Figure 2). The frequency of leaf lengths in *L. eximia* expands across a wider range of intervals (4 mm-12 mm) than *L. pygmaea* (4 mm-10 mm). The frequencies of each leaf length are also more variable in the *L. eximia* population across its range as opposed to the more continuous frequencies in *L. pygmaea* (Figure 2).

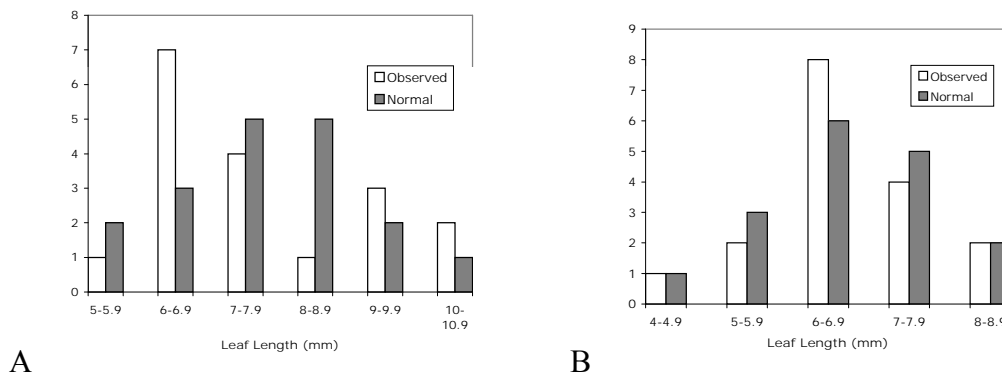


FIGURE 3. Observed and normal frequency distribution of average leaf lengths (mm) for A) subsets of 23 individuals of *Lepanthes eximia* and B) 17 individuals of *Lepanthes pygmaea* in Monteverde, Costa Rica.

When comparing the average leaf lengths of individuals of *L. eximia* and *L. pygmaea*, the frequency distribution of average leaf length per individual of 23 individuals of *L. eximia* appeared nonnormal and to have two or three modes (Figure 3A). The modes demonstrate subsets in *L. eximia* with leaf lengths between 5 mm and 7.9 mm, 8 mm and 9.9 mm, and 10 mm and 10.9 mm (Figure 3A). The frequency distribution of average leaf length per individual of 17 individuals of *L. pygmaea* was normal (Figure 3B) (Bartlett's test, $F\text{-ratio} = 2.47$, $df = 3$, $p = 0.0598$).

DISCUSSION

The purpose of this study was to determine whether *L. eximia* is self-pollinated and to examine the morphological variance in an inbred species compared to an outbred species. Few pollinators have been described or observed for *Lepanthes* and low fruit production indicates that the genus is pollinator-limited. In two studies performed by Tremblay

(1997) and Tremblay et al. (1998) over the course of two years on populations of *L. caritensis*, zero fruits were observed even though adult plants flowered continuously during the period of observation. In another study on *L. glicensteinii* in the Monteverde Orchid Garden, frequent pollination visits by *Bradysia floribunda*, a fungus gnat, yielded no fruits (Blanco and Barboza 2005). Contrary to this trend of low fruit production that was considered to be characteristic of all *Lepanthes* species, *L. eximia* displays a high fruit set despite having no observed pollinators. The columns in Figure 1 demonstrate that a total of 23 new and previously produced fruits were found on the 26 individuals of the capped *L. eximia* and seven new and previously produced fruits were found on the 23 individuals of the exposed group. When comparing the observed fruits of *L. eximia* with those of *L. caritensis* and *L. glicensteinii* in previous studies, *L. eximia* demonstrates high fruit production.

Initially, Figure 1 appears to show a high number of plant parts in each developmental stage in the capped *L. eximia*, but the ratio of the sum of the number of new mature buds and previously produced buds to the number of new fruits was 10 buds to 3 fruits for the exposed *L. eximia* and 33 buds to 12 fruits for the capped *L. eximia* (Figure 1). About the same percentages, 33% and 36%, of new fruits produced were produced from a combination of new and previously produced mature buds in both the exposed and capped groups. Although somewhat crude, this assessment indicates that the exposed group did not produce any more fruits than the capped group. Therefore, although the exposed group could have been both pollinated and self-pollinated and in so doing produce even more fruits, this did not seem to happen. Similar ratios for new mature buds that were observed producing new fruits suggest the same conclusion. One new fruit was produced from four new mature buds for the exposed group and three new fruits were produced from nine new mature buds for the capped group (Figure 1). These results are consistent with the results of a study by Esquilín and Tremblay (1999), which found no quantitative differences in fruit production when comparing self-pollinated and cross-pollinated populations of *L. woodburyana*. This supports my prediction that fruit production in the *L. eximia* group exposed to potential pollinators will not vary significantly from fruit production in the capped *L. eximia*.

In addition to these observations and comparisons, I observed four flowers open in *L. eximia* unexposed to potential pollinators, three of which produced fruits within the time frame of the study. This is a clear indication that *L. eximia* is capable of self-pollination and implies that it is self-compatible. Self-compatibility implies that the seeds in the produced fruits are viable and will generate offspring. The viability of the seeds of these populations of *L. eximia* was not tested in this study, but may be a subject for further research.

After determining that *L. eximia* is self-pollinated, two comparisons of the variation in morphological traits were made. In the first comparison, the variation in leaf length between all leaves in the exposed group of *L. eximia* (n = 94) and in the group of *L. pygmaea* (n = 117) was tested to compare the variation of morphological features for an inbred and an outbred species (Figure 2). It was expected that both populations would display a unimodal normal curve, but that the curve of *L. eximia* would have a narrower range of leaf lengths (Figure 4A and 4B). *Lepanthes pygmaea* did display a normal curve, but *L. eximia* displayed a wider range of leaf lengths than *L. pygmaea*. The inbred population of *L. eximia* demonstrated greater variation in morphological traits when

compared to the outbred population of *L. pygmaea* (Figure 2). One possible explanation for this unexpected result could be that each leaf was examined and tested as if it were an individual, when each leaf is actually connected closely to another leaf on the same plant. The scale of the comparison may have been wrong; therefore a second comparison on a different scale was performed.

The second comparison examined the variation in average leaf length of individuals within *L. eximia* ($n = 23$) and the variation in average leaf length of individuals within *L. pygmaea* ($n = 17$). It was expected that both populations of individuals would display normal curves. The individuals in *L. pygmaea* would display a unimodal curve, but the curve of the *L. eximia* individuals would either be unimodal with a narrow range of average leaf lengths or multimodal with more variance overall (Figure 4A and 4C). *Lepanthes pygmaea* did display a unimodal normal curve, but *L. eximia* displayed multiple modes. There appeared to be three separate modes in *L. eximia* in terms of average leaf length (Figure 3A). Because it is possible to have high variation if the population consists of highly distinct subpopulations or lineages, the three modes may represent three different lineages (A, B, and C) of inbreeding (Figure 3A). It was expected that individuals sharing a gene pool and a similar environment would also have similar phenotypes, or physical characteristics, so the three separate lineages of *L. eximia* observed was unexpected (Tremblay 1997) (Figure 3A). These results did not fit the normal curve, which indicates high genetic variability across subpopulations and low levels of inbreeding in the population despite self-pollination (Figure 3A). The observed leaf lengths of *L. pygmaea* were similar to the expected leaf lengths of a homogeneous population (Figure 3B). When comparing the variance of average leaf lengths of individuals between Lineages A, B, and C of *L. eximia* and individuals of *L. pygmaea*, it cannot be concluded that inbred populations are more homogeneous than outbred populations because Lineage A, an inbred population, was more variable in average leaf length per individual than *L. pygmaea*, an outbred population.

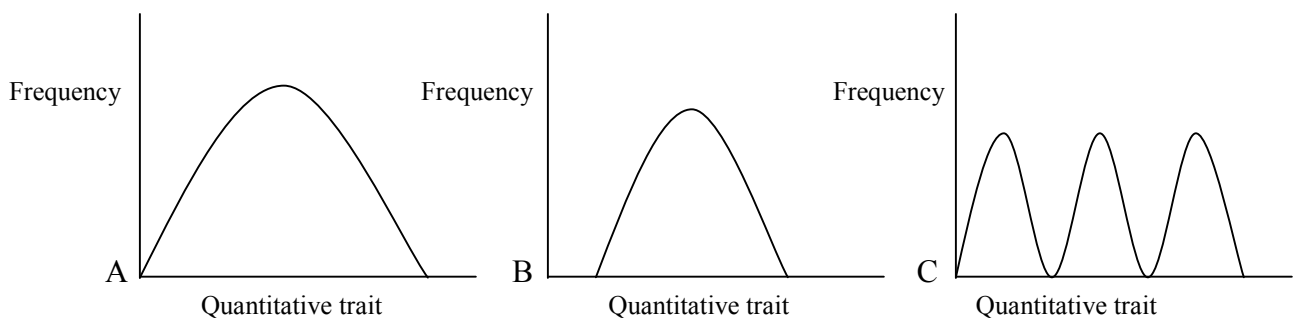


FIGURE 4. The expected curves for the quantitative traits in A) an outbreeding population, B) an inbreeding population, and C) a population with multiple subpopulations.

In a study examining morphological variation in tropical orchids, Tremblay (1997) found that morphological variation within and among populations in *Lepanthes* is large. He also found that gene flow among *Lepanthes* is low and that the majority of genetically distinct populations consisted of less than 20 individuals (Tremblay 1997). This is significant because it provides a possible explanation for the separate lineages in *L. eximia*. Tremblay's (1997) study supports that intraspecific morphological variation, between Lineages A, B, and C of *L. eximia* of my study, causes genetic sub structuring within one population. The concept of sub structuring as a result of morphological variance within a population is also supported by Walter (1983) who states that sympatric species of orchids can become reproductively isolated through morphologic adaptations.

The sub structuring of *L. eximia* could also be due to environmental differences in the microhabitats in which the individual plants spent most of their lives. The plants in the garden were collected from different locations in the cloud forest, and populations that are physically farther apart from each other are not as phenotypically or genotypically similar as those in closer proximity (Tremblay 1997). Another explanation could be that the age of the *L. eximia* individuals was not considered and may have been more varied causing differences in leaf length; one individual may have had more young leaves or more mature leaves. In order to further explore sub structuring, a future study could examine the variation in floral characteristics of *L. eximia* as opposed to leaf traits, which are more likely to be more influenced by the environment.

Although most morphological structures of orchids in *Lepanthes* favor cross-pollination, this study indicates that *L. eximia* utilizes self-pollination. With self-pollination as its reproductive mechanism, inbreeding and low genetic variability are expected to be expressed in the morphology of individuals in the population, but the results of this study do not support this prediction. It cannot be concluded that *L. eximia* demonstrates little morphological variation because of inbreeding, whereas an outbred species, *L. pygmaea*, demonstrates more variability. Additional studies on the physiological and ethological causes of sub structuring within *L. eximia* may be enlightening as to why high morphological variance is present in a self-pollinating population.

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