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ABSTRACT

Pollinators greatly influence the fitness of most tropical plants, and how effective plants are at attracting pollinators is key to their success. This investigation focuses on how inflorescence size of *Lantana camara* affects butterfly visitation. Naturally occurring inflorescence size was determined by counting how many flowers per inflorescence and how many inflorescences were in bloom per branch. The average number of flowers per inflorescence is 37 and the average number of inflorescences in bloom per branch is 2. To assess the impact of inflorescence size on butterfly visitation, five different sizes of *L. camara* inflorescences were created by bundling from one to five inflorescences, ranging from an average of 36 to 208 flowers per bundle. Butterfly visitation to each of these was recorded during sunny periods in the Monteverde Butterfly Garden, Monteverde, Costa Rica. As the size of the inflorescence increased, so did butterfly visitation. However, the proportion of butterfly visitors to number of flowers decreased as inflorescence size increased. Other traits (morphology, plant vigor, scent, etc.) impact attractiveness to butterflies. What is the point of the previous sentence? The interaction and balance of these other traits along with inflorescence size would reveal interesting results about how angiosperms create attractive inflorescences.

Animal-mediated pollination in the Tropics defines a plant's fitness, as species are normally rare and widely spaced. These tropical plants depend on insects for effective pollination. Over 90% of tropical plants rely on animals for pollination. Because plant species are generally rare and widely spaced, pollination is less likely. Therefore flowers have to be noticeable and attractive. To a pollinator, the attractiveness and pollination of a plant species generally depends on the characteristics of both the individual flower and how they are displayed in an inflorescence (Harder *et al.* 2004). Visual traits, such as bright red or orange color common sources of attraction for many pollinators, especially butterflies (Faegri *et al*. 1979, Lewis *et al*. 1990). Flowers are also able to increase their attractiveness using odor, reward and size (Stebbins 1970). One way of increasing reward and flower size is by increasing the number of flowers per inflorescence.

Inflorescences can be groupings of smaller flowers that can vary greatly in floral number. Flowers are expensive for plants to produce and for lowland tropical plants where the average floral lifetime is just one day. Therefore, the greater energy expense of larger inflorescences should translate into more visits by pollinators. It is possible that the number of visits could be additive, or larger inflorescences may mean a disproportionate increase of visits. In the latter case, natural selection should favor larger inflorescences, as a small increase in floral number increases the benefit greatly.

Past studies have shown that plants with large floral displays attract more pollinators than plants with only a few open flowers (Eckhart 1991). In one such study, bumblebees responded strongly to floral display size and generally preferred plants with numerous flowers

(Mitchell *et al.* 2004). With a larger inflorescence size, the size of the landing platform and the quantity of reward are increased, both contributing to the flower's attractiveness (Ohashi *et al.* 2001, Scheske 1976).

This study focuses on the common species *Lantana camara* (Verbenaceae). *L. camara* has a varying number of inflorescences of the same age located at each of its nodes. Each of these inflorescences has a varying number of bracteates, sessile dioecious flowers arranged spirally that develop from the center to the periphery (Knuth 1905, Mathur *et al.* 1986). Like many other tropical plants, they are insect pollinated, most commonly by butterflies (Faegri *et al.* 1979). This study investigates some of the naturally occurring characteristics of *L. camara* as well how butterfly visitation is effected through the manipulation of inflorescence size, from naturally occurring to larger sizes.

MATERIALS AND METHODS

This experiment was conducted at the Jardín de Mariposas (Butterfly Garden) located in Monteverde, Costa Rica, a lower montane wet forest. The Butterfly Garden consisits of four different gardens of varying size and each contained different varieties of butterflies. The garden used in this study is approximately 20 feet by 30 feet, fitted with plants that are naturally occurring in Costa Rica, and is covered with a roof allowing sun to enter, but does not allow for direct sunlight.

Flowers Per Inflorescence:

To determine the naturally occurring number of flowers per inflorescence, the number of flowers per inflorescence of 100 *L. camara* inflorescences was recorded. This data was then added and averaged. Although only yellow flowers present a reward (Scheske 1976, Mathur *et al.* 1986), the combination of red, orange and yellow colors plays a role in attractiveness for butterflies (Faegri *et al*. 1979, Lewis *et al*. 1990), so all flower colors were counted when taking samples.

Flowers Per Branch:

In order to determine the average number of naturally occurring blooming inflorescences per branch, 20 *L. camara* plants were sampled and the number of blooming inflorescences for each branch on the plant was counted. An average was then calculated for all 20 plants.

Floral Selection:

45 inflorescences were picked in the flower garden outside of the Butterfly Garden every morning. The process by which inflorescences were chosen was based on characteristics of similar size, color and number of open flowers. These inflorescences were then divided into five groups. Each group made up one ersatz inflorescence size. For any one ersatz inflorescence size, there were three replicates. The groups consisted of: one natural inflorescence, and bouquets of two natural inflorescences, three, four and, finally, five natural inflorescences put together for a total of 15 ersatz inflorescences (three of each of the five sizes). The number of flowers per bouquet was then counted. For those samples that had more than one natural inflorescence, the number of flowers per ersatz inflorescence was

added to get the total number of flowers per bouquet. This was done to determine the average number of flowers per ersatz inflorescence.

Nectar Injection:

Functioning as a reward for the butterflies, nectar was injected into the flowers. The same amount of nectar, a 20% sucrose solution, was added to each inflorescence through the use of a hypodermic needle in order to provide a reward. Nectar was only added to the yellow flowers because only the first-day flowers of *L. camara* produce nectar and the flowers change from yellow to orange to red in a 24-hour period (Scheske 1976, Mathur *et al.* 1986).

Site Description:

15 bamboo sticks of approximately the same height were placed in an area of the *Heliconius spp.* butterfly garden. This garden was chosen specifically at the suggestion of the Garden's staff because the *Heliconius spp.* butterfly garden has the most active butterfly population out of the gardens available. Sticks were placed approximately the same distance apart from each other in the same environmental conditions (all were in sun, no one was shaded by a nearby plant, etc.). At the end of each bamboo stick a small tube was attached to serve as a vase for water. The inflorescences were placed in a small vase with water. From these stations at the top of the bamboo sticks, butterfly visitation was visually observed and recorded. The bamboo sticks were switched to a new location in the garden everyday. This was done to reduce visitation patterns related to the previous day's floral display, as is exhibited by some species of butterflies (Goulson *et al.* 1997, Thomson *et al.* 2001).

Observation Methods:

Butterfly (*Heliconius spp.*) visitation, a butterfly landing on an inflorescence and staying for five seconds or longer, was observed visually twice a day for 20 minutes an hour for two consecutive hours. Observations went on for five days in total yielding a total of ten observation periods. To reduce the counting of the same butterfly that moved from one inflorescence to another, only the first visitation site was recorded. I was unable to account for re-visitation by butterflies after an amount of time due to the fact that I was unable to mark or tag the butterflies I observed making visitations.

RESULTS

The average number of flowers per inflorescence was found to be 37 flowers (SD +/- 8.819). The average number of inflorescences observed in bloom per branch was 2 (SD +/- .544) (or ~74 flowers in bloom per branch).

There was a general increasing trend of average number of butterfly visits as the inflorescence size increased (FIGURE 1). The average number of

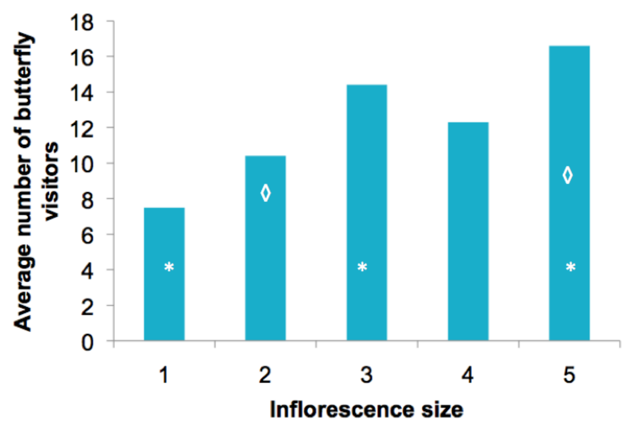

FIGURE 1. At the Monteverde Butterfly Garden, the average number of butterfly visitors was observed as the inflorescence size of *Lantana camara* was manipulated to be of larger size. * = a significant difference between the inflorescence size of 1 and 4, 5. ◊ = a significant difference between an inflorescence size of 2 and 5.

butterfly visits for an inflorescence size of one was 7.5, for a bouquet of 2 it was found to be 10.4 visits, for a bouquet of 3 an average of 14.4 visits was recorded. For the bouquet size of 4, the average number of visits was 12.3 and for a bouquet of 5, it was 16.6 visits. This overall trend was found to be statistically significant (Friedman test, χ^2=23.860, P < .0001, df=4). The values that were found to be statistically significant (P < .05) were between an inflorescence size of 1 and an inflorescence size of 3 (q=3.4648), 1 and 5 (q=4.3841), and 2 and 5 (q=3.0406). These values still support the overarching trend.

A decreasing trend of the average proportion of butterfly visits to number of flowers is apparent as the inflorescence size of *L. camara* increased (FIGURE 2). The average proportion of visitors for a naturally occurring inflorescence size of 1 is .212, for a bouquet of 2 it was calculated to be .137, and for a bouquet size of 3 the average proportion was .132. The average proportion for a bouquet of 4 was determined to be .077 and for a bouquet of 5, it was calculated to be .086. The proportion of butterfly visits to the number of flowers in an inflorescence as it is related to inflorescence size was also found to be statistically significant (Friedman test, χ^2=32.560, P < .0001, df=4). The values that were statistically significant (P < .05) were between the inflorescence size of 1 and 4 (q=4.6669), 1 and 5 (q=4.1012), 2 and 4 (q=3.2527), 2 and 5 (q=2.9698), and between an inflorescence size of 3 and 4 (q=3.2527). These values support the decreasing trend of proportion of visitors to number of flower size as inflorescence size increases.

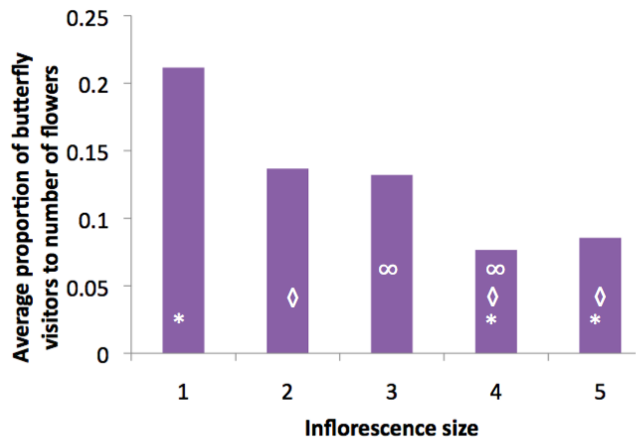

FIGURE 2. The average proportion of butterfly visitors, recorded at the Monteverde Butterfly Garden, to the number of flowers present in a *Lantana camara* manipulated inflorescence as the inflorescence size increased. * = a significant difference between the inflorescence size of 1 and 4, 5. ◊ = a significant difference between the inflorescence size of 2 and 4, 5. ∞ = a significant difference between the inflorescence size of 3 and 4.

<u>Additional Observations:</u>

During many observation periods there was cloudy or overcast weather that differed from other days where the weather was sunny. On these days of cloudy, overcast weather, butterfly activity, including visitation, decreased. Therefore, a Friedman Test was used to compare groups. This statistical test keeps all data from a single observation period together, negating the impact of weather between observation periods.

DISCUSSION

The general trend of increasing butterfly visitation as inflorescence size increases supports the hypothesis that larger inflorescences solicit higher butterfly visitation. This could have been the result of the fact that with increasing flower size, the quantity of reward is also increased, which tends to attract more visitors (Neff *et al.* 1990). A proposed idea that supports the findings of this study states that pollinators visit larger floral displays in order to reduce their energy expenditure per floral visit on expensive interplant flights (Kudo *et al*. 2005). In this study, in the case of the inflorescence size of four, it follows the general trend but not to the extent that it was expected. There is no known reason for this recorded outcome. From this general trend, it can be concluded that *Lantana camara* would benefit from a having larger inflorescence size. More butterfly visitation translates into a higher probability of pollination and higher reproductive success.

In contrast to the before mentioned results, the average proportion of butterfly visitors to the actual number of flowers in an inflorescence decreases as the inflorescence size increases. This trend conveys that with increasing floral number per inflorescence, the likelihood that any single flower will be visited decreases. Therefore, it appears that, while larger inflorescences have more visits overall, from an investment perspective, there are diminishing returns. A likely mechanism is that butterflies fill up on few flowers and then move on. If they stayed on the larger inflorescence the pollinator would have access to more possible rewards. Although not measured in this study, length of visitation and the number of flowers probed per inflorescence by butterfly visitors may be an important aspect of butterfly foraging techniques that is mirrored in specific floral traits. In regards to the findings of this study, in the case with any inflorescence size, the pollinator (butterfly) may only take a certain amount of time to search for a reward or fill up on a few flowers and then move on. In this case, the size of the inflorescence would not matter; the same number of flowers in a small inflorescence would be probed as in a large inflorescence. This being said, although a larger inflorescence size will attract more pollinators, it does not necessarily mean a higher reproductive success (more flowers per inflorescence pollinated) for the plant.

The advantage for having more visits for a larger inflorescence is that there is more pollen removed, but the consequence being that there are diminishing returns on a per flower basis. *Lantana camara*, although a common tropical forest plant, is not a typical tropical forest plant. Typical tropical forest plants may be so rare that they need to overcompensate in order to just get a few visits. Another discrepancy between the experiment performed using *L. camara* and typical tropical forests is that this study was performed in a pollinator-enriched garden. In nature, getting just a few visitors may require lots of flowers even though the chance that one of them gets visited goes down. In the case of *L. camara*, a commonly occurring plant, a larger inflorescence would increase the likelihood of within plant pollination. For this reason, the naturally occurring size is the most beneficial. A plant's investment in larger inflorescence size all depends on whether the benefits (pollinator attraction) outweigh the costs (expenditure of energy).

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LITERATURE CITED

ANDERSSON, S., AND H. DOBSON. 2003. Behavioral foraging responses by the butterfly *Heliconius melpomene* to *Lantana camara* floral scent. *Journal of Chemical Ecology* 29(10):2303-2318.

DENNIS, R. AND T. SPARKS. 2006. When is a habitat not a habitat? Dramatic resource use changes under differeing weather conditions for the butterfly *Plebejus argus*. *Biological Conservation* 129(3):291-301.

ECKHART, V.M. 1991 The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology* 5:370-384.

FAEGRI, K., and L. VAN DER PIJL. 1979. The principles of pollination ecology. Pergamon Press, Oxford.

GOULSON, D., J. OLLERTON, AND C. SLUMAN. 1997. Foraging strategies in the small skipper butterfly, *Thymelicus flavus*: When to switch? *Animal Behavior* 53:1009-1016.

HARDER, L.D., C.Y. JORDAN, W.E. GROSS AND M.B. ROUTLEY. 2004. Beyond floricentrism: the pollination function of inflorescences. *Plant Species Biology* 19:137-148.

KNUTH, P. 1905. Handbuch der Bluetenbiologie Band 3, Verlag Wilhelm Engelmann, Leipzig.

KUDO, G. AND L.D. HARDER. 2005. Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Functional Ecology* 19 :245-254.

LEWIS, A.C. AND G.A. LIPANI. 1990 Learning and flower use in butterflies: Hypothesis from honey bees, ppp. 95-110, *in* E.A. Bernays (ed.). Insect-Plant Interactions. CRC Press, Boca Raton, Florida.

MATHUR, G. AND H. MOHAN RAM. 1986. Floral biology and pollination of *Lantana camara*. Phytomorphology 36(1/2):79-100.

MITCHELL, R.J., J.D. KARRON, K.G. HOLMQUIST AND J.M. BELL. 2004. The influence of *Mimulus rigens* floral display size on pollinator visitation patterns. *Functional Ecology* 18(1):116-124.

NEFF, J.L. AND B.B. SIMPSON. 1990. The roles of phenology and reward structure in the pollination biology of wild sunflower (*Helianthus annuus* L. Asteraceae). *Israel Journal of Botany* 39:197-216.

OHASHI, K. AND T. YAHARA. 2001. Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. *Cognitive Ecology of Pollination* (eds L. Chittka & J.D. Thomson), pp 274-296. Cambridge University Press, New York.

ROBERTSON, A.W. AND M.R. MACNAIR. 1995. The effects of floral display size on Pollinator service to individual flowers of *Myosotis* and *Mimulus. Oikos* 72:106-114.

SCHESKE, DOUGLAS W. 1976. Pollinator Specificity in Lantana camara and L. trifolia (Verbenaceae). *Biotropica* 8(4):260-264

STEBBINS, G.L. 1970 Adaptive radiation in reproductive characteristics in angiosperms, I:Pollination mechanisms. *Annual Review of Ecological Systems* 1:307-326.

THOMSON, J.D. AND L. CHITTKA. 2001. Pollinator individuality: when does it matter? *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution* (eds L. Chittka & J.D. Thomson), pp. 1991-213. Cambridge University Press, New York.