November 2000

The influence of leaf shape of Passiflora biflora in Heliconius spp. butterfly oviposition

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The Influence of Leaf Shape of *Passiflora biflora* in *Heliconius spp.* Butterfly Oviposition

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ABSTRACT

Between species the leaf morphology of *Passiflora* (Passifloraceae) differ greatly in leaf shape. These differences may be attributed to the presence of *Heliconius* larvae, who feed exclusively on *Passiflora*. Adult females may use leaf shape as an oviposition cue, thus the importance of the changing leaf morphology. This study was conducted in an enclosure in the Monteverde Butterfly Garden where I studied three species of Heliconius butterflies: *Heliconius hecale*, *H. charitonius* and *H. erato*. I studied the oviposition behavior on *Passiflora biflora* due to the varying leaf shapes present, which are two and three lobed leaves. Egg placement showed that Heliconius tend to deposit about half of their eggs on objects other than leaves, such as tendrils (49%) and other miscellaneous objects (8%), without regard to the nearest leaf. The remaining eggs, 43%, were deposited on leaves, with a majority of these deposited on newly unopened leaves. With these unopened leaves, ovipositing females were unable to use leaf shape in discriminating oviposition sites. Of the remaining 35% of the leaves, 2/3 was two lobed and 1/3 were three lobed. Comparing the relative frequencies of two lobed and three lobed showed no preference for one (X^2 = 93.73, D.F. = 1). Therefore, *Heliconius* butterflies do not rely on leaf shape to locate host plants and determine oviposition sites, at least in this study. Leaf shape may be a major factor in a more complex habitat with increased rates of other factors such as competition, climate and resource variability. Alternatively, leaf shape variability may be used for other purposes such as to prevent shading of lower leaves and predator avoidance by confusion.

RESUMEN

Se estudió los efectos de la forma de las hojas en *Passiflora biflora* con la frecuencia que especies de mariposas del género *Heliconius* depositan sus huevos en las hojas. Este estudió se llevó a cabo por quince días en el Jardín de Mariposas en Monteverde, Puntarenas, Costa Rica. Los datos sugieren que las mariposas prefieran zarcillos y hojas nuevas. Estos datos sugieren que la forma de las hojas no es un factor importante en donde las mariposas depositan sus huevos. Tal vez, mis datos sugieren que esta es una aproximada adaptación por la carrera de la evolución entre las mariposas y la *Passiflora*. Quizás, hay diferencias entre las formas morfológicas de las hojas para evitar los depredadores o no obscura las hojas más bajas de la misma planta. Aproximadamente en medio del tiempo las mariposas depositan en objetivos que no son hojas, sin importar la forma de la próxima hoja.

INTRODUCTION

Coevolution is the examination of patterns of interaction between two major groups of organisms with a close and evident ecological relationship, such as Plants and insects (Ehrlich et al. 1964). These two organisms demonstrate a close relationship where each show specific adaptations to one another, leading to increased specialization. This specialization is attributed to the selective pressures that are present between the two organisms. One such example is that of *Papilio glaucus* and its host plants. *Papilio* *glaucus* has escaped, at least partially, from the chemical constrains that limit the host range of most other Papilionians (Feeny 1991). Apparently, *P. glaucus* is able to feed and grow successfully on the mature foliage of trees that have reduced concentrations of toxins. The Monarch butterfly, *Danaus plexippus* and its hostplant, *Asclepias curassavica* have developed an interesting relationship regarding both the larvae and the adult butterfly. The larvae feed on the leaves and accumulate cardenolides in their bodies, granting toxicity to both the caterpillars and the adults (Scott 1986). *Asclepias curassavica* benefits from this relationship because the adult butterfly serves as important pollinator. Each is very specialized in their needs for each other, influencing the interaction of coevolution.

The host plants of butterflies in the genus *Heliconius* belong exclusively to many genera in the family Passifloraceae, therefore creating a close relationship of coevolution between the two organisms (Benson et al. 1975). *Heliconius* butterflies undergone a series of adaptations, which allow the butterflies to continue to utilize *Passiflora* plants for host plants. This relationship between the plant and the herbivore has resulted in a complex of traits. The population dynamic interactions of heliconiines and their hosts are directly correlated with their different behaviors (Gilbert 1991). These behaviors include pollen feeding and ovipositing on new shoots. *Heliconius* butterflies possess well developed vision and learning ability, which were demonstrated in studies by Weiss (1995). With these advanced behaviors, the act of probing and searching for a perfect oviposition site is enhanced. These also aid in the detection of false eggs produced by *Passiflora*, egg predators and other *Heliconius* eggs and/or larvae, although mistakes can be made. The presence of other eggs and/or larvae may deter oviposition due to the fact that *Heliconius* larvae are cannibalistic (Gilbert 1975).

Plants in the genus *Passiflora* exhibit complex floral and vegetative characteristics which are believed to be the products of coevolution between *Passiflora* and its principle herbivore, *Heliconius* larvae (Gilbert 1991). *Passiflora* plants have evolved many mechanisms for deterring oviposition by *Heliconius*. Some of these defenses include, but are not limited to, the production of toxic chemicals (cyanogenic glycosides, alkaloids and saponins for instance), foul odors, toughness of leaves as they age, the presence of hooked hairs (trichomes), filiform stipules which resemble small tendrils, the production of egg and larval mimics, a variable leaf morphology and the presence of extra-floral nectaries to attract predatory ants (Benson et al. 1975 & Gilbert 1975). The broad range of chemical defenses are intended to deter predators and parasites. This defense is not practical for their primary herbivore, *Heliconius* butterflies, who produce their own cyanogenic glycoside system to counter that of the plant (Gilbert 1991). Hook-like trichomes are used to deter herbivores which are present on the surface of some *Passiflora* species (Gilbert 1971). The vast majority of *Passiflora* species possess extra-floral nectaries on petioles, leaves, or bracts. These glands secrete nectar which helps in maintaining a defense force of ants, vespid wasps and other egg parasites (Gilbert 1975). *Passiflora* produce structures that mimic eggs of *Heliconius* butterflies to prevent oviposition and were found to reduce egg laying in *Heliconius* butterflies (Gilbert 1991).

In addition, *Passiflora* has variable leaf shape, which has been attributed to coevolution with *Heliconius*. Because other butterflies use leaf shape as an oviposition cue, it is important to notice that heliconians may be confused by the variable leaf shape, thus protecting *Passiflora* from herbivory (Gilbert 1991). One of the most effective defenses against herbivory may be the diversity in leaf shape. Neto (1991) suggests that a leaf shape image is important as an oviposition cue for *Mechamitis lysimnia* and *Thyridia psidii* butterflies Gilbert (1975) suggests that this has been attributed to coevolution with *Heliconius* butterflies. This variable leaf shape may perplex the females during oviposition, and may cause deterrence of the butterfly, protecting *Passiflora* from herbivory. Passifloraceae are characterized by the plethora of unusual leaf shapes (Gentry 1993). This may be attributed to abiotic factors such as sunlight and temperature.

To examine the relationship between selection of oviposition sites by *Heliconius* females, I addressed the following questions: Does leaf shape influence oviposition of *Heliconius spp*. butterflies on *Passiflora biflora*? If so, is one shape preferred over another? If not, where are they depositing their eggs and why?

MATERIALS AND METHODS

I conducted my study at the Jardin de Mariposas in Monteverde, Puntarenas, Costa Rica, during October and November, 2000. This study was conducted in a greenhouse that simulated a mid-elevation habitat of Costa Rica. Mid-elevation regions (700-1,600m) include the following life zones: tropical moist, lower montane, and premontane (Holdridge 1967). The garden was chosen because of the abundance of *Passiflora biflora* and *Heliconius* butterflies, which include *H. erato*, *H. hecale* and *H. charitonius*. These three species use *P. biflora* as their host plants (De Vries 1987).

Eggs that were deposited on *P. biflora* were collected for 17 days and placed in numbered plastic containers. For each egg collected, I recorded the location of the egg. The locations of the eggs that were collected were classified in several groups in relation to leaf shape or placement on other objects (Table 1). Eggs that were deposited on leaves where distinguished between two and three lobed leaves (Fig. 5 & 6).

All data were analyzed using chi-square tests to determine the difference in the distributions of the oviposition sites. The following factors were each compared: eggs deposited on leaves and objects other than leaves, unfolded leaves that are undistinguishable and old leaves that have defined shape, two-lobed leaves and three lobed leaves, and tendrils in relation to other leaves. To obtain an expected value for the chi-square tests, I counted leaves and tendrils on a *P. biflora* plant to acquire a proportion of what type of foliage is present. This is relative to the proportion of foliage that is present for the butterflies to choose from.

RESULTS

From a total of 234 eggs that were collected, 101 had been deposited on leaves and 114 had been deposited on tendrils (Table 1). There were more eggs deposited on non-leaves, 49% of those being tendrils (Fig 1). There is a significant difference in that *Heliconius* lay their eggs on something other than leaves ($X^2 = 46.34$, d.f. = 1). Of those eggs that were deposited on leaves, 65% of them were on young leaves, defined by a folding leaf that is indistinguishable (Fig.2). Most were found on leaves that were too young to tell the shape due to folding ($X^2 = 94.00$, d.f. = 1).

The lack of difference in leaf shape has an influence in oviposition which is indicated with 51% of the eggs laid on the two lobed leaves and, similarly, 49% of the eggs were laid on three lobed leaves (Fig. 3). There is little variation in the choice of leaf shape, this being a significant result, indicating there is no preference for one shape over another (X^2 = 93.88, d.f. =1). Most eggs were laid on tendrils (Fig. 1). Furthermore, of those eggs which were laid on tendrils, most tendrils were not located near another leaf (Fig 4). There is a significant difference for tendrils that were not located near another leaf (X^2 = 184.34, d.f. = 3).

DISCUSSION

The findings of this study do not support the hypothesis that *Heliconius* oviposition is influenced by leaf shape in *Passiflora* plants. *Heliconius spp*. in this study clear do not use leaf shape as an important cue in choosing oviposition sites. Instead most females deposited eggs off leaves mostly on tendrils. Of the eggs that were found on leaves, unopened leaves were preferred. These leaves were too young to tell the difference in leaf shape due to folding. Of the eggs deposited on two and three lobed leaves, the females show no preference for one. A majority of the eggs were deposited on tendrils where there was no difference in the relation to two or three lobed leaves.

These results differ from those of Chew and Robbins (1984) and Benson (1975) who found that in *Heliconius*, ovipositing butterflies select sites by leaf shape in *Passiflora*. Benson (1975) did extensive field studies with *Heliconius* adult females in his study to summarize the coevolution of plants and herbivores, specifically with *Heliconius* butterflies. This study found there to be numerous other butterflies that deposited eggs on leaves such as *Heliconius sapo-sara* and *Heliconius doris*. Benson (1978) suggested that leaf shape image is used by other species of butterflies such as *Mechamitis lysimnia* (Neto 1991). These results may not accurately reflect trends as seen in this study due to the larger scale of the study and the area. A complex lowland rainforest is much larger and more diverse than a small garden with controlled conditions. Searching for the correct host plant in a huge forest could prove to be much more difficult than in a garden. Another explanation for the differences in results could be the increased learning ability in *Heliconius* butterflies. They may be able to learn where the best sites are for oviposition and continue to go there again and again. This behavior has been examined by Weiss (1995) with *Agraulis vanilla* butterflies where associative color learning was studied. The studies showed that the *A. Vanillae* did learn to associate color with nectar rewards.

The leaf shape of *Passiflora* does not seem to be influenced by oviposition of *Heliconius* in this study. There must be other factors influencing the leaf morphology of *Passiflora*. Gilbert (1975) suggests that leaf shape is related to physical factors or mimicry with other tropical trees and vines. This may be supported by the similarities in two South African plant genera where the two genera demonstrate strong convergent mimicry each, as stated by Gilbert (1975). Physical factors may include competition for sunlight, water or other abiotic factors, where there is a demand for diverse leaf morphology (Castellanos et al. 1986). Highly lobed leaves allow light to pass through to the new growth to leaves below, maximizing the use of solar energy. These ideas could be further researched to understand the other environmental factors that play a role in the diversity of leaf shape in *Passiflora*.

Oviposition sites that are not on the leaves may be in response to predator avoidance by organisms such as attending ants that are often found on *Passiflora*. Due to the presence of extrafloral nectaries, which attract ants and other parasites, *Heliconius* butterflies may be depositing their eggs on the tendrils to maximize the distance from the extrafloral nectaries. Smiley (1985) found that mortality in *Heliconius* larvae was sufficiently higher on host plants on which there were ants attending the extrafloral nectaries. From his findings, one could presume that the same trend of mortality would be seen with *Heliconius* eggs. The increased distance from the extrafloral nectaries could maximize chances of survival.

An additional explanation may be the apparent “mistakes” that females often make when oviposition sites are chosen. This behavior is considered normal by Chew and Robbins (1984) who give examples of mistakes, such as oviposition on non-plant substrates, withered or unsuitable plant parts, and plants that are of insufficient size to support complete development by the larvae. These mistakes may occur for a variety of reasons and often yield normal larval behavior with no severe consequences. The larvae still have a chance to search for their hostplant and survive.

This study has demonstrated that leaf shape does not influence oviposition in *Heliconius* butterflies and there may be other factors influencing this behavior. Further studies may help to explain why there was a higher frequency of eggs deposited on tendrils and not leaves. There may be other aspects of the *Passiflora – Heliconius* relationship in regard to oviposition site that could be further researched. These may present new findings regarding the relationship of how habitat heterogeneity affects the behavior of oviposition. Another aspect of this relationship that needs further research is the advanced learning behavior in *Heliconius* and the effects of the behavior of oviposition.

ACKNOWLEDGEMENTS

I would like to thank the staff and volunteers at the Jardin de Mariposas for lots of enduring days with nothing but smiles. Special thanks are given to Jim and Marta Wolfe, who let me play with their eggs in my effort to rear larvae, at which I failed miserably. I am grateful to Tim Kuhman and Andrew Rodstrom, thanks for the extra effort and time. To Dr. Alan Masters, I think we’ve learned that communication is the key to success. Thanks for the walk in the woods. Furthermore, I am thankful to everyone and everything here in Monteverde. We are in a world of our own here.

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Table 1. Location of *Heliconius* oviposition sites on the hostplants, *Passiflora biflora*. Data of sites shows that most eggs were deposited on tendrils. More specifically, eggs were deposited on tendrils that were not located near another leaf.

Location of Eggs	**Number of Eggs Found**
Tendril near new leaf	15
Tendril near two-lobed leaf	7
Tendril near three-lobed leaf	13
Tendril near no leaf	79
Sub-total tendrils and non leaves	114
Folded New leaves	66
Two-lobed leaves	23
Three-lobed leaves	12
Sub-total leaves	101
String	6
Dead leaf	4
Stem	8
Old Branch	1
	TOTAL: 234

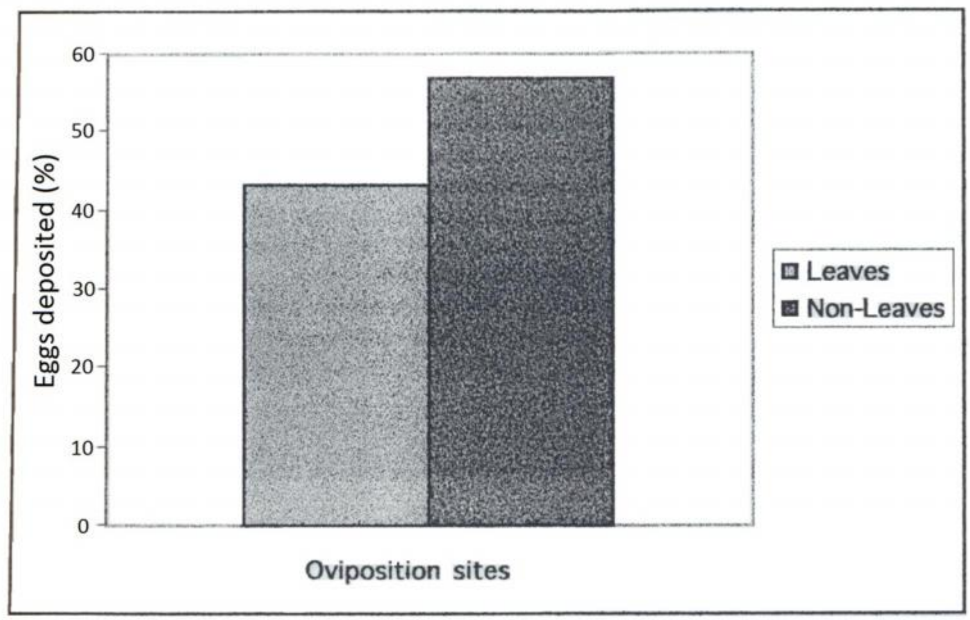


Figure 1. Oviposition sites by *Heliconius* on their hostplant *Passiflora biflora*. Differences show that 57% of the eggs found were deposited on non-leaves and 43% of eggs were deposited on leaves. Non-leaves includes tendrils, string, dead leaf, stem and old branch. These differences were significant (X^2 = 46.34, d.f = 1).

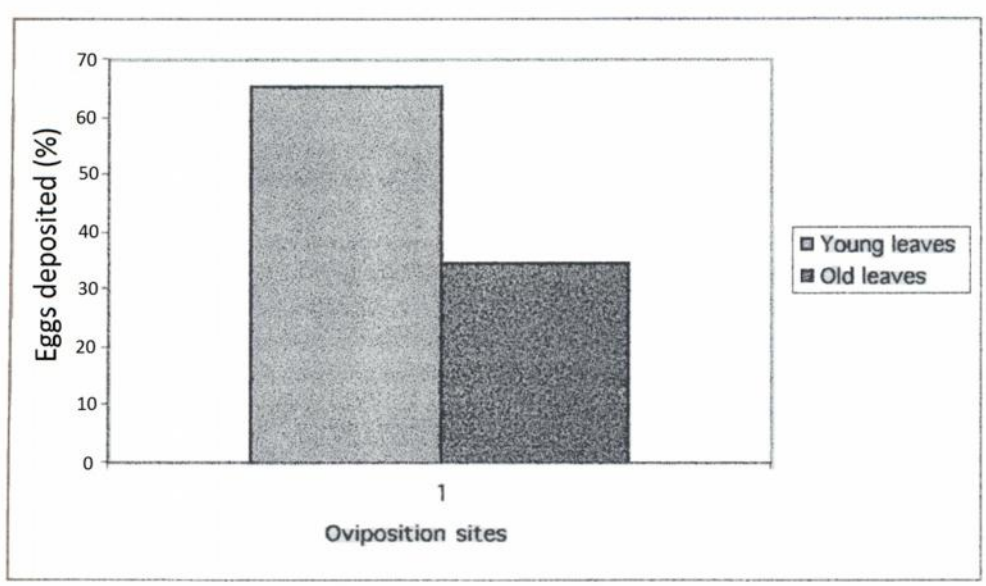


Figure 2. Oviposition sites by *Heliconius spp*. butterflies on their hostplant *Passiflora biflora*. There were 65% of the eggs found on young leaves, defined as having no shape due to the folding of the new leaf. In relation to these leaves, shape is not influencing oviposition due to the leaves being undistinguishable (X^2 = 94.00, d.f. = 1).

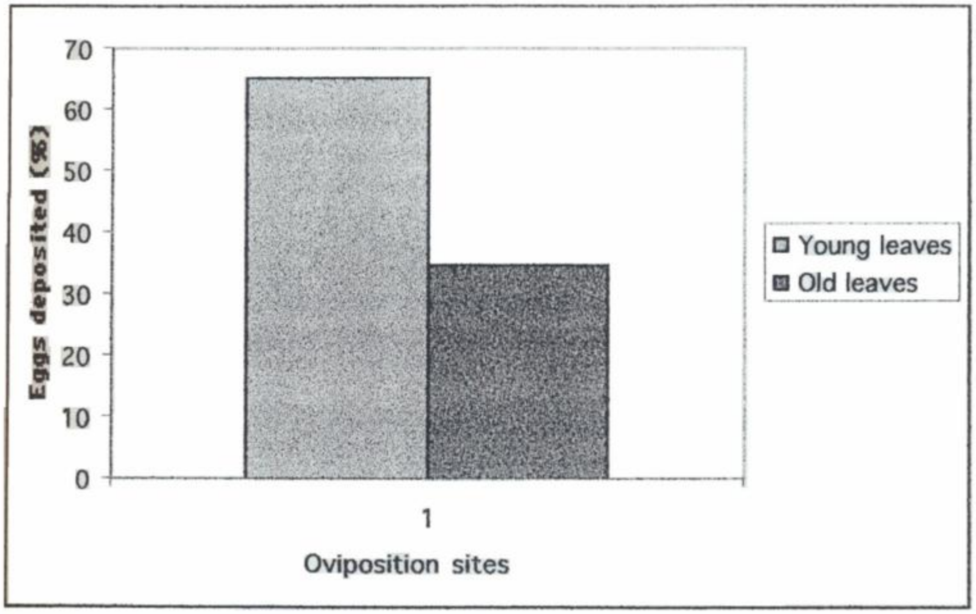
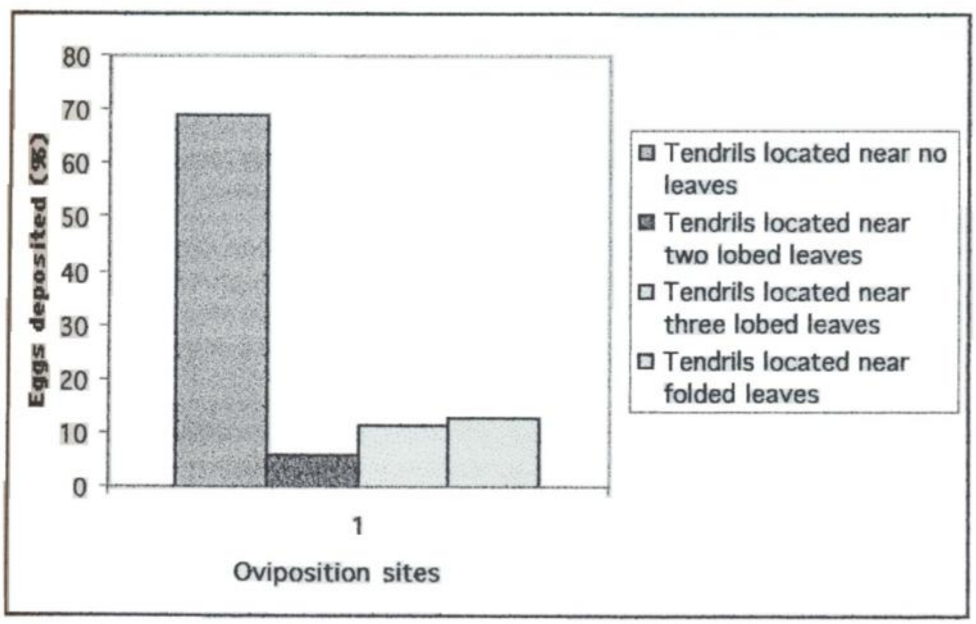


Figure 3. Oviposition sites by *Heliconius. spp*. butterflies on the hostplant *Passiflora biflora*. Differences show that eggs were deposited on young leaves 66% of the time, young leaves being defined as folded with no distinguishable shape. This indicates that there was no influence of leaf shape on oviposition ($X^2 = 93.73$, d.f = 1).



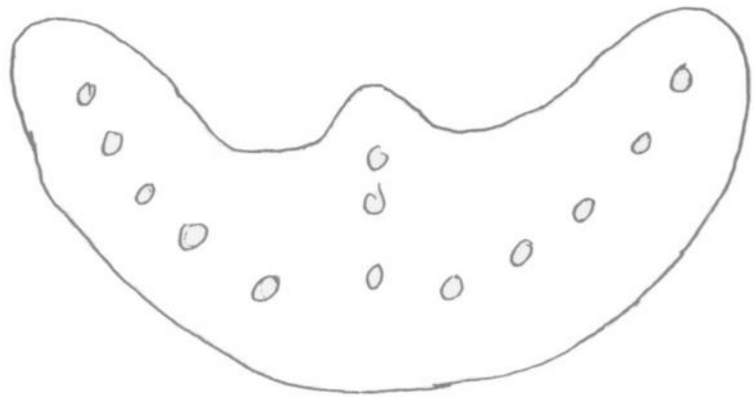


Figure 5. Leaf shape variation among *Passiflora biflara*; an example of a three-lobed leaf.

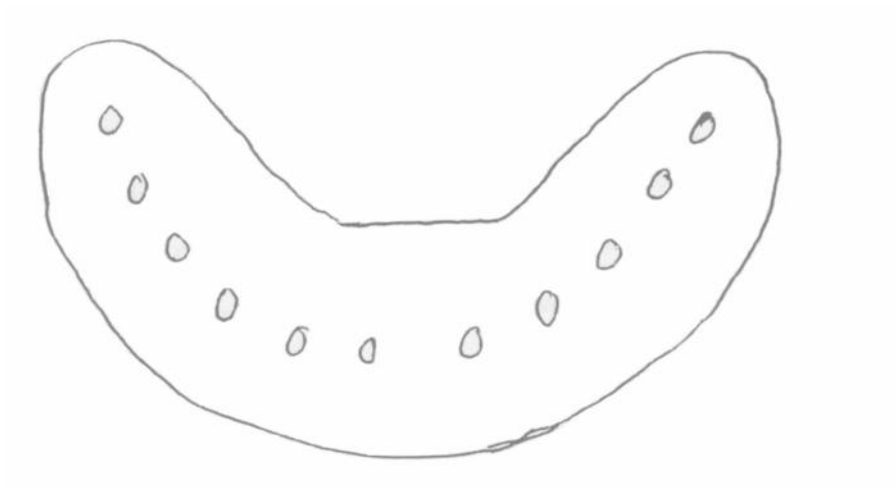


Figure 6. Leaf shape variation among Passiflora biflora; an example of a two-lobed leaf.