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Asymmetry of floral structures and the possibility of self-pollination in Oerstedella exasperata (Orchidaceae)

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ABSTRACT

The purpose of this study was to investigate the causes of the pervasive asymmetry found in the Neotropical orchid *Oerstedella exasperata*. Previous studies have found that high levels of asymmetry decrease reproductive success, and can be caused by a high amount of homozygosis within a population. This study tested the ability of *O. exasperata* to self-pollinate, a condition that indicates the possibility of a high level of homozygosis. In addition, the relationships between three degrees of asymmetry and floral color, floral mass, plant size and sun exposure were investigated to determine whether higher asymmetry is correlated with lower reproductive success or reduced overall plant fitness. Results showed an overall trend suggesting that self-pollination is possible, which implies that *O. exasperata*'s high level of asymmetry could be caused by homozygosis. Floral asymmetry was not related to any of the other examined factors, implying that it is a neutral trait that does not affect the overall fitness of this species of orchid. These results contradict other studies' findings that asymmetry is detrimental to plant health, indicating that *O. exasperata* could be an exception to the general rule that symmetry indicates superior genetics in plants.

RESUMEN

El propósito de este estudio fue investigar las causas de la asimetría en la orquídea neotropical *Oerstedella exasperata*. Estudios anteriores han reportado que niveles elevados de asimetría bajan el éxito reproductivo, y pueden ser causados por homozigosidad alta en una población. Este estudio probó la capacidad de *O. exasperata* de auto-polinizarse, una condición que indica la posibilidad de un nivel alto de homozigosidad. También, las relaciones entre tres grados de asimetría y color floral, masa floral, tamaño de la planta y exposición al sol fueron investigados para determinar si la alta asimetría se correlaciona con un bajo éxito reproductivo. Los resultados mostraron una tendencia que surgiere que la auto-polinización ocurre, lo que implica que el alto nivel de asimetría en *O. exasperata* puede ser causado por homozigosidad. La asimetría floral no fue relacionada a ningún otro factor examinado, lo implica que puede ser un carácter neutro que no afecta la salud general de esta especie de orquídea. Estos resueltos contradicen otros estudios que dicen que la asimetría es mala para la salud de plantas, e indica que *O. exasperata* pueda ser una excepción a la regla general que simetría indica una genética superior en plantas.

INTRODUCTION

Symmetry is a characteristic used by many organisms to evaluate the genetic quality of other individuals. Potential mates with relatively high levels of fluctuating asymmetry have been shown to be less fit than more symmetrical individuals, and therefore tend to be selected against (Palmer and Strobeck 1986). Symmetry is also an important factor in plant-pollination systems, as flowers provide a visual signal to pollinators in order to disperse their pollen. Various studies have shown that pollinators prefer symmetrical flowers over asymmetrical ones, and that bees in particular show a preference for symmetrical flowers independent of pollinator rewards, floral color, odor and size (Møller 1995, Møller and Eriksson 1995, Møller and Sorci 1998). Giurfa *et al.* (1999) also note that flowers with lower levels of fluctuating asymmetry enjoy higher visitation rates, which result in greater pollen removal and deposition, as well as the receipt of pollen of superior quality.

Given these findings, it is surprising that the Neotropical montane orchid *Oerstedella exasperata* shows a persistently high level of fluctuating asymmetry, both within and between individuals (Snayd 2007). However, Snayd (2007) has found that asymmetry does not have a negative effect on the reproductive success of *O. exasperata* as measured by pollinia removal. In addition, Snayd found that there was no correlation between the degree of bilateral asymmetry and the degree of fringe symmetry, which contradicts earlier studies (Møller 1995 and Neal *et al.* 1998) that indicate that asymmetry in flowering plants results in an overall decrease in fitness, which is the ability to survive and transmit genes to the next generation (Jenkins 1990). It is possible, therefore, that asymmetry is a selectively neutral trait for *O. exasperata*, and its persistence in the species is simply due to a lack of natural selection against it.

It is also known that individuals showing high levels of homozygosity due to inbreeding can be prone to higher levels of fluctuating asymmetry (Møller and Eriksson 1995). Higher levels of homozygosity are found in organisms that are capable of self-pollination, and evidence of selfing ability in *O. exasperata* would be an important step toward confirming homozygosity as a cause of its persistent asymmetry. In addition, resource constraints have been found to affect flower production in the Neotropical orchid *Brassavola nodosa* (Murren and Ellison 1996), so it may be possible that *O. exasperata* also expresses asymmetry as a response to environmental conditions.

The purpose of this study was to shed light on the cause or causes of the high asymmetry seen in *O. exasperata* by determining whether the orchid is capable of the self-pollination that would imply inbreeding and homozygosity, and whether floral asymmetry is correlated with different floral characteristics, plant size or sun exposure. I hypothesized that, like many montane species, *O. exasperata* would show self-compatibility because of low pollinator ability due to harsh environmental conditions. I predicted that floral asymmetry would not be correlated with other floral traits, as implied by Snayd’s previous study. I also hypothesized that asymmetry would be correlated with plant size and sun exposure, because of past studies that suggest that environmental factors affect floral development.

METHODS

Study Organism

Oerstedella exasperata is a terrestrial or epiphytic orchid distributed throughout the mountains of Costa Rica and Panama between 1000 and 2500 m, and is common along roadsides. It is one of the largest orchids in Costa Rica, reaching up to three m in size (Zuchowski 2005). It has adventitious roots and tolerates various conditions of elevation and rainfall, which helps account for its ubiquity. Its stems are highly branched, and can reach over one cm in diameter at the base, with leaves ranging from five to fifteen cm long. Its petals are usually greenish at the base fading to yellow toward the terminal end, and are more oblique in shape than the oblanceolate sepals (Hágsater 1992). The column and labellum are flanked by prominent, lobate fringes, which have a variable number of lobes. The white fringes, labellum and callus are usually highlighted with purple markings, and the area of this coloration is also highly variable.

Study Site

This study was conducted between October 30th and November 16th, 2008 near the Monteverde Cloud Forest in Costa Rica. I examined nineteen *O. exasperata* plants growing along the TV Tower Road leading to Cerro de los Amigos. All individuals were found in the clay bank of the west side of the road. Plants were marked with flagging tape and given a unique identification letter.

Self-Pollination Experiment

I selected 40 young flowers with intact pollinia on nine *O. exaperata* plants on which to conduct my self-compatibility experiment. I self-pollinated 20 flowers and cross-pollinated 20 flowers, using a pin to remove pollinia from the pollinaria and deposit them onto the adhesive surface within the column. I then put pollinator bags around the pollinated flowers to prevent pollinator interference. Pollination was performed from November 2nd to November 4th. I removed the pollinator bags on November 13th and recorded the presence or absence of a swollen pedicel and column that would presumably indicate fruit formation and therefore fertilization. I entered the data into a contingency table and analyzed my findings with a chi-square test.

Asymmetry Experiment

I sampled 124 individual flowers from 19 plants at the study site, labeling each flower with its parent plant letter and a unique number. I evaluated fringe asymmetry and bilateral asymmetry following Snayd's methods of taking the absolute value of the difference between the number of fringes on each side and the lengths of each side of the labellum. I also evaluated petal asymmetry by taking the absolute value of the difference in the lengths of the petals. I measured the length of each side of the labellum and the lengths of each of the petals using calipers. In addition to the three asymmetry measurements, I created a scale to measure the amount of relative coverage of purple on the labellum, callus and fringes, and rated each flower from one (minimal purple) to five (saturation). I also weighed each flower to determine its mass. In addition to the floral characteristics, I determined the size of each plant by counting the number of leaves and measuring the length of the longest leaf (Murren and Ellison 1996). To evaluate the effects of sun exposure on plant development, I categorized each plant as being in the shade or sun, "shade" being defined as having other plants growing above it and preventing it from absorbing as much light as an uncovered plant. Bivariate regressions were performed to evaluate the relationship between the three different degrees of floral asymmetry, floral mass and color; between the average floral asymmetry, size and number of flowers on each plant; and between the number of flowers and plant size.

RESULTS

None of the pollinated flowers showed swollen pedicels, but as of the eleventh day of the experiment, 20 flowers did show swelling in the column, of which 35% were flowers that had been selfed. This implies that outcrossing may be a more common occurrence. The numbers of outcrossed flowers showing swelling and selfed flowers lacking swelling both exceeded the expected value of ten, with thirteen flowers each. Seven selfed flowers had swelling and seven outcrossed flowers lacked swelling, both of which are lower numbers than the expected ten flowers. However, these differences are not statistically significant (Figure 1). There was no significant difference between the average floral asymmetry of plants growing in different sunlight conditions, though fringe asymmetry was considerably higher than lip or petal asymmetry (Figure 2). Based on statistical tests, asymmetry is not related to any of the plant characteristics tested (Table 1), nor are the three degrees of asymmetry correlated with each other (Figure 3). In addition, no correlations were found between the three degrees of floral asymmetry and flower mass (Figure 4) or flower color (Figure 5). However, larger plants were observed to produce significantly more flowers than smaller ones (Figure 6).

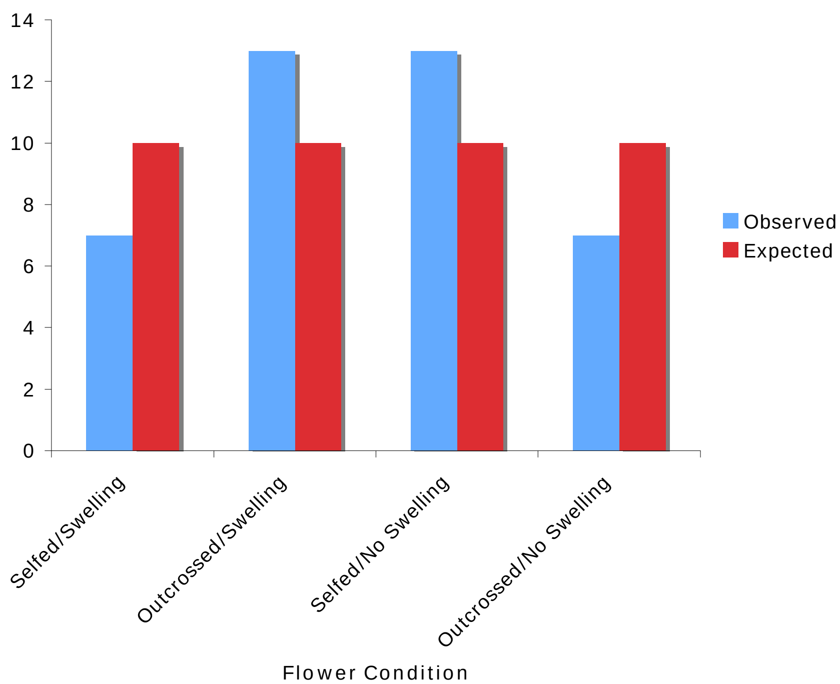


Figure 1: Difference between observed and expected values for presence or absence of swelling in the columns of selfed and outcrossed *Oerstedella exasperata* flowers in Monteverde, Costa Rica. Expected value for all = 10. N = 40 manually pollinated flowers. Chi-squared value = 3.6; df = 1; critical value = 3.84.

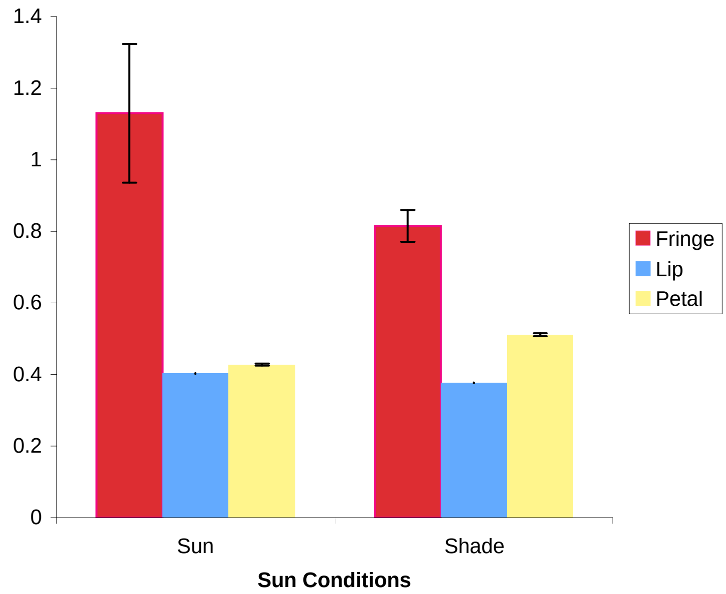
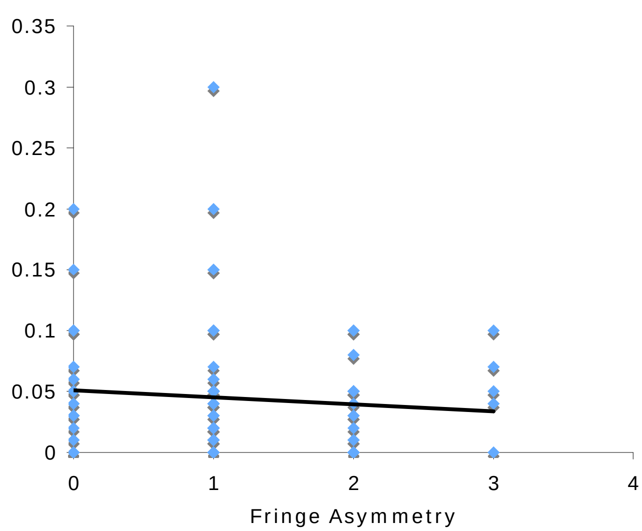
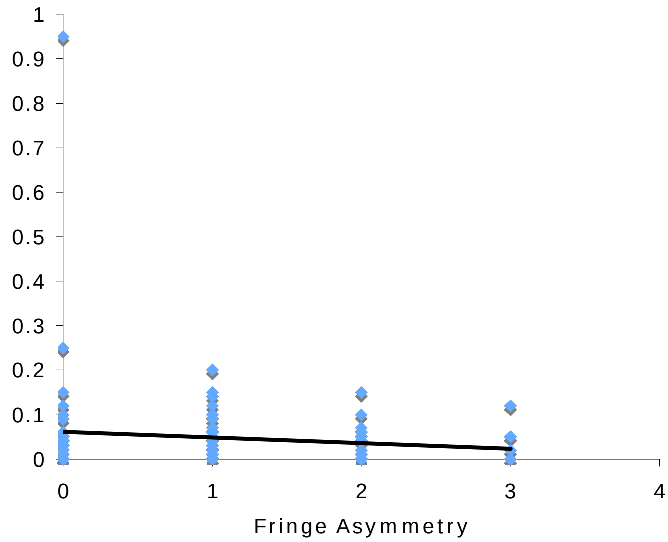


Figure 2: Mean value and standard error of three degrees of floral asymmetry with respect to sun conditions. Lip and petal values and corresponding error bars were multiplied by 10 to improve the resolution. N of sun plants = 11, n of shade plants = 8.

Table 1. *Statistical results of bivariate regressions comparing fringe, lip and petal asymmetry to flower mass, flower color, size of plant and number of flowers. Values are F (p value) and* R^2.

	Fringe Asymmetry	Lip Asymmetry	Petal Asymmetry
Flower Mass	2.259 (0.135) 0.018	0.752 (0.388) 0.006	1.559 (0.214) 0.013
Flower Color	0.281 (0.597) 0.002	0.202 (0.654) 0.002	1.134 (0.289) 0.009
# of Leaves	2.589 (0.126) 0.132	0.160 (0.695) 0.009	1.547 (0.230) 0.083
Longest Leaf	3.490 (0.079) 0.170	1.252 (0.279) 0.069	0.778 (0.390) 0.044
# of Flowers	0.0002 (0.988) 0.00001	1.758 (0.202) 0.094	0.126 (0.728) 0.007





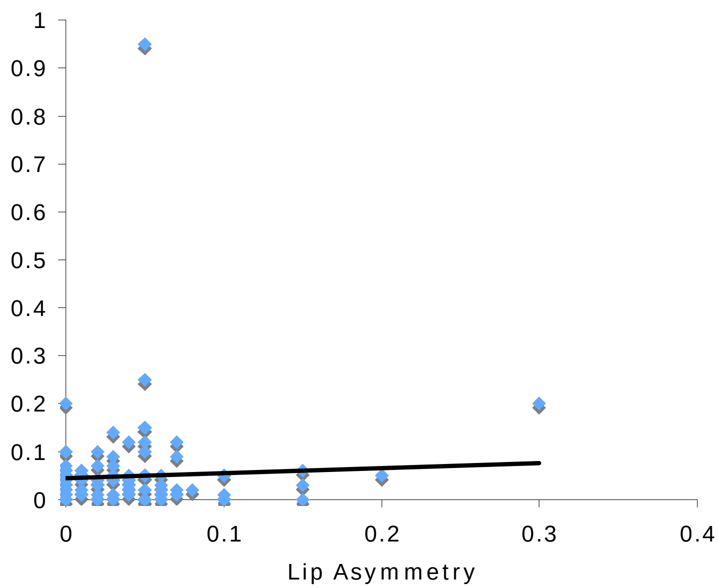
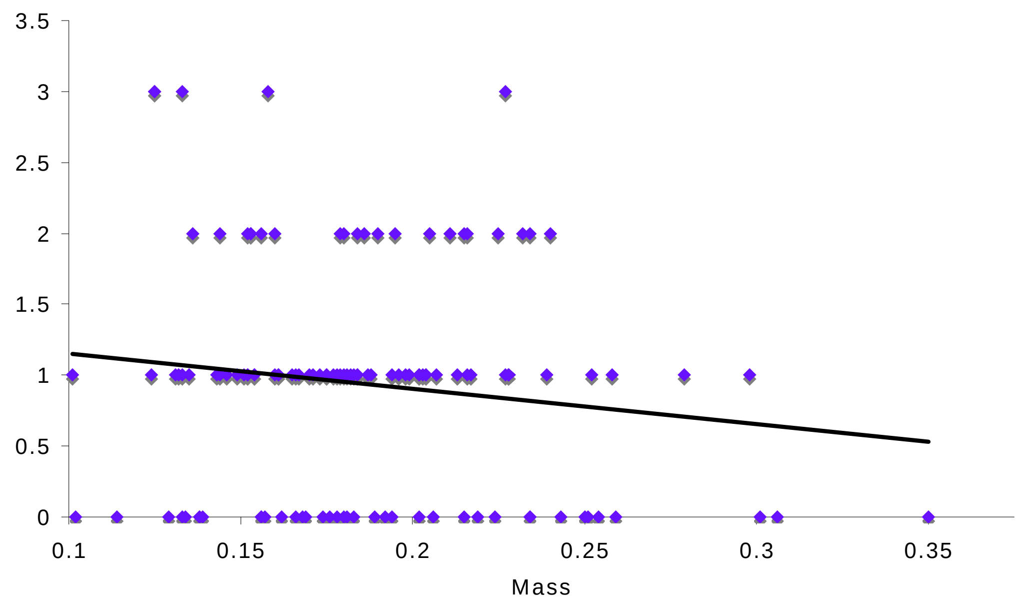


Figure 3: Three regressions showing: (a) relationships between fringe asymmetry (absolute value of [# of fringes on left side – number of fringes on right side]) and lip asymmetry (absolute value of [length of left side of labellum – length of right side of labellum]) $R^2 = 0.010$, $p = 0.265$; (b) relationships between fringe asymmetry and petal asymmetry (absolute value of [length of left petal – length of right petal]) $R^2 = 0.012$, $p = 0.220$; (c) relationships between lip and petal asymmetry $R^2 = 0.003$, $p = 0.580$. For all, n = 124 flowers.



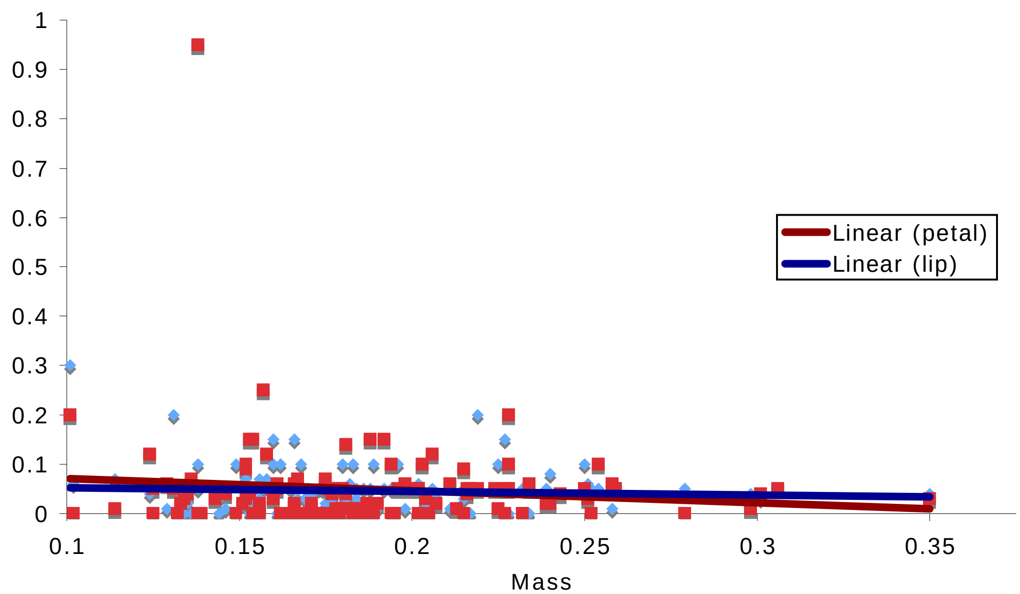
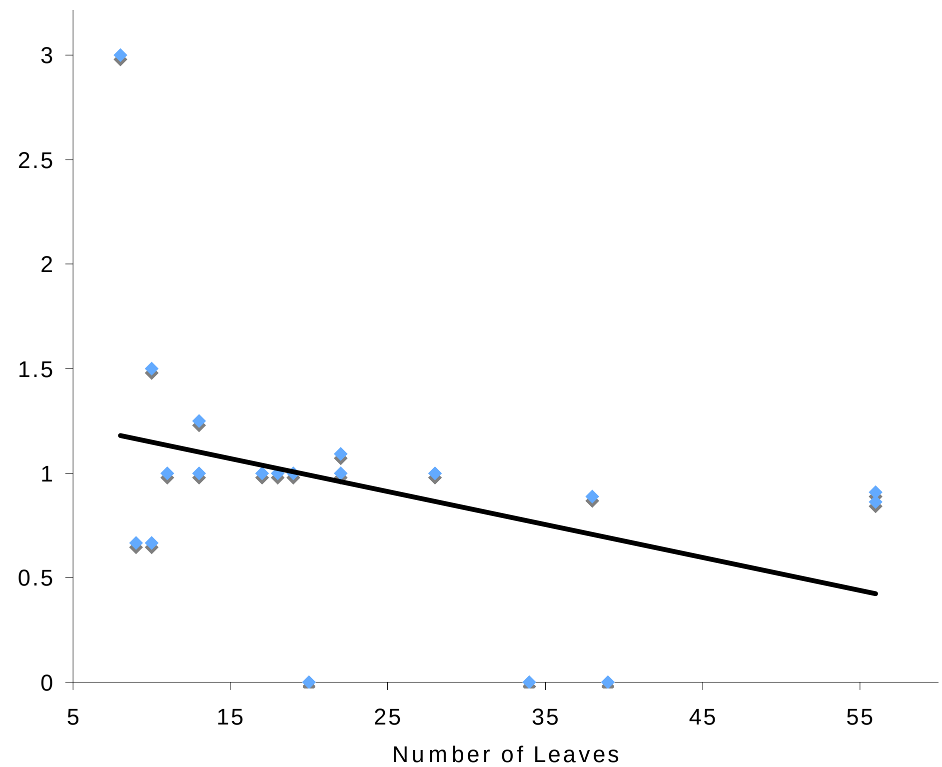


Figure 4: Mass of flower related to: a. fringe asymmetry (R^2 = 0.018, p = 0.135); b. lip (R^2 = 0.0049, p = 0.338) and petal asymmetry (R^2 = 0.0127, p = 0.214) related to floral mass. N = 124 flowers.



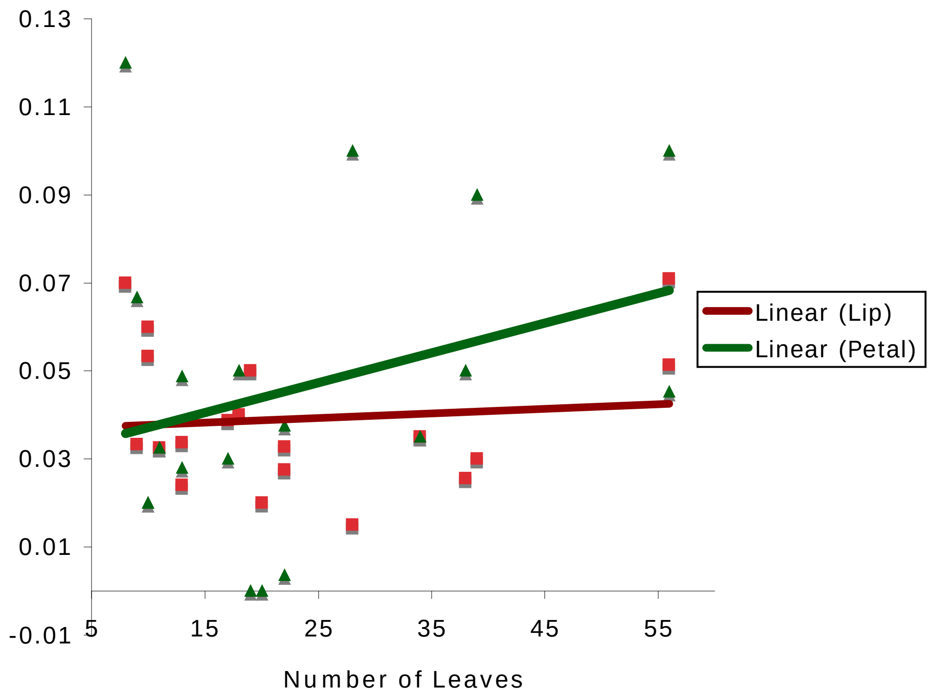


Figure 5: Relationship between number of leaves and: a. average degree of fringe asymmetry (R^2 = 0.132, p = 0.126; b. average degrees of lip (R^2 = 0.009, p = 0.695) and petal asymmetry (R^2 = 0.083, p = 0.230). N = 19 plants.

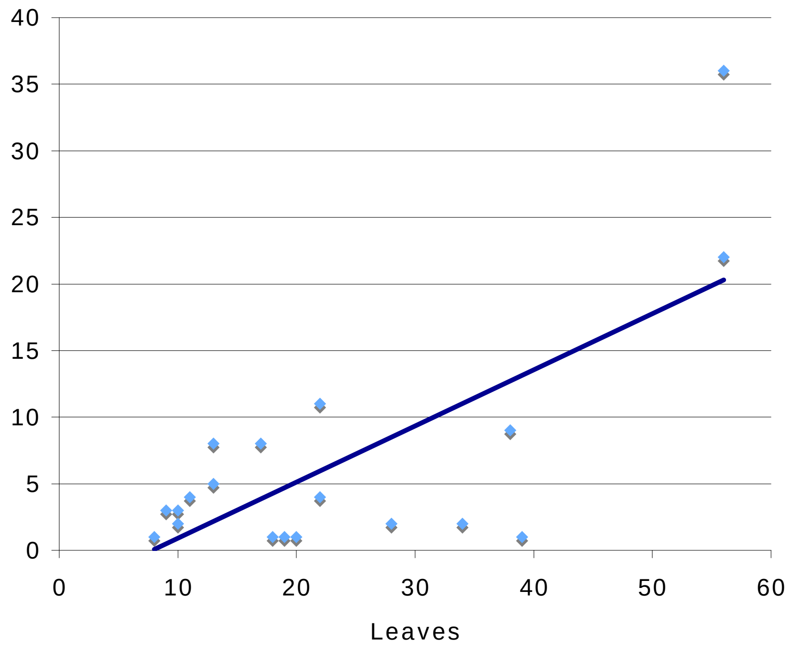


Figure 6: Relationship between number of flowers and number of leaves per plant. R2 = 0.509**, p <0.05**. N = 19 plants.

DISCUSSION

This study is the first to document manual pollination attempts on the Monteverde population of *O. exasperata*, as well as the first to examine the effects of sunlight on its floral morphology. Although the pollination data were not statistically significant, seven selfed individuals did show swelling, which suggests that *O. exasperata* is indeed self-compatible, though outcrossing seems to result in higher pollination success. Given that this species is usually found in montane conditions at mid- to high elevations, it could be that pollinators are often in short supply, and therefore self-pollination is utilized by this species when outcrossing is not possible. A repetition of the experiment with a larger sample size may provide significant support of this trend.

It is of interest to note that the observed swelling did not occur in the ovary area of the pedicel as expected, but rather in the column itself. This characteristic may be a precursor of fruit formation, and the time constraints of this study did not allow for the observation of subsequent swelling in the pedicel. However, it may also be that the swelling of the column is a response of the plant to successful pollination, but that no fruit developed because fertilization is prevented by self-incompatibility, in which case *O. exasperata* would not be capable of self-pollination (Richards 1996). The pollination system of this species is still poorly understood, and deserves more in-depth study.

In order to avoid pollinating flowers that had already received pollen, only flowers with intact pollinia were used in this experiment, as the previous arrival of a pollinator would probably have dislodged the pollinia. However, they were not bagged as buds and then allowed to flower, which would have eliminated the chance of previous pollination. Future studies should employ this method to obtain more accurate results.

If *O. exasperata* is found to be self-incompatible, it may still experience a high level of homozygosis due to its adventitious roots, which allow vegetative reproduction. This could result in patches of individuals who are essentially clones of each other, which would reinforce homozygosis if its pollinators generally forage in a small area. In this case, outcrossing and selfing would both contribute to an increased level of homozygosis, as there would be no effective genetic variation between neighboring individuals. It would be beneficial to perform genotyping on this population to determine whether it does indeed have the high level of homozygosis that can result in increased fluctuating asymmetry.

The fact that floral asymmetry is not correlated with floral color or mass implies that it does not have a negative impact on the reproductive success of *O. exasperata*, as more colorful and larger flowers would presumably be more attractive to pollinators. This finding contradicts past studies on other species of flowering plants but agrees with Snayd's previous study on the same population. It could be that this species of orchid or this population specifically, is an exception to the general rule that symmetrical flowers confer a reproductive advantage. Perhaps its primary mode of reproduction is vegetative, and therefore it does not invest energy in producing symmetrical flowers to attract pollinators. It could also be that its pollinator, as yet unknown, is a species or genus that is not attracted by floral symmetry.

Neal et al (1998) state that the environmental factors that trigger asymmetry may result in overall reduced fitness. If this condition were true for *O. exasperata*, decreased sunlight would be expected to result in both smaller plant size and increased asymmetry, as a plant starved of solar energy would not be able to invest extra resources in growth and the production of symmetrical flowers. This would lower this species' fitness in terms of reproductive ability and overall health. The strong correlation between the number of leaves and flowers present on plants indicated that plant size was a good measurement of both overall fitness and potential reproductive success. However, floral asymmetry was not correlated with sun exposure or plant size, which implies that higher asymmetry, is not an indicator of poor plant quality, nor is it affected by environmental conditions. Additional studies evaluating the effects of other environmental factors such as substrate, rainfall and elevation on asymmetry are needed to confirm this prediction. In addition, as Snayd (2007) observed, one would expect a correlation between all measures of asymmetry if environmental factors do affect plant health. However, this study found no correlations between fringe, lip and petal asymmetry, which further suggests that increased asymmetry does not decrease this species' overall fitness.

In conclusion, floral asymmetry in *O. exasperata* appears to be a neutral trait, showing no negative, predictable relationship with other floral characteristics, plant size or levels of sunlight. This conclusion contradicts previous studies that have found that symmetrical flowers have higher reproductive success, which implies that *O. exasperata* may be an exception to the general rule that floral asymmetry is a detrimental trait in plants. The high level of asymmetry in this population could be perpetuated due to a high level of homozygosis caused by either self-fertilization or low levels of genetic variation as a result of vegetative reproduction. Future studies should focus on the pollination system of this orchid, its genetic makeup and the effects of different environmental conditions on floral asymmetry.

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LITERATURE CITED

Giurfa, M, A. Dafni and P. R. Neal. 1999. Floral Symmetry and Its Role in Plant-Pollinator Systems. International Journal of Plant Sciences 160: S41-S50.

Hágsater. 1992. *Oerstedella exasperata*. In: Orchids of Costa Rica, Part 2, D.E. Mora de Retana and J.T. Atwood eds. The Marie Selby Botanical Gardens, Sarasota, FL, pp.1460-1461.

Jenkins, J. B. 1990. Human Genetics. Harper and Row, New York, NY. As cited on Webref.org. <http://www.webref.org/biology/biology.htm>

Mitton, J.B. and M. C. Grant. Associations Among Protein Heterozygosis, Growth Rate and Developmental Homeostasis. Annual Review of Ecology and Systematics 15: 479-499.

Møller, A.P. 1995. Bumblebee Preference for Symmetrical Flowers. Proceedings of the National Academy of Sciences of the United States of America 92: 2288-2292.

Møller, A.P. and G. Sorci. 1998. Insect Preference of Symmetrical Artificial Flowers. Oecologia 114: 37-42.

Møller, A.P. and M. Eriksson. 1995. Pollinator Preference for Symmetrical Flowers and Sexual Selection in Plants. Oikos 73: 15-22.

Murren, C.J. and A.M. Ellison. 1996. Effects of Habitat, Plant Size, and Floral Display on Male and Female Reproductive Success of the Neotropical Orchid *Brassavola nodosa*. Biotropica 28: 30-41.

Neal, P.R. *et al*. 1998. Floral Symmetry and Its Role in Plant-Pollinator Systems: Terminology, Distribution, and Hypotheses. Annual Review of Ecology and Systematics 29: 345-373.

Palmer, A.R. and C. Strobeck. 1986. Fluctuating Asymmetry: Measurement, Analysis, Patterns. Annual Review of Ecology and Systematics 17: 391-421.

Richards, J.A. 1996. Breeding Systems in Flowering Plants and the Control of Variability. Folia Geobotanica & Phytotaxonomica 31: 283-293.

Snayd, M. 2007. Relationship between Floral Advertisement and Pollinia Removal in *Oerstedella exasperata* (Orchidaceae). Estación Biológica Monteverde, Costa Rica. Biology. Fall 2007, pp. 35-41.

Zuchowski, W. 2005. A Guide to Tropical Plants of Costa Rica. Zona Tropical, Miami, FL, pp. 420-421.