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Reproductive Strategies of a Population of *Besleria princeps* in the Monteverde Cloud Forest

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Abstract

In this study I looked at reproductive strategies of *Besleria princeps* (Gesneriaceae). I took population sex ratios from 100 randomly chosen plants over a nine day period and found that individual flowers as well as the population as a whole were protandrous. There was a significant difference in the ratios of males to females from day one to day two. This protandrous system promotes outbreeding thereby reducing the negative effects of inbreeding. In a separate group of plants, I bagged 94 individuals including 162 buds. When the buds opened, I noted their sex and separated them into groups in which I either probed for nectar, removed pollen, or left them alone. I monitored them daily, noting when the sex change occurred from male to female to determine if the plants responded to simulated visitation by pollinators. The group that I probed for nectar switched sex sooner than the control group, which suggests that the flowers switch after their pollen has likely been removed and therefore the chance of inbreeding is reduced. There was a trend among flowers whose pollen was removed to switch sex earlier than the control but it was not statistically significant.

Resumen

En este estudio, estudie las estrategias de reproducción en *Besleria princeps* (Gesneriaceae). Tomé proporción de sexos de una población de 100 plantas que yo elegí al azar en un plazo de nueve días y encontré que flores individuales y también la población eran protandria. Hubo una diferencia entre las proporciones de machos y hembras de día uno a día dos. Este sistema de protandria promueve entre cruce reduciendo los efectos negativos de endogamia. Se embolsaron 94 plantas individuales con 162 botones y cuando se abrieron yo hice nota de sus sexos y los separé en grupos en los que revise por néctar, quité polen, o los deje solos. Los revisé cada día, haciendo notas de cuando se dio el cambio de sexo de macho a hembra para determinar si las plantas respondieron a la visita simulada de polinizadores. El grupo que revisé por néctar cambio de sexo más rápido que el grupo control, lo que sugiere que las flores cambian después que su polen ha sido removido y por lo tanto el riesgo de endogamia se reduce. Hubo una tendencia entre las flores en donde el polen fue removido a cambiar de sexo más temprano que el control pero no hubo diferencia estadística.

Introduction

A plant's success is measured by its ability to reproduce. Different species have evolved unique strategies to maximize fitness, such as various modes of sexual reproduction, fragmentation (that is, the vegetative propagation of broken stems), vegetative

reproduction (i.e. reproduction via rhizomes) or a combination of these strategies. Most plants rely on sexual reproduction, namely outcrossing, as their major mode of reproduction. Outcrossing is defined as the production of offspring resulting from fertilization of gametes from different individuals (Endress, 1994). Consequently outcrossing promotes genetic variability, whereas in most cases inbreeding causes inbreeding depression (Charlesworth and Charlesworth 1987). Inbreeding depression results from increased homozygosity and the expression of deleterious alleles which reduces fitness (Charlesworth and Charlesworth, 1987). Therefore, as Darwin suggested, many plants have adopted reproductive systems that ensure outcrossing, thus avoiding inbreeding and its costs. The phenomenon in bisexual flowers when pollen is exposed before stigma receptivity (Endress, 1994), known as protandry, is one strategy often used to promote outbreeding (Wiklund and Solbreck, 1982). Plants that rely on biotic pollinators may face uncertainty in whether or not outcrossing will occur. Therefore it may be advantageous to reproduce asexually as well as sexually. Asexual reproduction occurs when the offspring are cloned from their parent plant. Vegetative propagation is one mode of asexual reproduction that does not result in the reduction of heterozygosity like inbreeding does.

In this study I observed *Besleria princeps* (Gesneriaceae). It is characterized by flowers with brilliant orange tubular corollas and leaves that are dark green with dense pubescence. This plant employs both sexual and asexual means of reproduction. It reproduces sexually through hummingbird pollination as well as asexually through rhizomes and fragmentation. What makes this plant so intriguing to study is its conspicuous sexual dimorphism. When the flower is in the male phase, the stigma is hidden behind the anthers, which are dehiscing pollen. In the female phase, the anthers dip to the base of the flower exposing the stigma and allowing pollination. This prompted me to look at breeding mechanisms in this plant, especially those that promote outbreeding.

I examined the sex ratios of individual's plants as well as the sex ratios of an entire population. I hypothesized that individual flowers would be protandrous to deter inbreeding. Further, if protandrous flowers may be triggered to change sexes in response to particular cues. I hypothesized that pollen removal, nectar removal or both would speed up the sex change process. I reasoned that probing a flower's nectar glands should simulate being visited by a hummingbird, as should removing pollen from anthers. If so, flowers may then switch sex to female since the risk of receiving their own pollen should be minimized (Koptur, 1990).

The timing of nectar production, nectar volume and concentration, may affect inbreeding and outbreeding rates (Baer, 1999). I assumed that nectar production would take place on the first day that the flower opens. The faster that a flower is able to be pollinated, the less chance it has to be killed by insects or chance events. The plant's main goal is to outcross whereas the hummingbird's main goal is nectar, so if nectar is produced on the first day, the flower increases its chance of being visited. By studying the reproduction of *B. princeps* I hoped to better understand its abundance and apparent success as a species.

Materials and Methods

This study on *Besleria princeps* was conducted in the forest behind the Estación Biológica, Monteverde, Puntarenas Province, Costa Rica from November 8, 1999 to November 20, 1999. In one population of *B. princeps*, which I did not manipulate or bag, I took sex ratios five times every other day over a nine day period. The first day that I took these sex ratios and every successive inventory was termed as day one, day three, day five, etc. On each day I sampled 100 plants, not taking into consideration which plants had been sampled the previous day. The flowers were placed into three categories: functional male, non-functional male, and female. The functional males have pollen on their anthers whereas the non-functional males have no pollen on their anthers. I did not consider the non-functional males to be truly one sex or the other because at this point in their life cycle they can perform neither as male nor female. Therefore I did not analyze them in my data analysis. Every day, I tested to see if there was a 50: 50 ratio of functional males to females. Each successive day I tested to see if the ratios were significantly different than the previous day. These analyses were done using a Chi-squared test. On the plants with multiple flowers, I also noted how many of these plants were of a single sex and how many were of multiple sexes. These results were then graphed.

In a separate group of plants, a total of 94 plants containing 162 buds were covered with breathable bags and numbered. Throughout the study any flower that was attacked by insects or had evidence of nectar robbery (a hole in the base of the corolla or calyx) was discarded and not used for the study. Every day I noted which buds had opened, recorded their sex, and observed them every day thereafter. In this case, the day that the buds opened was termed day one and each successive day was given the term day two, day three, etc. These sex data were used to determine whether or not this species at the level of the individual flower is protandrous. Each newly opened flower was assigned into a different treatment. One group of 20 flowers was assigned to the treatment of nectar removal. I tried to extract nectar from the flowers, using capillary tubes, on a daily basis to determine when nectar was produced. The second group, consisting of five flowers, was assigned to the pollen removal treatment. I removed pollen from the anthers using a capillary tube on the first day that the flower opened. The third group contained 11 flowers and was my control group, to which I did nothing. After I administered the treatments I watched the flowers daily until they switched to the opposite sex. I observed all of the flowers that I used in my analysis for at least ten days, and for those flowers which I did not have time to observe until completion. I assumed that the sex change would occur on the day after they were last checked. These data were analyzed using a Kruskal-Wallis test to determine whether pollen removal, nectar removal, or no manipulation affect the average day of sex change.

Results

All of the 88 flowers that I observed on the first day open were male. On day one through five, the percent's of functional males to total were 78%, 64%, 60%, 54%, and 48%

respectively (Figure 1). Days one, two, and three had significantly different ratios (male biased) than 50: 50 ($\chi^2 = 44.09$, $\chi^2 = 9.63$, $\chi^2 = 4.68$, $p < 3.81$), but by day four the sex ratio was not significantly different from 50: 50 ($\chi^2 = .833$, $p < 3.81$), becoming less male-biased.

The ratio of bisexual to unisexual plants did not differ from 50: 50. Day one through nine had 24, 17, 16, 17, and 20 bisexual plants and six, 13, 12, 13, and 19 unisexual plants respectively (Figure 2).

The nectar treatment group produced very little nectar and the day that nectar was produced was not determinable. Only one of the 20 flowers produced nectar on the first day and in five other flower nectar was produced on various later days.

The mean day in which sex change occurred from male to female for the control, nectar, and pollen groups were approximately 11 (sd = .894), 8 (sd = 1.447), and 10 (sd = 1.643) respectively (Figure 3). The Kruskal-Wallis test showed that there was a significant difference between at least one of these values ($H = .0001$, $p < .05$). The difference occurs between the control group and the nectar-removed group.

Discussion

Individual flowers as well as entire populations avoid inbreeding depression by employing mechanisms that promote outbreeding. Therefore, evolution via selection has favored flower systems that reduce inbreeding (Feldman and Christiansen, 1983). Protandry has been suggested as one possible mechanism to increase outbreeding (Wilklund and Solbreck, 1982). On an individual level, all the *Besleria princeps* flowers that I observed were protandrous. They began as males and after an average of ten days, switched to female. All flowers complete this cycle before they die, whether or not they are visited by a pollinator. The different sexual phases have different reproductive structures (Figure 4). In addition to the male and female phases described earlier, there is also an intermediate stage which lasts a day or two where the anthers are still placed in front of the stigma but do not have pollen on them. This loss of pollen occurs due to visitation by a pollinator or if visitation does not occur, the anthers drop their pollen. This protandrous cycle is an evolutionary mechanism to inhibit setting. In one visit or even successive visits in a relatively short period of time, the pollinator is unable to pollinate the same flower from which it receives pollen.

On the plant level, however, *B. princeps* does not exhibit protandry to promote outbreeding. The occurrence of bisexual plants are almost as common as unisexual plants (Figure 2). New buds are continuously opening and the sex change of the other flowers on the same plant are occurring on different schedules. This may suggest a tradeoff between reducing inbreeding depression and maintaining local adaptation (see below).

At the level of the population, protandry is again apparent. The sex ratios of successive days showed a significant reduction in the number of males and increase in the number of females (Figure 1). I started taking sex ratios with the first flowering period of the season and only went through approximately one complete cycle of sex changes. It would be interesting to see if this oscillating pattern would continue or if the ratios would even out with time. I expect the latter, due to the constant emergence of new buds and flushing of old females, however more investigation is needed.

When I tried to take nectar from the flowers, I found that very little nectar was produced. Only 1 of the 20 flowers produced nectar on the first day which leads me to conclude that this species exhibits delayed nectar production. This phenomenon is also seen in *Columnea microcalyx*, another Gesneriaceae flower (Baer, 1999). In *B. princeps*, nectar was produced on various later days in five other flowers. Others produced enough nectar to be tasted but not collected in capillary tubes and still others did not produce any at all. Also there did not seem to be any vegetative cues, such as difference in flower size or color, to which flowers produced nectar or not.

My data support my prediction that nectar removal promotes sex change. The flowers in the nectar treatment on average changed sex sooner than the control. When nectar is removed, the plant senses that it has been visited by a pollinator, pollen has been removed, and the chances of self-pollination are thereby reduced. The plant thus responds by becoming female (Koptur 1990). This is another mechanism by which *B. princeps* promotes outbreeding. There was also a trend toward the flowers in the pollen treatment changing sex sooner than the control, but it did not seem to be statistically significant. Two possible explanations account for this statistical non-significance. First, it is possible that due to my small sample size I was unable to detect the difference. Second, it is possible that it is easier for a given flower to detect nectar removal than pollen removal. The anthers which are more exposed to insects, rain, wind and other environmental conditions may be less sensitive to disturbance than the more protected area around the ovary.

There are two possible mechanisms, not mutually exclusive, to explain the phenomena seen in this plant. First, the flower is trying to promote outcrossing by employing floral mimicry. Due to external cues such as color or fragrance, the pollinator visits this flower expecting a reward, however the mimic does not provide a reward and pollination occurs through deceit (Haber, 1984). In the case of *B. princeps* the flower possess the right floral characteristics to be a hummingbird pollinated plant however does not produce very much nectar. The plant reduces its costs of making nectar and at the same time forces the hummingbirds to visit many flowers to acquire nectar, thus promoting outcrossing (Feinsinger, 1983). The idea that *B. princeps* wants to promote outcrossing also explains its protandrous nature at the level of the individual and population and its response to visitation by a pollinator. It does not however explain why *B. princeps* is not protandrous at the level of the individual plant. Also because the flower does not produce very much nectar and the hummingbird is forced to visit many flowers, possibly on the same plant, to find nectar, floral mimicry may be an inefficient way to reduce inbreeding.

The second explanation is that *B. princeps* relies more on vegetative propagation rather than pollination by hummingbirds, but it retains ancestral traits from a time when outcrossing was necessary that occasionally result in outcrossing. This would explain the showy flowers with little to no investment in nectar production. It also explains the occurrence of protandry and why at the level of the individual plant protandry is relaxed/nonexistent. This explanation also makes sense because this population of *B. princeps* lives in an environment where abiotic factors make vegetative reproduction possible, and in fact occurs the majority of the time. Populations of *B. princeps* sampled near my study site, reproduced more often by fragmentation than seed germination, with

only five of 40 plants sampled developing from seed germination (Leamy, 1999). This is not surprising since this area is of high moisture and high disturbance, such as falling lianas, limbs, or trees. This highly cloudy and moist environment may also not be very conducive to hummingbirds. Of the females that I sampled, few had obvious signs of pollination (pollen on the stigma). Hence vegetative reproduction may be an important method of survival for understory plants such as these that occur in areas of high disturbance (Garner 1989). Although vegetative propagation does not promote genetic variation, it does not reduce heterozygosity like inbreeding does. Whatever amount of heterozygosity that the parent plant has is preserved in offspring through vegetative propagation. A plant that is well suited for its environment wants to promote these adaptive genes and clone itself. This explanation does not however explain very well why the flower is stimulated to switch sex by visitation by a pollinator. Neither of these two explanations is perfect but it seems as if outbreeding, when possible, is a better alternative, however when nectar production is costly or pollinators are limited, asexual reproduction via fragmentation may be a better alternative.

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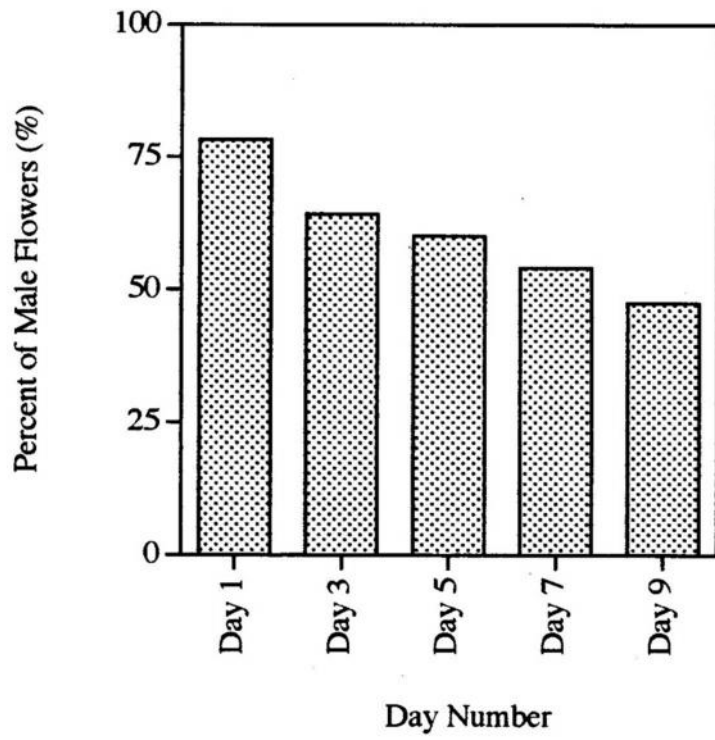


Fig. 1 Percent of male flowers of all flowers open in the population over a nine day period.

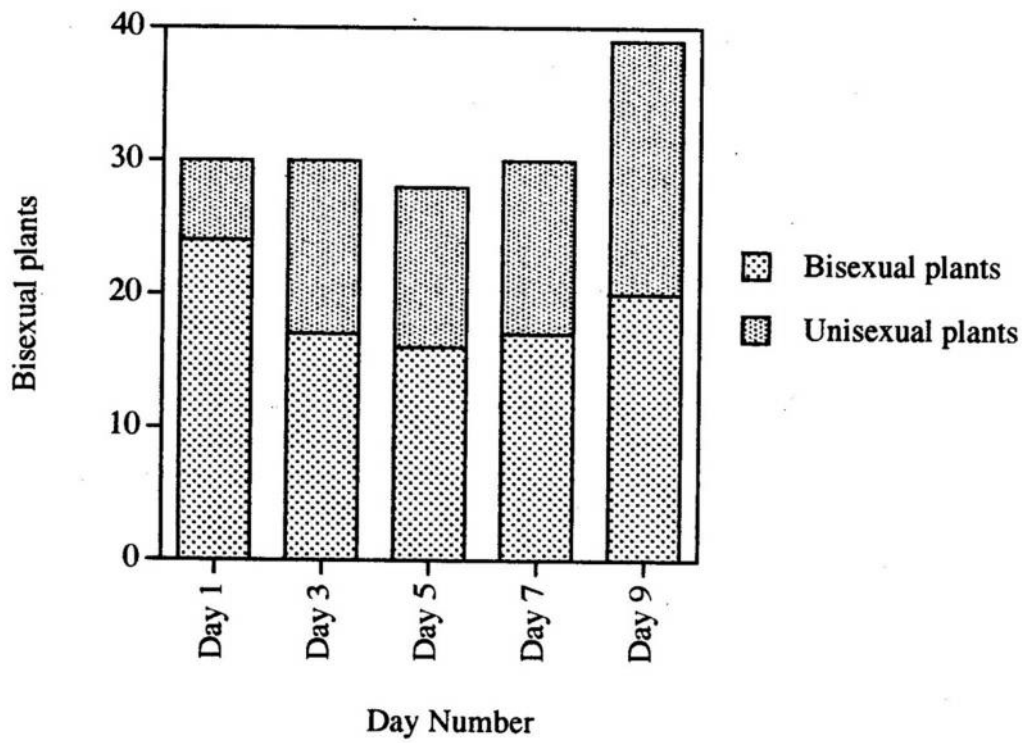


Fig. 2 Number of bisexual and unisexual plants in a population over a ten day period.

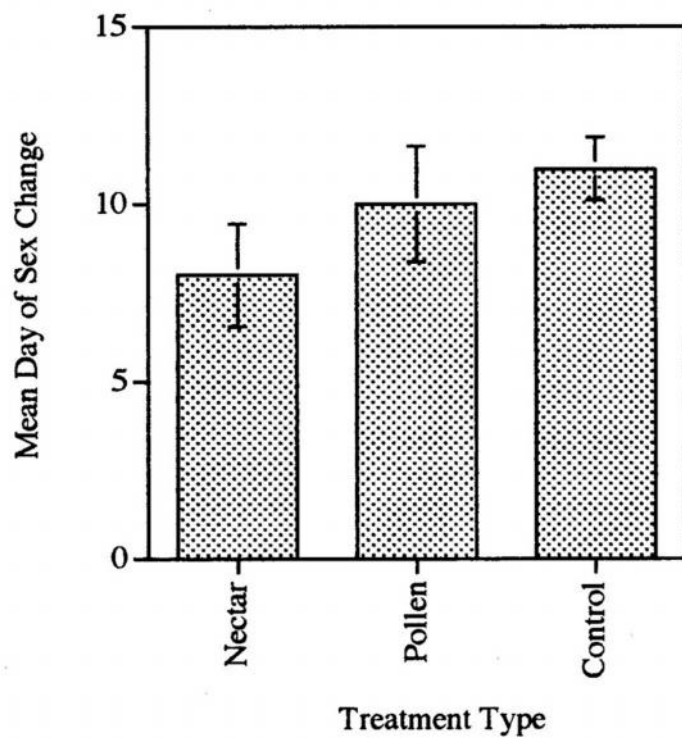


Fig. 3 Mean day in which sex change occurred between the three treatment types.

Male Phase

Female Phase

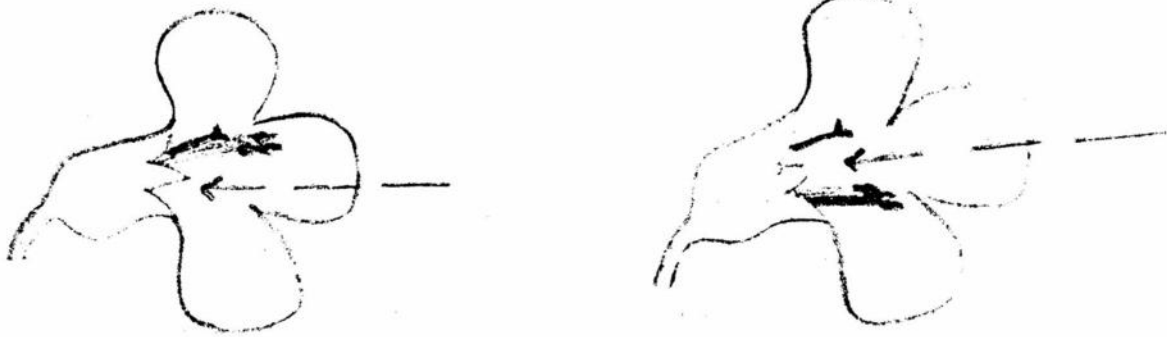


Fig. 4 Female versus male floral structures in *Besleria princeps*. Flowers in the male phase have their anthers raised against the roof of the corolla hiding the stigma. Flowers in the female phase have their anthers lowered to the bottom of the corolla allowing the stigma to be available for pollination.