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Relationship between floral advertisement and pollinia removal in Oerstedella exasperata (Orchidaceae)

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ABSTRACT

The purpose of this study was to examine how the floral displays and morphology of *Oerstedella exasperata* affect reproductive success. This species of orchid is typified by extreme variability in both inflorescence and floral characteristics. In particular there is a wide distribution regarding the degree of symmetry observed in the labellum. The degrees of symmetry are characterized by variations within the fringe symmetry and bilateral symmetry. Furthermore, the surface area of the labellum varies greatly within a population. These remarkably diverse phenotypic morphologies and the summation of the floral traits can enhance or reduce the reproductive success of particular species. To test the relationship between floral advertisement and reproductive success, each flower was examined and grouped according to pollinia presence or removal. Results indicated that pollinia removal was not affected by the degree of bilateral or fringe symmetry or by the size of the labellum. Furthermore, within the 100 individuals examined, the asymmetrical trends were considered directional for bilateral symmetry but nondirectional for fringe symmetry.

RESUMEN

El objetivo de este estudio fue examinar como los patrones de asimetría en los rasgos morfológicos florales de *Oerstedella exasperata* afecta el éxito reproductivo de esta especie. Esta especie de orquídea se caracteriza por la extrema variabilidad en las características de la flor y de la inflorescencia. El grado de simetría se caracteriza por variaciones entre la simetría radial y la simetría bilateral. En otras palabras, el área superficial del labio varía ampliamente dentro de una misma población. Esta remarcable diversidad en la morfología fenotípica adicionado a los rasgos florales puede incrementar o reducir el éxito reproductivo de esa especie. Para determinar la relación entre las características florales y el éxito reproductivo, cada flor fue examinada y agrupada de acuerdo a la presencia o ausencia de los tubos polínicos. Los resultados indican que la remoción de los tubos polínicos no se vio afectada por el grado de simetría bilateral o la radial, o por el tamaño del labellum. Dentro de los 100 individuos examinados, de rasgos asimétricos fueron considerados direccionales para la simetría bilateral y no direccionales para la simetría

INTRODUCTION

The Orchidaceae family is an extremely diverse group of angiosperms that consist of over 20,000 species. Among these species and within individual species, various advertisement mechanisms are used to promote successful pollinia removal. Pollinia are coherent waxy masses of pollen grains and their removal is an indirect measure of reproductive success (Nilsson & Fritz 1996). In order to ensure removal, pollination ecology and the advertisement strategies of orchids have been co-evolving for millions of years. Orchid species have evolved fine-tuned suites of advertisement strategies to further capture the attention of pollinators. The advertisement strategies of flowers rely on intense displays of color, symmetry, size, shape and inflorescence size (number and arrangement of stalks, buds and open flowers). One of the predominant cues that orchids use to lure their pollinators is the attractiveness and exploitation of their labellum, which is a modified median petal that is often critical in attracting, trapping, or providing a landing pad for pollinators. The labellum is further characterized by fleshy lumps, ridges, keels, fringes and lace (Dressler 1981). In addition to these phenotypic traits, the production of nectar, scents and oils act as rewards to entice and create consistent pollinators. These individual characteristics and the summation of the displays provide various stimuli that promote pollinia removal and reproductive success (Murren & Ellison 1996).

The majority of orchids are dependent on specific types of pollinators to ensure pollinia removal and reproductive success. However, due to the sheer number of orchid species, there is limited knowledge regarding each pollinator. There is an array of taxa that pollinate orchids; however bees are the most common. Bees are attracted to orchids that have a sweet honey smell, nectar guides and lack a pure red coloration (van der Cingel 1996). Within this bee pollination syndrome, there is a tendency for bees to visit preferentially different forms of flowers, even among intraspecies forms (Kiester et al. 1984). A general pollination syndrome has been applied to many understudied orchid species as a result.

The tropical montane orchid, *Oerstedella exasperata*, is one of many understudied orchid species. Based on its morphological characteristics and its tendency to follow the bee pollination syndrome it is assumed to be pollinated by bees. The morphological tendencies of *O. exasperata* show extreme variability in both inflorescence and floral characteristics. These remarkably diverse phenotypic morphologies may affect pollination success. The flower is characterized by three brown valvate sepals with a pale yellow outline along the edge. The two petals tend to have a pale green base which fades into a white/yellow color. The labellum is a predominately ornamental median petal that is white with distinct purple markings. Based on morphological observations of the various labella within *O. exasperata* there are apparent differences amongst the coloration patterns, coloration intensity and surface area. Furthermore, there is obvious asymmetry in terms of the number and size of the labella fringes. Degrees of symmetry are based on differences between the right and left side of the labella. These quantitative measurements reveal that the labella contained within *O. exasperata* populations have varying degrees of symmetry.

Prior studies indicate that morphological variation among intraspecies flowers directly affects their reproductive success and ability to attract pollinators. The morphological variation observed in terms of the symmetry and area of the *O. exasperata* labellum, raises the question of whether these variations also increase or decrease the reproductive success of the individual. In relation to the fluctuating asymmetric tendencies of the labella, studies have shown that in many species of flowers there is assortative pollination, in which the pollinators preferentially visit symmetrical flowers (Møller 1995). This relationship was further examined using artificial flower models and bumblebees; the results also indicated that bumblebees preferred to visit the larger, symmetrical flowers (Møller & Sorci 1997). Correlations between morphological flower variations and reproductive success have also been analyzed in terms of surface area of floral structures**.** It has been shown that butterflies, honeybees and bumblebees preferentially visit and pollinate flowers with longer outlines (Dafni 1992). Based on these previous studies, morphological characteristics on the flower level play a key role in advertisement and reproductive success.

The morphological characteristics and advertisements such as color intensity, symmetrical arrangements, inflorescence size and labellum size have most likely developed evolutionarily in conjunction with their corresponding pollinator. The morphological variations within *O. exasperata* should directly affect the percentage of pollinia removal. The specimens with the more attractive morphological variations should represent the specimens that have a greater percentage of pollinia removal. Therefore flowers with larger, more symmetrical labella should have a higher percentage of pollinia removal. The intraspecies variability within the *O. exasperata* population gives an unusual opportunity to test predictions regarding the effects of flower morphology on pollinator behavior.

MATERIALS AND METHODS

Oerstedella exasperata is a predominantly terrestrial orchid that flowers between October and March from Costa Rica to Panama at an elevation of 1000-2,200 meters (Zuchowski 2005). This investigation was conducted between October 23, 2007 and November 18, 2007. The study site was located near the Monteverde Cloud Forest of Costa Rica at an elevation of approximately 1800 meters. All *O. exasperata* examined were found on the banks of Cerro Amigos heading towards the Monteverde radio tower.

Analysis focused on the labellum morphology; however additional observations were made regarding inflorescence structure and size. The sample size included 100 recently senesced flowers which were taken from 19 individual plants. These older flowers were chosen in order to ensure that all flowers had an equal opportunity of being pollinated. The older flowers are characterized by a pale yellow coloration within the lip, rather than the white lip of the younger flowers.

Each of the 100 flowers were marked, assigned a number and labeled with a letter indicating the parent plant. The number of stalks and the number of flowers in bloom per plant were counted. For each labellum, the number of fringes on the right and left side were quantified. The difference in the number of fringes observed on the right and left side of the labellum was used as a parameter to determine the degree of symmetry. The absolute value of the difference between the number of fringes on the right and left side was considered the degree of fringe asymmetry. The second parameter for measuring degree of symmetry was based on bilateral symmetry in terms of the length of each side. The length of the right and left side of the lip was measured in millimeters using calipers. The absolute value of the difference between the two sides was considered the degree of bilateral asymmetry. Width measurements were also recoded; these measures were taken at the widest region of the labellum. Pollinia removal (PR) was determined by probing the inner column; if the flower had not been pollinated then four pollinia were present (PP) in the column. The last observation was made by removing the labellum from the column and measuring the area (cm^2) using the Leaf Area Meter. This device will not take accurate area measurements if the sample is too translucent, therefore prior to taking measurements it was necessary to color evenly the labellum with a permanent marker.

The morphological characteristics of the labellum were analyzed separately and in relation to pollinia removal. Frequency distributions were performed in order to determine the degrees of asymmetry among the sampled individuals. Shapiro-Wilk

Normality tests were run on these distributions to adjust the distribution. A Sign test was conducted using the absolute values of the two degrees of asymmetry to determine if the trends in asymmetry were directional or non-directional. A simple regression analysis was run in order to determine if the two measures of symmetry were correlated. Simple regressions were also conducted in order to determine if significant correlations existed between labellum size (width or area) and symmetry. Width and area were run individually against three measures of symmetry. The first two symmetry measures used were the previously calculated degree of fringe symmetry and degree of bilateral symmetry. The third measure was considered the index of asymmetry and it was calculated for each flower by multiplying the absolute value of the fringe symmetry to the absolute value of the bilateral symmetry. Finally, six t-tests were performed to establish if individual morphological traits (symmetry, area and width) affected pollinia removal.

RESULTS

The results here indicate that there is a great degree of asymmetry in the population studied, in terms of both fringe and bilateral symmetry (Figs. 1 and 2). In the case of fringe symmetry, it is nondirectional, i.e., it fluctuates between having greater numbers of fringes on the left and right equally resulting in an average symmetry of nearly zero. The nondirectional symmetry was further supported by the results of a Sign test. In the case of the length of the two sides of the lip, however, the symmetry is directional: there is a tendency for the lip to be slightly longer on the left side. A Sign test reinforced these results of directional symmetry seen in Figure 2. There is no trend relating the degree of bilateral or fringe symmetry (Figure 3), or between the area (cm^2) of the lip or any measure of symmetry (Figure 4). Furthermore, there was not significant regression between the width of the labellum and the degree of symmetry (bilateral or fringe; Figure 5). Out of the 100 senesced flowers sampled, 55 had their pollinia removed (PR) while the remaining 45 had pollinia present (PP). However, there was no significant difference in the morphological features of the flowers with pollinia removed versus those with it still present in terms of their symmetry, lip width, or area (Figs. 6-8).

DISCUSSION

This study is the first to document the amount of labella asymmetry found among *O. exasperata* individuals in the Monteverde population. A gradient of bilateral asymmetry was not only observed within the number of labella fringes but, also in terms of the length of the right and left side. On each flower *O. exasperata* has lip fringes that number from two to six, often in unequal numbers, resulting in high asymmetry. The degree of fringe symmetry fluctuates randomly, such that the overall mean for the population is near zero. Fluctuating asymmetry is thought to be a result of either genetic or environmental factors (Neal et al. 1998). Studies indicate that individuals with a higher level of fluctuating asymmetry tend to be less fit in terms of reproductive success because they are discriminated against by pollinators (Neal et al. 1998). Based on preliminary studies with *O. exasperata*, results did not coincide with the above findings, indicating that asymmetry does not necessarily result in reduced fitness for this species.

Regarding the second measure of asymmetry, results indicated high bilateral asymmetry, but in this case, it is directional (with the left side usually larger). This may be due to how the flower twists while developing in the bud. *O. exasperata* has a resupinate labellum (on the bottom) and therefore only twists once, just prior to opening (Dressler 1981). This process of twisting may be directional, and if so, during this process the left side may tend to elongate.

Asymmetry tends to reflect "developmental instability", i.e., a mismatch in the growth stages that the two sides of a bilaterally symmetrical organism experiences in early development. Sometimes it arises among highly homozygous individuals, or otherwise genetically impaired individuals. Furthermore, the environment can have an impact on whether development proceeds as it should. According to Neal et al. (1998) the factors that trigger asymmetry may result in overall reduced fitness. This infers that one developmental obstacle can result in subsequent reductions of other attractive traits. If this were true for *O. exasperata* then one would expect a correlation to exist between the measures of all asymmetry. However, results indicated that there was no correlation between the degree of bilateral asymmetry and the degree of fringe asymmetry (Figure 3). Perhaps the asymmetry in the two traits arises due to developmental problems that occur at different stages or under the influence of differential environmental factors. If the traits are genetically decoupled or under environmental influences then it is understandable that a correlation does not exist between the two degrees of symmetry.

In a recent study, Strauss (1997) indicated that fitness is also based on other morphological characteristics (*e.g.* surface area) that attract pollinators. If asymmetry truly affected the fitness of *O. exasperata* then one could expect other morphological characteristics such as the area and width of the labellum to be affected as well. However, the asymmetrical tendencies observed within the individual labella did not correlate to the corresponding widths or area. These findings counter past studies performed by both Møller (1995) and Neal et al. (1998) who indicated asymmetry results in an overall decrease of fitness. Despite the non-significant correlations observed between asymmetry and surface area, future studies may want to consider the relationship between asymmetry and other more attractive traits not examined in this study, for example, lip color, nectar concentration, or floral scent.

Both symmetry and surface area as floral displays are considered signals of phenotypic and genotypic quality (Møller 1995). According to Møller (1995), bumblebees preferentially visited and pollinated flowers that were either large or symmetrical. Based on these findings it was reasonable to study the effects of labellum symmetry and labellum area on pollinia removal. However, results were not consistent with previous findings. Results indicated that flowers that had pollinia removed were no more symmetrical, nor did they have larger or wider lips, than the flowers whose pollinia were removed. The contradictory results may be due to the quantity and quality of *O. exasperata* nectar production. The studies that have shown that insect pollinators preferentially pollinate symmetrical or large flowers had indicated that nectar production was greater for these individuals (Møller 1995). In terms of symmetry alone, this signal and the high quality reward has exerted selective pressures on those flowers that are not as symmetrical (Giurfa et al. 1999; Møller & Sorci 1997). Due to the fact that *O. exasperata* is extremely understudied it is not known whether nectar production correlates with symmetry or any other morphological trait. If morphological signals are not signaling greater rewards, than there is no reason why the degree of symmetry or degree of one morphological trait would be uniform throughout the population. On another note, pollinia removal may not have been dependent on symmetry or flower size due to the possibility that the pollinators of *O. exasperata* are simply more enticed by overall plant and inflorescence size rather than the parameters examined.

A greater understanding of the reproductive success of *O. exasperata* is dependent on future studies taking into account other morphological parameters. These parameters must include plant location, plant density, inflorescence size, and flower density seeing as these attributes are more than likely responsible for initially attracting pollinators. Studies have indicated that reproductive success is not only affected by morphological variations on a flower level but it is also influenced by the morphology on a plant level (Lehtila & Strauss 1997; Larson & Larson 1990; Bierzychudek 1981; Flores-Palados & Garcia-Franco 2000; Todzia 1983). Pollinator behavior and the reproductive success of flowers have also been linked to chemical attractants (Galen et al. 1987) and environmental factors (Johnson & Bond 1992), therefore these two dynamics are worthy of investigation as well. Furthermore, in order to obtain a better grasp on the morphological trends and reproductive success within the population of *O. exasperata*, studies must focus on determining who in fact pollinates this species. Assumptions will hinder further understanding between floral displays and reproductive success because the sensory capabilities may be very different between bees, flies and butterflies.

In terms of reproductive success and pollinia removal the parameters tested proved to be an oversimplification of pollination ecology. Although this study explored floral characteristics that have been deemed important in other species; the lack of such importance here implies that future *O. exasperata* studies will require a multifactorial approach. This multi-dimensional approach along with the acknowledgement that many cues operate simultaneously on the pollinator will enhance our understanding of pollination systems, floral morphology and reproductive success.

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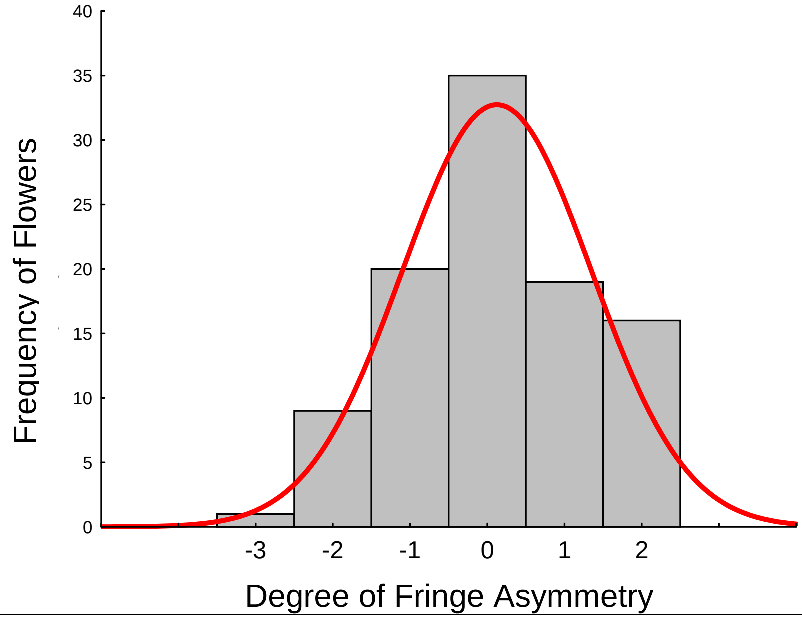

Figure 1: Frequency distribution of the degree of fringe asymmetry on the labellum of *Oerstedella exasperata* in Monteverde. Degree of fringe asymmetry = (# of fringes on right of the labellum)-(#of fringes on left of the labellum). Shapiro-Wilk Test of Normality; SW-W = 0.92, $P < .0001$, n = 100 recently senesced flowers.

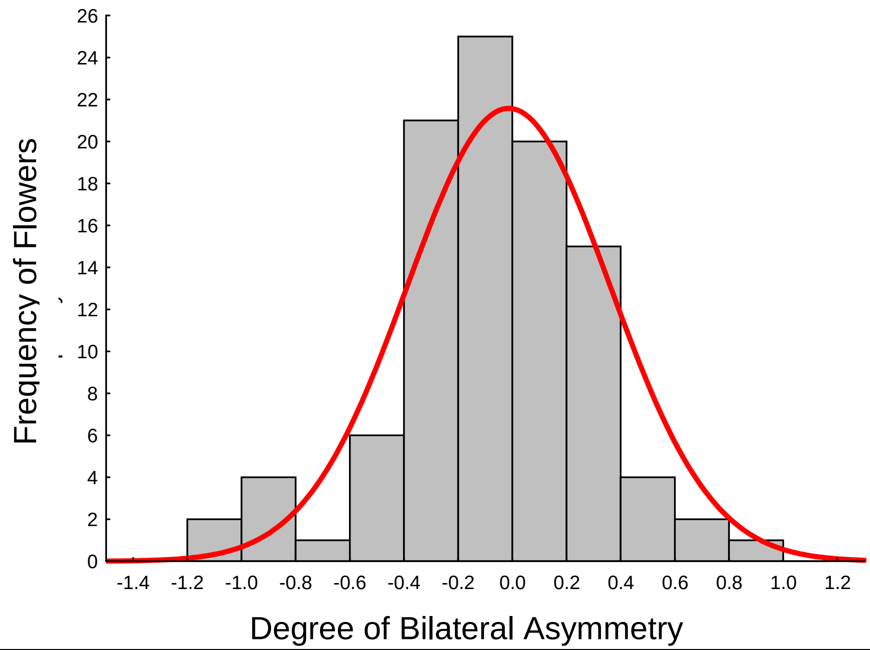

Figure 2: Frequency distribution of the degree of bilateral asymmetry on the labellum of *Oerstedella exasperata* in Monteverde. Degree of bilateral asymmetry = (length of right)-(length on left). Shapiro-Wilk Test of Normality; SW-W = 0.97, P = 0.01, n = 100 recently senesced flowers.

(NOTE: here we have a case of *directional* asymmetry: the left side tends to be longer than the right. This is evident by the p value, and the high frequency of negative values.)

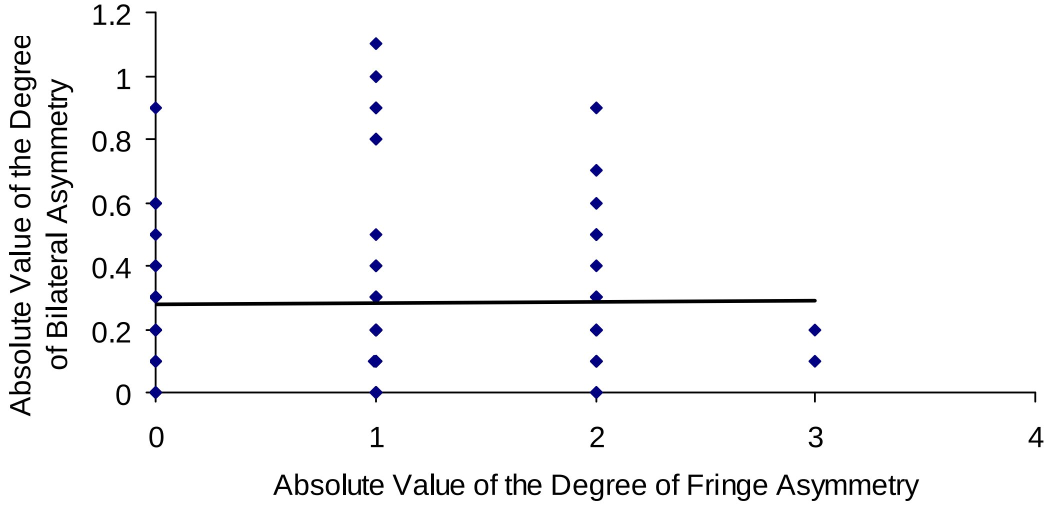

Figure 3: Relationship between the degree of bilateral symmetry and fringe symmetry. $R^2 = 0.00$, $P = 0.92$, $n = 100$ recently senesced flowers.

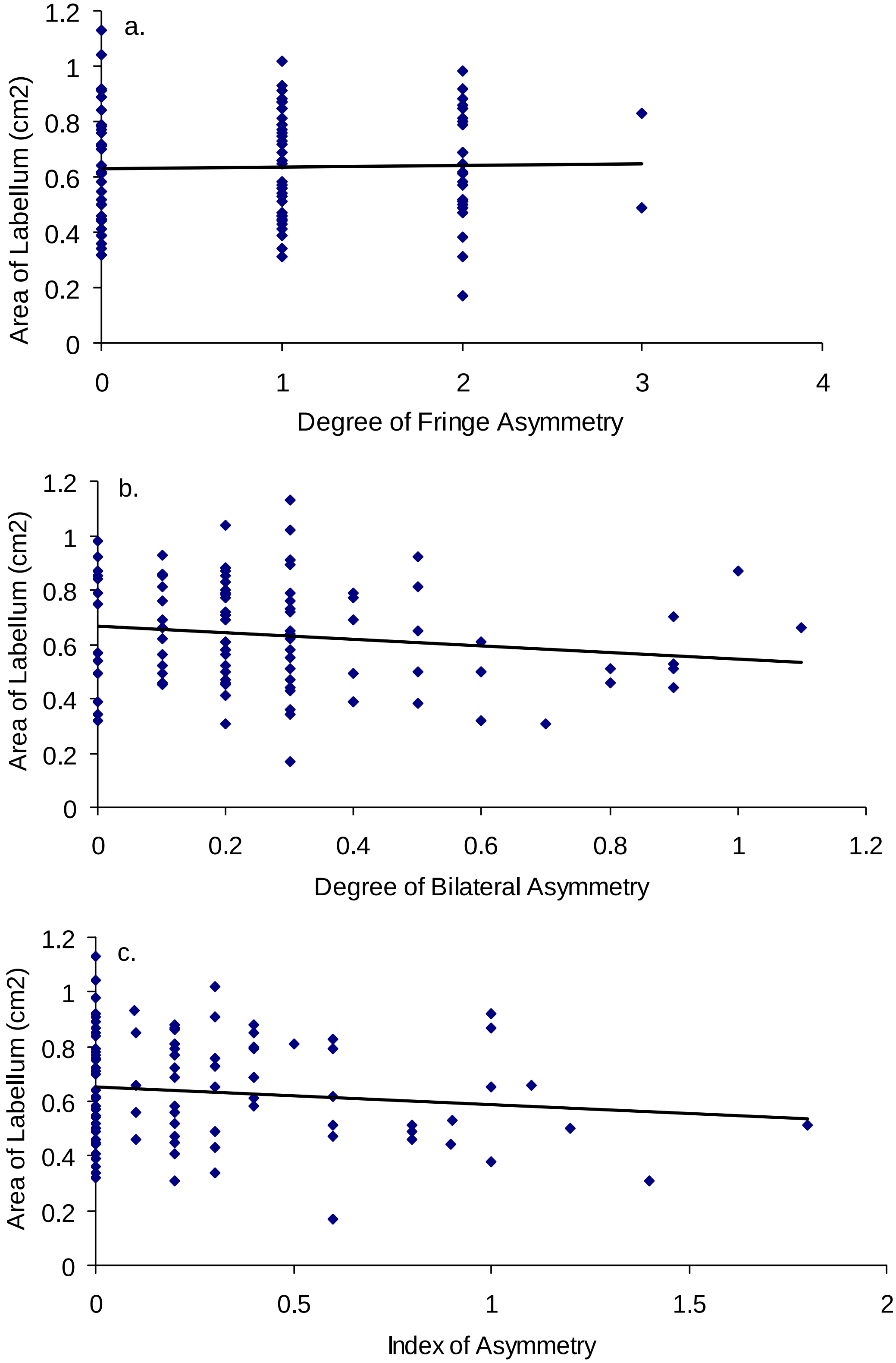

Figure 4: Simple Regressions illustrating - a. relationships between lip area and degree of fringe symmetry R^2 = 0.00, P = 0.78. b. relationship between lip area and degree of bilateral symmetry R^2 = 0.02, P = 0.14. c. relationship between lip area and index of asymmetry (abs fringe asymmetry x abs bilateral asymmetry) R^2 = 0.01 , P = 0.24. For all, n = 100 recently senesced flowers.

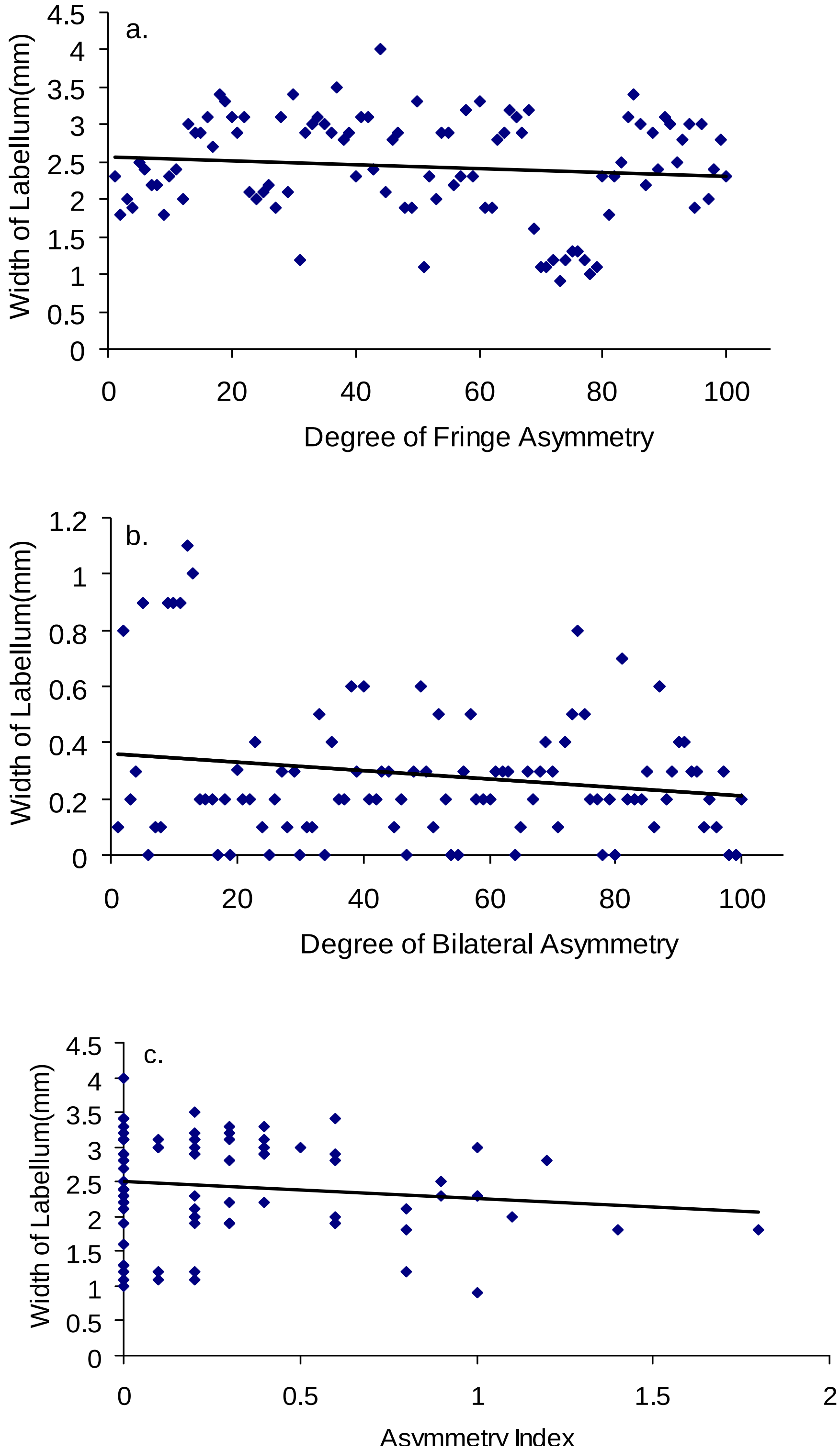

Figure 5: Simple Regressions illustrating - a. relationships between width and degree of fringe symmetry R^2 = 0.01, P = 0.49. b. relationship between width and degree of bilateral symmetry R^2 = 0.03, P = 0.05. c. relationship between width and index of asymmetry (abs fringe asymmetry x abs bilateral asymmetry) R^2 = 0.02, P = 0.19. For all, n = 100 recently senesced flowers.

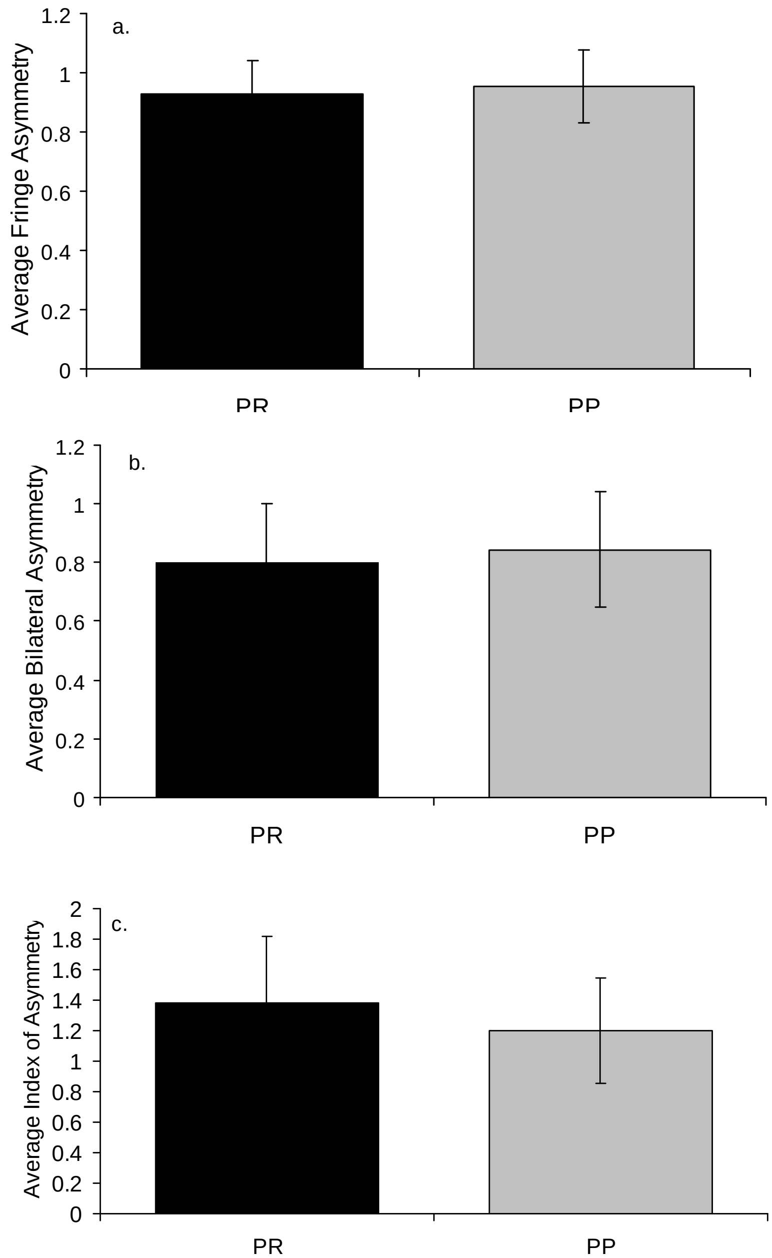

Figure 6: Relationship between pollinia removal (PR) pollinia presence (PP) and symmetry. a. relationship between pollinia removal and degree of fringe asymmetry, t = 0.17, P = 0.87. b. relationship between pollinia removal and average bilateral asymmetry, t = 0.16, P = 0.88. c. pollinia removal and index of asymmetry, t = -0.33, P = 0.75. For all, n = 100 recently senesced flowers.

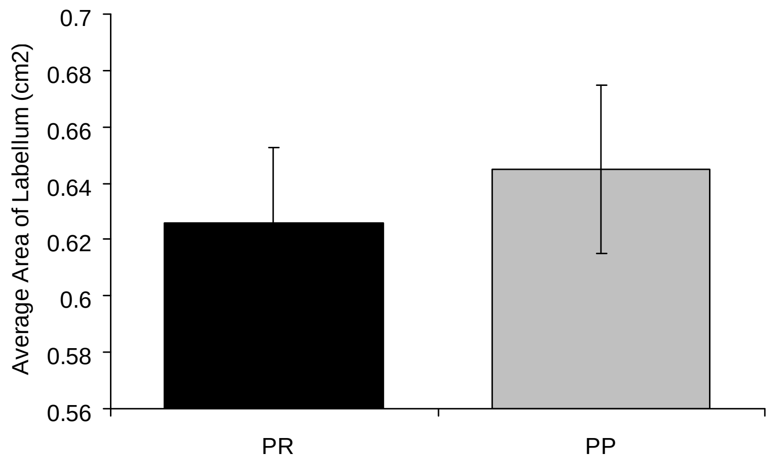

Figure 7: Relationship between pollinia removal (PR) pollinia presence (PP) and lip area, t = 0.48, P = 0.63, n =100.

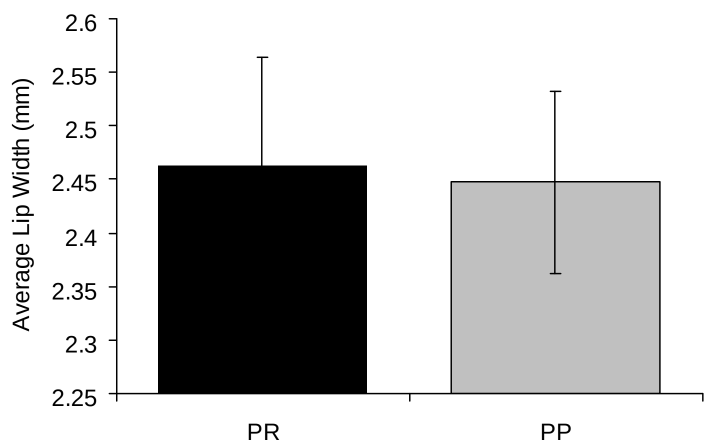

Figure 8: Relationship between pollinia removal (PR) pollinia presence (PP) and lip width, t = 0.12, P = 0.91, n = 100.