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The effect of flower angle on bat pollination of Mucuna urens (F. Papilionaceae)

Laura Grieneisen

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Department of Biology, The College of William & Mary

ABSTRACT

The purpose of this study is to examine the relationship between bat pollination and flower angle in *Mucuna urens* (F. Papilionaceae). To determine the natural variation among *M. urens*, the angles of 100 *M. urens* flowers were measured with a protractor to the nearest 5°. The mean angle was -82.45° from the horizon, the mode was -90°, and the range was from -45° to -105°. Approximately one-third of the flowers were tied so that they opened at 90° greater than the natural angle, one-third were tied to open at 90° less than the natural angle, and one-third were left at the natural angle. Over several nights, the pollination status of 383 mature *M. urens* flowers was observed. More flowers than expected were pollinated at the natural angle and fewer flowers than expected were pollinated at the positive and negative angles. (X^2 = 63.96, $p<0.001$, df = 2). This suggests that natural *M. urens* flower angles are more accessible to bats than other angles.

RESUMEN

El propósito de este investigación es examinar la relación entre la polinización por murciélagos y el ángulo de la flor en *Mucuna urens* (F. Papilionaceae). Para saber la variedad natural sobre *M. urens*, se midieron los ángulos de 100 M. urens flores con un transportador al grado cinco más cercano. El promedio del ángulo fue -82.45º del horizonte, la moda fue -90º, y el rango fue de -45º a -105º. Se amarraron aproximadamente un 1/3 de las flores para que se abrieran 90º más que el ángulo natural, se amarraron también un 1/3 para que se abrieron 90º menos que el ángulo natural, y 1/3 se mantuvieron al ángulo natural. Durante algunas noches, se observó el estado de polinización de 383 flores maduras de *M. urens*. Más flores de las esperadas se polinizaron al ángulo natural y menos flores de las esperadas se polinizaron a los ángulos positivos y negativos. (X^2 = 63.96, $p<0.001$, df = 2). Los resultados sugieren que los ángulos naturales de flores de M. urens son más accesibles para los murciélagos que otros ángulos.

INTRODUCTION

Bats offer an efficient method of long distance pollination in the Neotropics, as a single bat may travel several kilometers per night searching for flowers (Von Helversen and Reyer 1984). Over six hundred species of Neotropical plants are pollinated by bats (Dobat 1985). It is estimated that two-thirds of chiropterophilous plants in Costa Rica are pollinated by nectivorous bats in the subfamily Glossophaginae (F. Phyllostomidae) (Winter and von Helversen 2001). Glossophagine bats hover in front of flowers much like hummingbirds, and extend their long tongues into flowers to lap nectar (LaVal and Rodriguez-H 2002). Because they require large amounts of nectar, Glossophagine bats are known as high-cost pollinators. They have a metabolic rate that is 15% higher than that of other bats, or similar to that of birds (Arends et al. 1995). Flower species whose primary pollinators are Glossophaginae often produce 100 microL of nectar per flower (Winter and von Helversen 2001). Because of the high nectar investment on the part of the plant and high energy expended by a foraging bat, it is to the advantage of both that flowers be easy to find and access.

Although bats use olfaction and sight to find desired flowering plants, they determine a flower's exact position via echolocation (Stich and Winter 2006). Bat flowers are structured to be acoustically distinct from surrounding foliage. When a bat is flying and projects a vocalization, a leaf might return only one call (Simon et al. 2003). A specially curved bat flower will return echoes from multiple locations for several calls in a row—+/-50^{o} in any direction in the case of *Mucuna holtonii*— as a bat changes position when it flies (von Helversen et al. 2003). Many flowers are cauliflorous or on stalks away from the rest of the plant to reduce acoustic interference from vegetation and have thicker petals to better amplify echoes (Simon et al 2006, von Helversen et al 2003).

A plant genus that includes chiropterophilous flowers with unique echolocation and reproductive structures is *Mucuna* (F. Papilionaceae). *Mucuna* species in the Neotropics appear to have evolved for Glossophagine echolocation because they are morphologically different than those that are pollinated by birds or non-echolocating bats in the palaeotropics (Winter and von Helversen 2001). Previous studies conducted by von Helversen & von Helversen (2003) on *Mucuna holtonii* have shown that bats identify a mature flower by its raised vexillum, a concave petal that reflects echolocation calls to form an "acoustic nectar guide." (Figure 1). Other studies have shown that removing, tilting, blocking, or rotating the vexillum reduces the chance a bat will pollinate that flower and substituting an artificially large vexillum increases the chance (Macedo 2000, von Helversen and von Helversen 1999, von Helversen and von Helversen 2003). Other factors that have been examined, such as variations in inflorescence height and flower symmetry, have not shown a significant effect on the number of flowers that bats visit in other *Mucuna* species (Vincent 2003).

Figure 1: An immature *M. urens* flower (top) and mature flower with raised vexillum (bottom). Photograph by Laura Grieneisen.

Although several studies have changed the angle of the vexillum, none has studied the impact of changing the angle of the entire flower, which is the purpose of this study. Since the orientation of vexillum has been shown to affect the ability of bats to locate and pollinate flowers of *M. holtonii*, I predict that changing the angle of orientation in *M. urens* will result in a difference in pollination. Specifically, I predict that an extreme deviation from the natural mean flower angle will lower the visitation rate. Such a finding would suggest that certain angles are easier to access than others and that there may be an optimal angle for *Mucuna* flowers.

METHODS

Study sites

This study was conducted from 22 April to 1 May 2008 at eight flowering *Mucuna urens* patches along the trails at El Centro de Educación Creativa (elevation 1479 meters) and the Estación Biológica de Monteverde (elevation 1550 meters) in Monteverde, Costa Rica. The seven patches at La Creativa were located in secondary growth tropical lower montane wet forest within 10 meters of a trail. The patch at la Estación was located in front of the Biological Station on the edge of secondary growth tropical lower montane wet forest.

Study organism: *Mucuna urens*

Mucuna urens (F. Papilionaceae) is a woody Neotropical liana that is common in secondary growth forests in the Monteverde region (Zuchowski 2005). It is also called ojo de buey—ox eye— for its large black seeds. The liana can grow up to 70 m long and drape from ground level to 20 m into the canopy (O'Dell 2000). *Mucuna urens* has chandelier-like inflorescences that hang from a ~0.5m long stem. Its flowers are pale green and are bat pollinated. It blooms for several weeks, with one to eight of the 20 or so flowers on each inflorescence flowering each night. This maximizes the spatial memory of bats and allows them to return to the same plant many times (Von Helversen and von Helversen 2003, Winter and von Helversen 2001).

Each flower is composed of two fused keel petals, two wing petals, and an upper vexillum petal (von Helversen and von Helversen 1999). A Glossophagine or other Phyllostomidae bat will fly around to inspect different flowers before hovering in front of a mature flower and pressing its snout against the front slit on the keel petals. This causes the keel petals to split, the reproductive parts to explosively pop out and dust the bat with pollen, and allows the bat to reach the nectar (von Helversen and von Helversen 2003). This also makes it easy to differentiate between pollinated and unpollinated flowers.

Determining average normal angle

The angles of 100 unaltered *M. urens* flowers were measured with a protractor to the nearest 5^{o} to determine the average natural angle of *M. urens*. The flowers were from 25 different inflorescences from all eight patches. They had all matured within the previous 24 hours (vexillum petal had been raised fully) but none had been pollinated. The angle of every mature flower that was found over the course of three days was measured until a sample size of 100 was reached.

Angle alteration

At each patch of *M. urens*, the mature flowers were determined by checking if the vexillum was raised. A raised vexillum would indicate the flower was mature. Between 3 and 18 flowers (depending on the number mature) in each patch each night were manipulated such that an approximately equal number were oriented at each angle. The angles used were natural, positive (90^o greater than the natural angle), and negative (90^o less than the natural angle). Odorless white cotton string was used to tie the flowers to face the negative and positive angles. (Figure 2). The state—immature, mature, or pollinated— of all the flowers on an inflorescence that contained mature flowers was recorded. Each inflorescence was assigned a number and letter and labeled by tying yellow flagging tape approximately 1 meter away. The following morning each inflorescence was examined and whether the individual flowers were pollinated was recorded. This procedure was followed every night until it either started to wilt or was pollinated (Appendix Figure 5). Flowers that broke off of the main stem or were missing the next day were not counted towards the flower total. A total of 383 flowers were documented: 105 flowers at the positive angle, 103 flowers at the negative angle, and 175 flowers at the normal angle. Flowers ranged in height from 15 cm to 2.5 m from the ground.

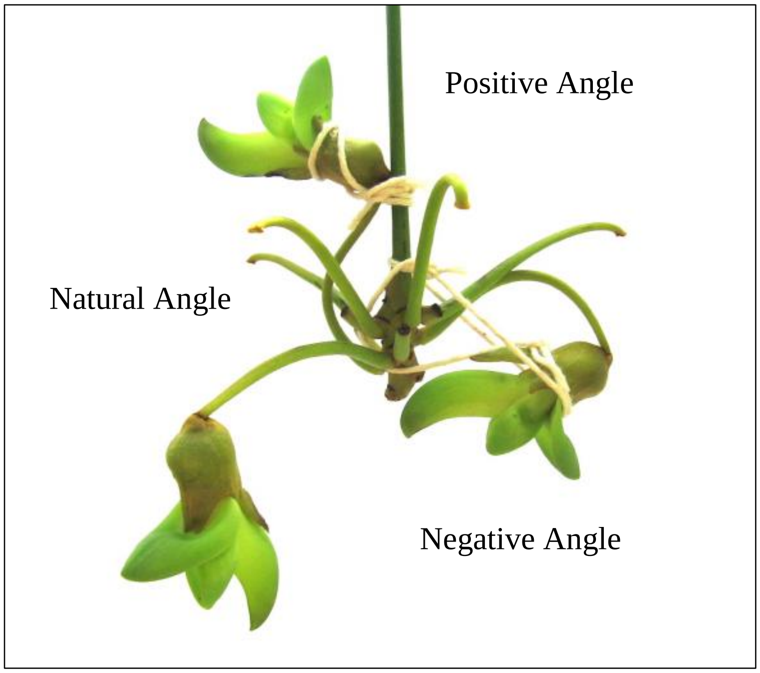

Figure 2: The three angles of *M. urens* compared in this study and how the positive and negative angles were tied. Photograph taken by Laura Grieneisen.

RESULTS

Average natural angle

Of the 100 unaltered *M. urens* flowers whose angles were measured, the mean angle was -82.45^o from the horizon. The most common angle was -90o, which composed 48 out of 100 flowers. Angles ranged from -45^o to -105^o (Figure 3).

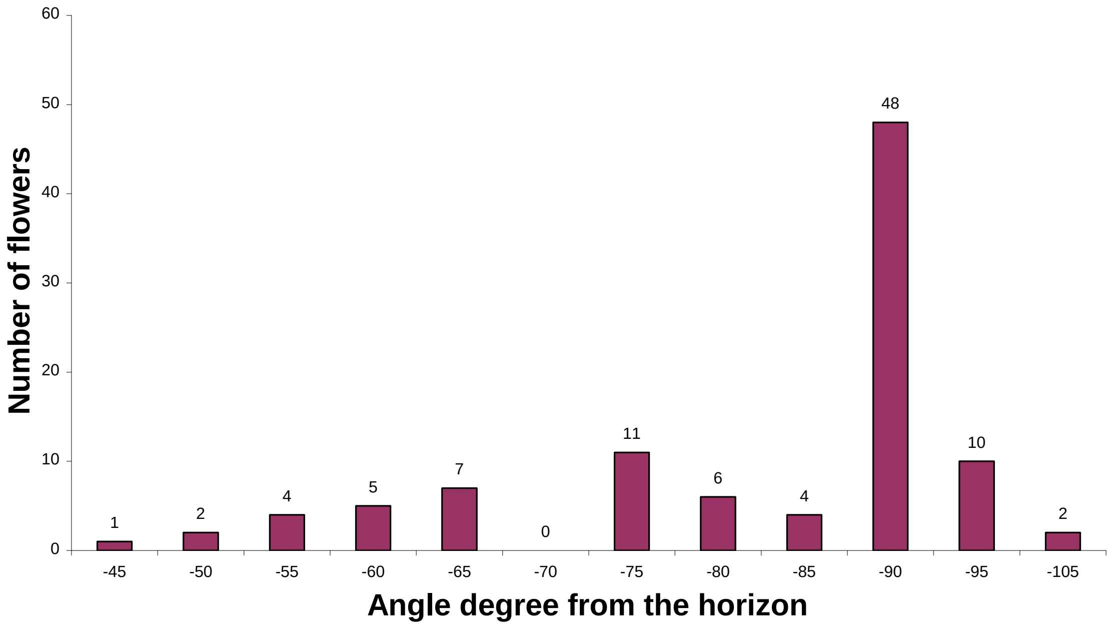

Figure 3: The natural angles of 100 *M. urens* flowers. -90º is a flower that faces the ground when mature. The bars refer to the number of flowers at a given angle.

Angle alteration

Eleven out of 105 flowers of the positive angle were pollinated, 4 out of 103 flowers of the negative angle were pollinated, and 87 out of 175 flowers at the natural angle were pollinated. More flowers than expected at the natural angle were pollinated and fewer flowers at the positive and negative angles were pollinated than expected ($X^2 = 63.96$, $p<0.001$, df = 2) (Figure 4).

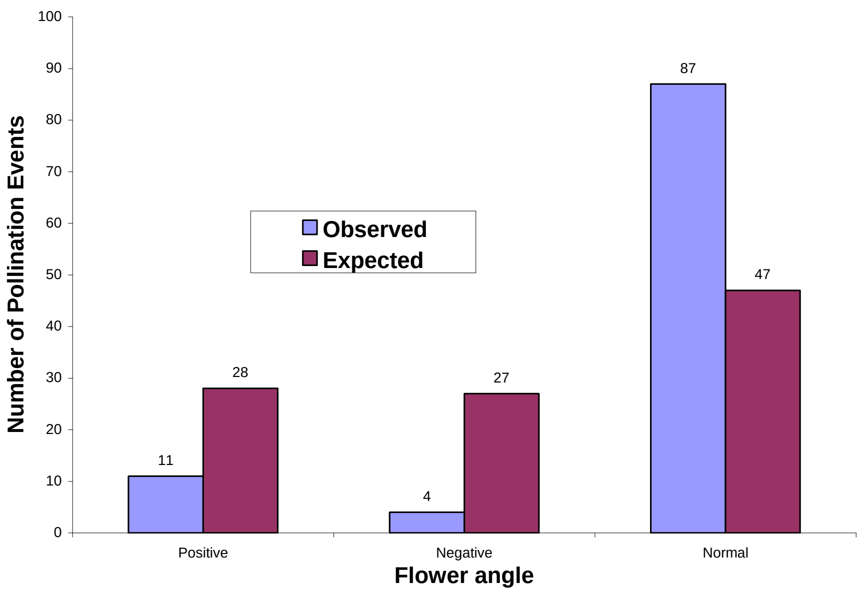

Figure 4: The expected versus observed number of pollinated *M. urens* flowers for positive, negative, and natural angles. The bars represent the number of flowers pollinated. (chi-square = 63.96, p<0.001, df = 2).

DISCUSSION

The angles of *Mucuna urens* had a 60 ^{o} range, but over half of the flowers had an angle of -90^{o}. This natural variation suggests that although the plants is capable of producing flowers at a range of angles, there may be an optimal angle to maximize bat pollination. When flowers were manipulated, more flowers at the natural angle were pollinated and fewer flowers at the positive and negative angles were pollinated. This suggests that bats prefer natural angles to positive or negative angles and may indicate that certain angles are easier to access than others. Some positive and negative angles were opened, which shows that it is not impossible for bats to pollinate flowers with drastically different angles than natural *M. urens*. It is also possible that bats are accustomed to the natural angle, but if the same bats were presented with flowers of different angles for several nights in a row and became accustomed to handling them, they would pollinate an equal number of each angle. Further studies could try to determine why more natural angles were pollinated than positive or negative angles. This study could also be repeated using different angles.

Because bats must hover in front of *M. urens* in order to open it, it is likely that the change in angle made this more difficult. Bats may have been able to locate the flowers acoustically, but were unable to hover in front of them to open the keel. In a previous study in which the vexillum was rotated slightly, bats were able to locate the flower but were unable to open it successfully because they normally use the vexillum to guide them (von Helversen and von Helversen 2003). Although the vexillum was not altered in this study, opening an upside-down flower could likewise prove to be difficult.

The angle of *M. urens* flowers may reduce nectar robbing by other animals. Covering the buds and sepals in urticating hairs and angling the flowers down may discourage potential predators. On 26 April after I had finished tying a patch, I observed a hummingbird stick its beak into four positive angle flowers, but no flowers of other angles in the patch. None of the flowers were pollinated, although it is possible that the hummingbird obtained nectar. To stick its beak into a natural angled flower and reach the nectar, it would have had to tilt its head straight up.

The results of this study may help lead to a better understanding of the relationship between flower morphs and bat pollination and what impacts flower choice by bats. Although this study supports the idea that bats prefer flowers at natural angles to altered angles and several possible causes are suggested, the reason why is still unknown. Any future studies that could help to elucidate this would be fascinating. The shape of other chiropterophilous flowers have been studied, but very little research has been done on the angle of the entire flower. It would be interesting to study the flower angle of various bat flowers in Monteverde to see if there are certain angles that are more common than others.

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Appendix

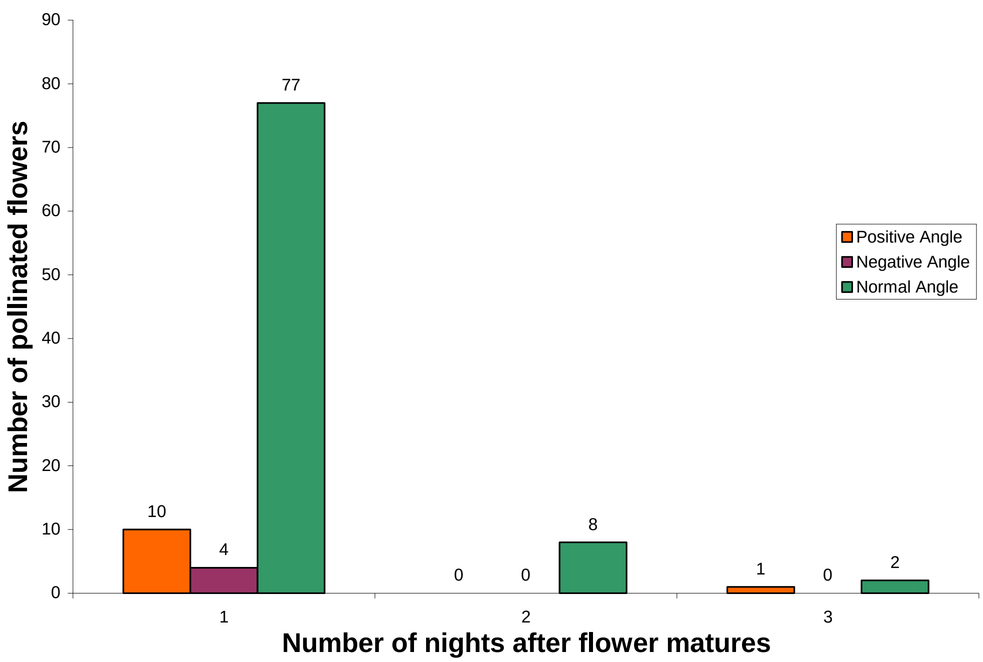

Figure 5: The number of positive, negative, and normal angle M. urens flowers pollinated each night after the flower matured. Because different flowers matured different nights, 'Nights 1,2, and 3' do not represent a specific date.