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The effect of reward and color pattern on butterfly foraging in Lantana camara (Verbenaceae)

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The Effect of Reward and Color Pattern on Butterfly Foraging in *Lantana camara* (Verbenaceae)

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ABSTRACT

Lantana camara has inflorescences composed of young, nectar-producing yellow flowers surrounded by older orange flowers that no longer produce nectar (Moldenke 1973). We examined the relationship between *L. camara* and its butterfly pollinators to see if their preference for yellow flowers (Peters 1998) is innate or learned. Further, we altered the position of flower color on the inflorescence to see if the normal yellow-centered bull's eye pattern increased visitation over other patterns. We collected data for butterflies foraging on *L. camara* with six patterns of yellow and orange flowers by painting these colors on the inflorescences. Next, we watched these same patterns with a 20% sucrose solution injected to all of the flowers on an inflorescence. Butterflies demonstrated an innate preference for yellow flowers as an addition of nectar to orange flowers showed no change in preference. Likewise, butterflies showed a preference for yellow flowers in all cases (df = 1, $x^2 = 4.734$, p = 0.02). Varying the pattern of yellow and orange flowers in an inflorescence showed that butterflies prefer a solid yellow inflorescence. Therefore, it appears that the pattern of flower color and butterfly preference are not coevolved to enhance visitation and hence, pollination. While butterflies benefit from innately visiting yellow flowers most frequently, *L. camara* may increase pollinator visits by keeping its older flowers yellow in order to create a solid yellow inflorescence.

RESUMEN

Las inflorescencias de *Lantana camara* están compuestas por flores jóvenes amarillas que producen néctar, y flores más viejas de color naranja que no producen néctar. Nosotras examinamos la relación entre *Lantana* y las mariposas que polinizan esta planta, para observar si la preferencia por las flores amarillas es un comportamiento innato o aprendido. Además, alteramos la posición del color de las flores en la inflorescencia para observar si el patrón del objetivo con las flores amarillas en el centro aumenta la visita sobre otros posibles patrones. Colectamos datos de mariposas que forrajean sobre plantas con seis patrones de colores pintando los colores en la inflorescencia. A continuación, observamos estos mismos patrones con un 20% de sacarosa inyectada a estas inflorescencias. Las mariposas demostraron un preferencia innata por las flores amarillas, y la adición de néctar a las floreas naranjas no mostró cambio en la preferencia. Asimismo, las mariposas muestran preferencia por las flores amarillas en todos los casos (df = 1, x2 = 4.734, p = 0.02). Al variar los patrones de coloración en las inflorescencias se muestra que las mariposas muestran una preferencia sólida por las flores amarillas. Por lo tanto, parece que el patrón de coloración no han cohevolucionado para mejorar las visitas y por lo tanto la polinización. Mientras el beneficio de las mariposas por visitar las flores amarillas más frecuentemente, *L. camara* puede aumentar las visitas de los polinizadores manteniendo las flores amarillas más viejas en orden de crear una inflorescencia amarilla sólida.

INTRODUCTION

Plants enhance their pollination efficiency by offering a reward to pollinators, and by evolving to most affectively attract the best pollinators. In addition, pollinators increase foraging efficiency by specializing on certain plants (Moldenke 1973). Over time, these plant-pollinator relationships become tightly coevolved since certain colors, patterns and rewards attract specific pollinators. In butterfly pollination ecology, favored flowers are red, orange and yellow, with short corollas that offer a reward (Moldenke 1973).

Lantana camara is a butterfly pollinated plant that has inflorescences that are arranged in a bull's eye pattern, with yellow flowers in the center and orange flowers on the outside (Moldenke 1973). The orange flowers are older and no longer produce nectar, while yellow flowers are young and nectar producing (Moldenke 1973). According to Peter's study in 1998, butterflies more frequently visit the yellow flowers, but it is unknown whether this preference is innate or learned. It has been theorized that orange flowers are retained on an inflorescence to increase the landing platform for butterflies and therefore increase the attractiveness of the inflorescence.

Butterfly pollinators may learn their visual preferences through experience with flower reward (Zurinskas 2003). *Pieris rapae*, cabbage butterflies, were studied regards to their flower consistency on 16 different taxa, and proved to stick with their normally preferred inflorescences, even when others were filled with nectar. She attributed this to a limited memory capacity of butterflies (Lewis 1989). Restricted memory capacity in butterflies may cause them to innately prefer yellow flowers, or they may choose to frequent yellow *L. camara* flowers because they learn through experience that yellow flowers are signals for efficient foraging (Zurinskas 2003).

We hypothesize that yellow flowers on a bull's eye pattern will be innately preferred. Considering this, we investigate whether butterfly preference for yellow is innate or preferred by seeing if their preference changes when orange flowers are rewarding. Further, we study how efficient the bull's eye inflorescence is in attracting butterflies by varying color contrasts and inflorescence arrangement to see if they prefer the natural pattern.

METHODS

This investigation took place at Gold Mine Adventure in La Sierra, Costa Rica, from November 4th to November 11th. There are several bushes of *Lantana camara* located there that we observed during sunny hours ranging from 7:00 am to 2:00 pm. *L. camara*, originally from the West Indies, is a common weedy shrub in Costa Rica that thrives in the sun (Zuchowski, 2007). It flowers and fruits throughout the year. The bull's eye formation is made of young, yellow, nectar-producing flowers in the center. Surrounding the yellow center are older, orange flowers that no longer produce nectar. *L. camara* flowers change from yellow to orange over a 24-hour period and will remain on the inflorescence for three days (Moldenke 1973).

Nectar variation—We observed foraging behavior of the butterflies *Anartia fatima*, *Anartia jatrophae*, and the long-tailed skipper, *Urbanus proteus* on *L. camara* to establish color preference. The smallest inflorescence on the shrub had 20 flowers, and we controlled for size by removing flowers from inflorescences with more than twenty. We removed yellow and/or orange flowers so there was about a 2:1 ratio respectively. We used a syringe to fill both yellow and orange flowers with 20% sucrose solution to imitate nectar. We then observed butterfly foraging behavior on both orange and yellow flowers to see if the addition of nectar to both colors had an effect on butterfly color preference. We filled all twenty flowers on the eighteen inflorescences with the sucrose solution to make sure that they all offered an equivalent quality and quantity of nectar. Flower color choice was placed on a time line noting color of flowers probed per inflorescence for three 20-minute sessions to see if orange flower probing increased over time. Each inflorescence received a "rank" that indicated order of visitation. The same inflorescences were re-filled with nectar after every 20-minute session to assure that all flowers continued to reward visitors.

Pattern variation—Tempera paint was used on 18 size controlled inflorescences to create six different color patterns (three inflorescences of each pattern). The first group was an unpainted control. The second inflorescence type was painted, but the same bull's eye pattern that naturally occurs. This group acted as a second type of control to assure that paint did not affect foraging behavior. The remaining four inflorescence types were all yellow, all orange, inverted bull's eye (with orange inside and yellow outside), and a random mix of yellow and orange flowers on the same inflorescence. Number of visits to each flower color recorded for three 20-minute sessions. In between sessions, we moved to a new location of 18 inflorescences on the shrub, since the paint eventually caused the flowers to shrivel.

Combined variation—Finally, we combined the first two parts of our experiment to vary both color and reward aspects. We put the sucrose solution in all flowers and used the six color variations that were used in part two of our experiment. Activity was recorded on a time line for three 20-minute sessions, with 18 new inflorescences and a refill of sucrose solution after every session.

RESULTS

On the nectar variation, the percent of yellow flowers probed decreased over time, but not significantly (Figure 1). A t-test was used to determine the differences between the order of orange and yellow visits ($t = .829$, $df = 244$, $p = .4076$). The average order of the yellow flowers probed was 5.79 ± 3.06 ($N = 332$), and the average order of orange flowers probed was 4.43 ± 3.18 ($N = 114$). This means that yellow flowers were not initially preferred and then lost as more orange flowers were visited. Instead, yellow flowers were preferred throughout and orange flowers were just as likely to be visited earlier as later.

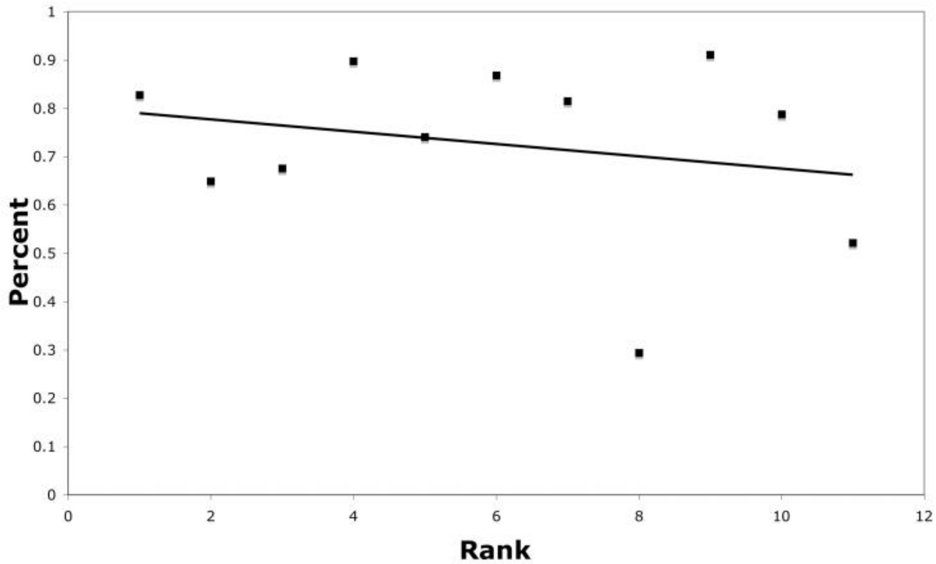

Figure 1. Trend line showing the percent of yellow flowers probed as order increased. The order of flower visits did not statistically differ over time when comparing means of orange and yellow flowers using a t-test (t =.829, df = 244, p = .4076).

When patterns were varied, yellow flowers were preferred in all cases, representing nearly all of the visits flowers, regardless of inflorescence type (see Figure 2). Demonstrating this trend most clearly are the control inflorescences, where 239 total flowers were visited, 233 of which were yellow flowers (df = 1, x^2=1.741, p=0.187). However, some types of inflorescences were more attractive to butterflies, as indicated here by the number of flowers probed per inflorescence. Yellow only inflorescences were significantly the most attractive with 289 visits, followed by the unpainted control with 239 visits (df = 1, x^2 = 4.734, p = 0.02), and the painted control with 211 visits. Inverted (151 flowers visited) and mixed inflorescences (144 flowers visited) had about half or slightly more than half of the floral visits to yellow only inflorescences. Orange only inflorescences were least favored, with 99 flowers visited. In this case, all flowers visited were orange.

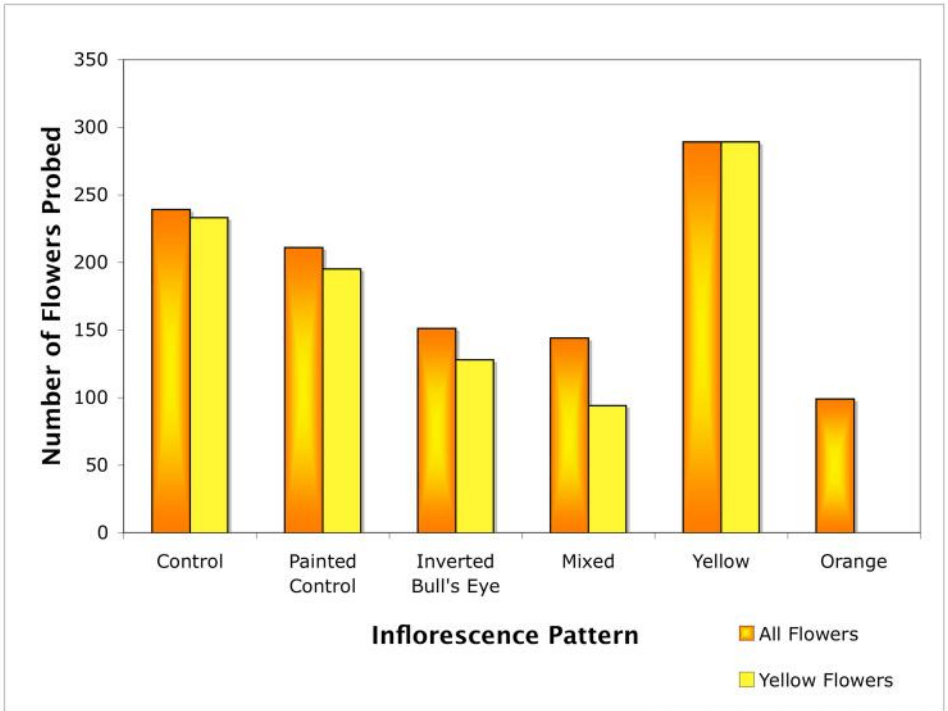

Figure 2. This shows number of flowers probed for each of the pattern variations. Orange bars indicate the total number of flowers (orange and yellow) probed for each pattern, and yellow bars show how many of the total flowers probed were yellow rather than orange.

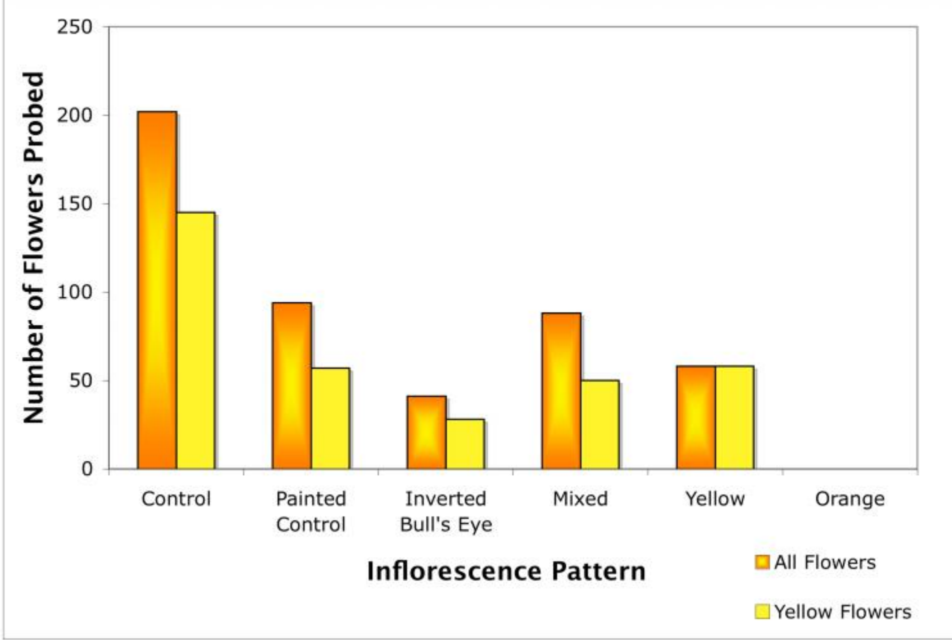

Figure 3. The number of flowers probed on each of pattern variations under the combined variation circumstances. Orange bars indicate the total number of flowers (orange and yellow) probed for each pattern, and yellow bars show how many of the total flowers probed were yellow rather than orange.

Control inflorescence flowers were favored when all flowers were enhanced with artificial nectar (df = 1, x^2 = 39.41, p = $3.44*10^{-10}$). All other inflorescences were visited about half the amount of times as the unpainted control, except for orange inflorescences, which had no visits. Also, orange flowers were visited more often than before, as there was a difference between the total amount of flowers probed, and just the yellow flowers probed on the unpainted control (df = 1, x^2 = 9.363, p = .002). The number of total flowers visited on the control inflorescence was greater
than the total flowers visited on the painted control inflorescence (see Figure 3; df = 1, x^2 = 9.363, p = .002). In general, fewer flowers were probed during combined variation sessions (N=483) than painted variation sessions (N=1,133).

In regards to the number of visits from each butterfly species, *A. fatima* were the most common pollinators. For the pattern variation, 963 flowers foraged on were *A. fatima* and the two other species were observed to visit 199 flowers.

DISCUSSION

The results demonstrated that butterflies have a preference for yellow flowers that does fundamentally change if orange flowers also offer reward. This suggests that the yellow preference is either learned early and unlikely to change, or it is innate. If the butterflies were capable of overriding their initial preference for yellow, they would have been expected to take more and more orange flowers once artificial nectar was placed in them. Instead, we noticed that butterflies tried each yellow flower multiple times before even checking the orange flowers to see if they contained nectar. We can therefore conclude from this that butterflies show a likely innate preference for yellow flowers, and did not necessarily learn that yellow flowers are the only nectar producing flowers on an *L. camara* inflorescence. Nonetheless, butterflies did occasionally visit orange flowers and with greater frequently when they had nectar.

For pattern variation, control and painted control comparisons showed us that butterflies did not mind foraging on flowers that were painted. We found that the bull's eye pattern is more effective with yellow in the center, rather than orange. Foraging pattern on the inverted bull's eye pattern was different than regular bull's eye pattern, in that butterflies still pollinated the "yellow" flowers, which were located on the outside ring around the orange. We believe that butterflies had a difficult time foraging on outside flowers because they were harder to reach, existed less than center flowers, and did not contain nectar. Therefore, they chose the inverted inflorescences patterns fewer times and were attracted to patterns in which yellow flowers were easily accessible. In addition to showing yellow flower preference on both bull's eye patterns, the all yellow inflorescence was visited more than the bull's eye, suggesting that butterflies prefer an all yellow inflorescence over the natural pattern.

Adding nectar to all flowers produced a surprising result. The number of flowers visited declined for all inflorescence types. This may be due to an increased foraging time on flowers that were filled with nectar and as a result less flowers visited during the allotted time. Beyond this, butterflies overwhelmingly chose the unpainted control. All other inflorescence types had steep drops in visitation compared to when artificial nectar was not added. Since butterfly foraging behavior was clearly affected by the paint on this variation and not on the variation where no nectar was added, we think that the butterflies could afford to be picky about their inflorescence choice when flowers were full of nectar. In the pattern variation, butterflies were forced to visit inflorescences other than the unpainted control in order to find nectar. We believe the reason that the yellow inflorescence was not probed the most in this variation is just because they did not want to visit painted inflorescences.

Our variations show that *L. camara* has not evolved in a way that maximizes butterfly visits. We have shown that it may be more beneficial for the inflorescences to keep their older flowers yellow instead of turning them orange, therefore creating a solid yellow inflorescence. Older flowers may turn orange due to an inevitable pigment change. If energy is expended by the plant in turning flowers orange, *L. camara* can save its energy in addition to attracting more pollinators by discontinuing this adaptation. Butterflies on the other hand, are foraging in the most efficient way. They have evolved to visit yellow flowers overwhelmingly more than orange because of their innate preference, and since the yellow flowers are the only nectar producing flowers, it would not be beneficial for them to forage on orange flowers. Since a majority of the butterflies that pollinated the shrubs we observed were *A. fatima*, the mutualistic relationship may be tightly coevolved between the two species. This means it is important for *L. camara* to keep catering to its most common pollinators by keeping older flowers yellow. Coevolution of plants and pollinator is shown to be a long process that can be affected by behavior in either plant or pollinator. In this case, the pollinator may cause the plant to evolve differently into the future. If it *L. camara* does not evolve differently to attract more pollinators, and a preference for all yellow inflorescences is maintained, the tight plant-pollinator relationship may be detrimental to *L. camara* if *A. fatima* become more attracted to other inflorescences that are all yellow.

Future studies could investigate the effect of a “hyper-reward system”, in which only the orange flowers are injected with nectar. Also, research could be conducted on inflorescences in which all of the orange flowers are pulled off of the inflorescence. This would create a solid yellow inflorescence that is more attractive to butterflies, but a smaller platform. Results of this study could tell whether or not retaining old flowers for a bigger platform is beneficial toward *L. camara* pollination.

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