

Male and female booths with separate entrances in the tiny flowers of *Guazuma ulmifolia* (Malvaceae–Byttnerioideae). I. Structural integration

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Dedicated to Prof. Dr. Stefan Vogel, the most esteemed academic teacher of the first author, on the occasion of his 80th birthday

Abstract

In spite of their tiny dimensions ($3 \times 5 \text{ mm}^2$), the strongly honey-scented flowers of *Guazuma ulmifolia* (Malvaceae–Byttnerioideae) provide six sexual chambers. A central female unit is surrounded by five interconnected male compartments. At the onset of anthesis the strap-shaped odoriferous petal appendages form an open star with the female chamber in the centre. Hereafter, they wilt, intermingle and finally lock access to the stigma. Meanwhile, wilting sepals liberate new entrances from the back between the petal claws. They give way to nectar-offering male chambers. In each of them a well-lit (but closed) window lures the visitor into a standard position for passive pollen uptake. Finally, the insects have to back out of one of the rear entrances. It remains unknown how the minute visitors (maximum height ca. 0.5 mm) – if ever – overcome the distance to another *Guazuma* tree.

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Introduction

In spite of the huge amount of literature relating to the reproduction of cocoa (*Theobroma cacao*) (see Young et al. (1987), and references therein), very little is known on the floral biology of other members of the Theobromeae (Malvaceae–Byttnerioideae). *Guazuma ulmifolia* Lam. belongs to this alliance and shows some similarity to the flowers of cocoa. It is a widespread and variable species, distributed from Mexico to northern Argentina and growing as a pioneer plant (Salazar and Jøker, 2000). It is a much-utilized multipurpose and

reforestation tree all over Latin America (Powell, 1997; Neto and Aguiar, 1999) as well as a commercial timber (Richter and Dallwitz, 2000). The tiny flowers appear in masses all over the year, and thus are easily accessible and ideal for study. The still unresolved problem is why (and which) animals visit the small and extremely complex flowers of the cocoa alliance. The present results may contribute to a solution of the pollination problems cocoa growers are confronted with.

Materials and methods

The plants used for analysis were from the Campus do Pici of the Ceará Federal University, Fortaleza, Ceará

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(Northeastern Brazil), 3.8°S; 38.5°W. The specimens differed in many aspects, they were obviously planted and apparently came from various origins.

Flowers were studied on the trees and in the laboratory. In the lab, we used a dissecting microscope WILD M5 with drawing device. Details were documented by drawings. Photos were taken using a Nikon F 90X camera with a Sigma 105 mm macro lens and a ring flash (Nikon SB 29). The receptive area of the stigma was demarcated by applying the peroxide (H₂O₂) test after Zeisler (1938).

Results

Flower morphology

Gynoecium

The whitish gynoecium consists of five fused carpels that form a star-shaped structure with rounded rays when seen from above. The ovary is barrel-shaped and well set off from the narrow non-branched style. On the surface it is covered by fine hairs that close the space between the ovary and the surrounding androecium consisting of stamens and staminodes (Fig. 2g).

Over most of its length, the style is covered by rows of equidistant papillae, only the base is devoid of them. In the upper third, there are free stigma arms, but they are secondarily fused to each other in a zipper-like manner by interconnected papillae, the ends of which are slightly swollen. As indicated by the peroxide test, receptive areas cover the whole length of the style and are found as well in the lines between the papillae. Thus, the entire structure usually referred to as the style is in reality a complex of stigmas.

Androecium

The androecium consists of some 20 stamens and staminodes (Fig. 2f). For their major part, they are fused into a tubular androphore with slightly diverging walls, with its tips reaching about the height of the style (Fig. 2g). The free ends of all members are bent outwards in two levels. Five large (antesepalous) inner staminodia (Fig. 2a, f) form the upper level and alternate with five (antepetalous) lower complexes of some three stamens each (Fig. 2f). When interpreting the fertile stamen complexes as single stamens with secondary partition, one might interpret the androecium as obdiplostemonous in relation to the surrounding perianth.

The five conspicuous staminodia dominate the complex. They represent flat reddish or greenish structures covered by simple hairs on their inner surfaces. These hairs only are responsible for the coloration. The triangular free tips are slightly bent outwards, and thus

partly occupy the gaps between the corollar cuculli (Fig. 2a). Hairs from both sides intermingle and close the spaces remaining between the petals and staminodia. At the beginning of anthesis, these attractive inner staminodes give the flower centre the aspect of a reddish eye, recalling those described by Endress (1984) for some primitive angiosperm families (Austrobaileyaceae, Degeneriaceae, Eupomatiaceae, Himantandraceae).

The whitish fertile stamens are set off from the androecial complex between and below the tips of the staminodes. They bend slightly outward, and then divide into 2–3 arms (two upper, one lower). Hereafter, they suddenly bend outwards in a right angle, the two upper arms forming distinct “knees” at the bend (Fig. 2g). In many flowers, one of the three stamens is fused to one of the others. Thus, instead of three pollen-displaying areas of two thecae each, in these cases one area of two and one area of four result. The small thecae are red (or even blackish) while closed and yellowish to off-coloured when open. They open sequentially and expose their few pollen grains in an outward-downward (extrorse) position into the free space, never in close contact to a petal.

When manually opening the connection (see below) between petal hood and androecium, the pollen is ejected as a cloud towards the petal when overcoming the resistance between the hood and the androecial clamp. Thus, when inspecting a petal, one regularly finds pollen on its inner side, which possibly led some authors (see Yeo, 1993) to suspect secondary pollen presentation. This, however, is clearly an artefact.

Corolla

The five petals are independent of each other, but secondarily connected via interlacing hairs. The petals are divided into four different segments (terms in parentheses according to Bayer and Hoppe (1990), Young et al. (1987)): a basal claw, a central hood (pouch, cucullus, concha), a connecting strap (ligule strap, Taenialsegment) and two distal band-shaped ligules.

The claw is a rounded (in transverse section) stiff structure that elevates the plate from the flower base, at right angles to floral axis and pedicel. It soon and abruptly widens into a hood-shaped blade that forms a semicircular (lengthwise and crosswise) cave, the so-called cucullus (Fig. 2d, c). Inside the hood, the strong major veins diverge from the claw in a fan-shaped manner (Fig. 2d). These veins are greenish in the beginning and change to reddish during anthesis. As they are elevated and the intercostal areas bulge to the outside (not depicted), they jointly form a structure like corrugated iron, thus stabilizing the hood. While the veins are opaque to dark red, the intercostal areas are translucent. This results in luminosity increasing from the base to the distal end of the hood, being by far most

brilliant next to the anther complexes. At the base, the rims of the blade are bent outwards and backwards (Fig. 2e), thus forming (jointly with the neighbouring petal) wide rear openings (entrance/exit holes). The blade is hairy on the outside. At the flanges, these stiff hairs intermingle with those of the neighbouring petal, thus linking these two organs laterally and leaving no passage (neither for entering nor for leaving) between them.

At the distal end, the hood is bent inwards and abruptly narrows into a strap-shaped final part that – after a U-turn – runs parallel to the final part of the hood (Fig. 2d, g). At the transition, strap and blade are folded in a V-shaped manner, forming “knees” with the upper end of the hood (Fig. 2d). These “knees” are stuck below the (antesepalous) staminodes on either side (Fig. 2a), while the stamen complex grips the petal from below, entering via the “V” formed at the transition. The different parts of the androecium thus represent a clamp that fixes the hood mechanically from above (staminodes) and below (stamen complex) and provide additional stability to its complex form. Petal hairs here also intermingle with those of the neighbouring staminodes; thus closing whatever passage between them.

The whitish strap flattens after its V-shaped beginning. After a short stretch, it divides into two ligules acting as osmophores (already mentioned by Vogel (1963)). These slightly widened bands are somewhat curled at the margins and also at their ends. They have a mat surface and are yellowish (reddish) at the beginning of anthesis, later on, they turn brownish or reddish brown. After unfolding, the ligules are bent at right angles relative to the floral axis at the beginning of anthesis, and thus at small angles with the remainder of the petal (the hood) (Fig. 2a). Together, they form a star-shaped structure, leaving the gynoecium visible and accessible in their centre (Fig. 2a). In other flowers (other trees) the (in this case reddish, Fig. 1) straps are \pm parallel at the beginning, only slightly bending outward. During anthesis, the (bases of the) ligules wilt more rapidly than the remainder of the flower, approaching and finally meshing with each other, thus barring the entrance to the flower centre (Fig. 2c).

Calyx

At bud stage, the calyx segments are valvate (Fig. 2b, left) and are fused to its neighbours over their total length. The lower and upper epidermises are continuous, with few layers of mesophyll between them. They thus form a closed ball, covered on the outer side by complex tree-like hairs. When the bud is forced open by the expanding corolla, the calyx splits between certain sepals, usually leaving groups of (3) and (2) joined (Fig. 2b, right). On rare occasions, a combination of (2)+(2)+1 is observed. The break lines assume a brownish coloration with time. When drying, the



Fig. 1. Flower of *Guazuma ulmifolia* in male stage, with rear entrances open. Note nectar (?) droplet glistening in one doorway and smooth interior of a (2)-sepal-complex in front of some rear entrances (lower left).

complex of two combined sepals curves backwards. With the compound of three this is more or less impossible, as the circumference of the opening line is smaller than the maximum diameter of the bud and thus the maximum diameter of the calyx hood of three (Fig. 2b). So, this complex dries curving inwards. As a consequence, the (2)-complex is removed from the corolla base and exposes its smooth interior (Fig. 1, lower left). The (3)-compound instead remains next to the petal insertion and exposes its hairy outer face. A manual splitting of the segments is easily possible, but only when gripping each segment with separate forceps. The tissues are already flabby and do not give sufficient stability to open the fusion when gripping only a single sepal. Obviously, an opening of more than the two lines (always the same or at random?) between two and three segments is not intended in this flower.

Function

Visitor attraction

Signalling of the flowers is more by scent than by colour, the greatest part of the blossoms is off-coloured (yellowish–greenish at best). In some cases, the osmophores are reddish (Fig. 1) or brownish, but not attractively coloured. Instead, there is an extremely strong (especially in relation to the size of the osmophores), even nauseating honey-like scent, sometimes perceptible at more than 10 m from the tree. The head-note is quite similar to that of the pseudo-flowers of *Euphorbia cyparissias* (Euphorbiaceae) infected by the rust fungus *Uromyces pisi* s.l. (Pucciniaceae, Basidiomycetes; for a life history of this interaction, see Pfunder and Roy (2000)). When approaching the flowers, there

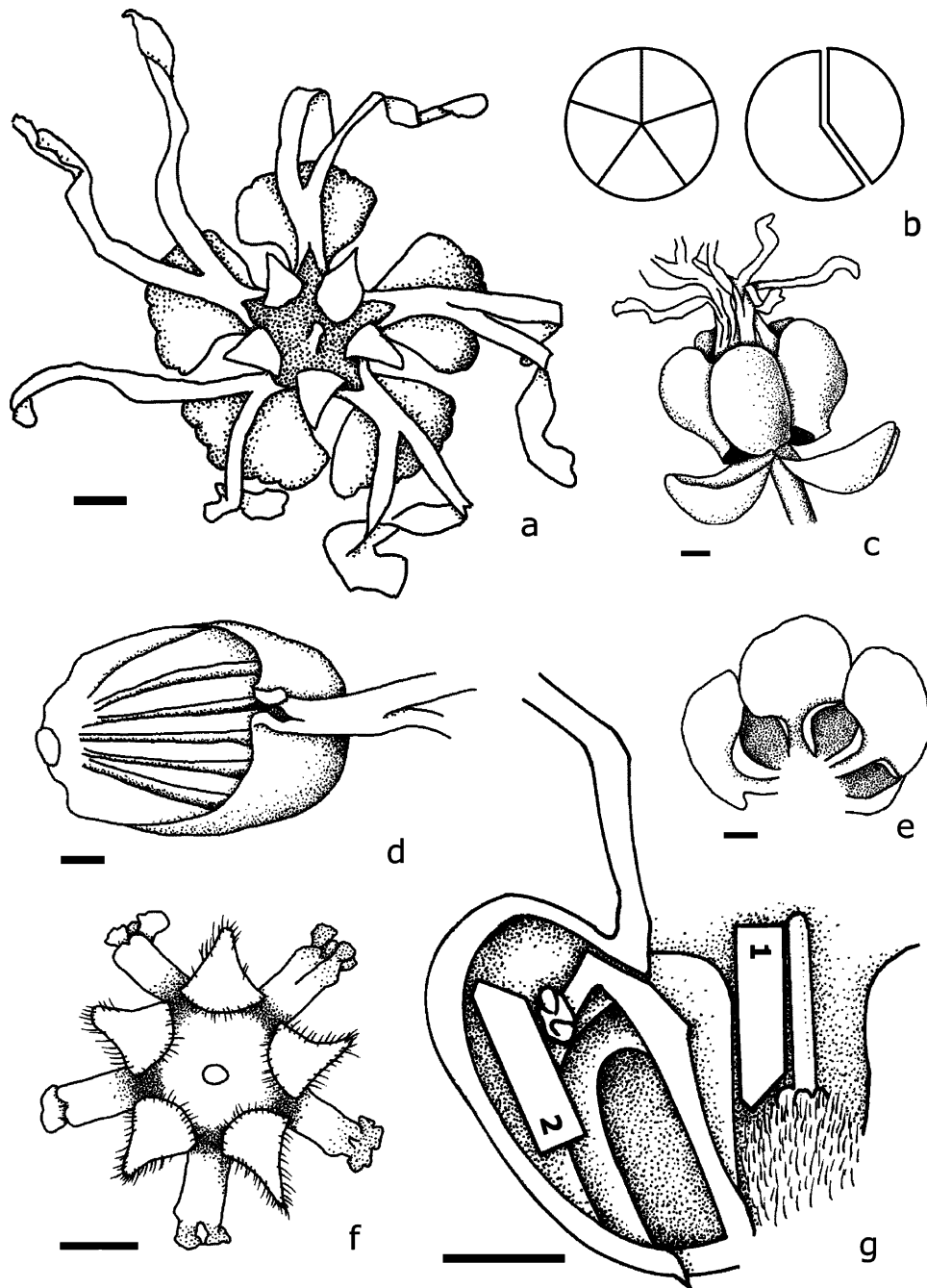


Fig. 2. Flower of *Guazuma ulmifolia*: (a) Entire flower, seen from above, with female chamber widely open. For the sake of clarity, the hair cover is not shown. (b) Diagram of calyx opening; left: five sepals fused, right: calyx split open leaving one complex of (3) and one of (2) sepals. (c) Entire flower in side-view: Front entrance closed by straps and intermingled ligulae, rear entrances open. (d) Petal seen from inside showing incurved hood with elevated veins and illuminated intercostals fields. Note the V-shaped transition to the strap that lodges above the stamen complex in the flower (compare (g)). (e) Corolla seen from below, showing the rear entrances towards the male booths opened by outward folds at the petal bases. (f) Androecium sheath and style, seen from above. Note upper level of triangular staminodes and lower level of fertile stamen complexes, each one ending in 3 anthers. (g) Neighbouring female (right) and male (left) chambers, cut lengthwise, with schematic visitors: arrow 1: standing on one of the staminodes, this visitor touches the style/stigma with its dorsum; arrow 2: after having entered through the rear entrance (at petal base) and attracted by the stimulus of the illuminated window pane, this visitor comes to a stop, impeded by the petal in front and the stamen complex above that contaminates its dorsal side with pollen. Note lateral connection to the next male chamber via exit of actual and entrance to next booth, both visible below stamen complex. Bar: 1 mm.

are (at least in some cases) reddish guide lines inside the female (staminodes) as well as inside the male chambers (reddish veins, staminode part of the androphore). Inside the flower, there is a scent different from that for long-distance guidance. It emanates from the androecial ring and is of a similar quality but less sweet, even slightly bitter.

Attractant is probably nectar, but no regularity was observed up to now as to when and where it is produced. In several instances, an immense (in relation to the sizes of the chamber and the visitor) drop was observed inside certain male chambers (Fig. 1). It was located in the region where the main veins diverge. As yet, a nectary has not been identified. While Young et al. (1984) reported the presence of nectaries, Vogel (2000) expressed his doubts on their presence in Theobromaeae. Further studies as to (nectar, scent) gland localization, structure and function are under way.

Female chamber

There is a single female chamber in the centre of the flower. It is formed by the androphore and the petal straps (Fig. 2a). In the female phase, it is widely open. The ligules serve as first landing sites and guidelines towards the interior. Later, the reddish inner staminodes that give the centre the aspect of a red eye, may serve for orientation. When walking to the interior on one of the staminodes, hairs on the latter help to keep the back of the visitor in contact with the central style and its stigmas. The maximal height of a visitor admitted is about 0.5 mm (Fig. 2f, g; arrow 1).

Male chambers

The five male chambers are connected to its neighbours laterally (see exit and entrance in Fig. 2g), thus forming a circle around the female chamber (Fig. 2a) – but not a walk-around blossom (“Umlaufblume” in the German literature). Each chamber consists of a petal hood and a part of the androphore with a stamen complex (Fig. 2g, left). As the petal margin at its beginning at the claw is folded outward (Fig. 2g, lower left), entrances are formed at either side of the petal base (Fig. 2e), jointly with the same structure of the next petal. Thus, theoretically, there exist five entrances between the chambers. Some of them, however, are inaccessible because of the (3)-sepal-complex that is folded inward towards the petal insertions and thus hides several petal bases. Even, if it leaves an entrance partially open, its exposed external surface is impassable because of its tree-like hairs. The (2)-sepal-complex, on the other hand, presents its smooth interior surface and thus offers a forecourt in front of the petal-base entrances (Fig. 1, lower left).

At the entrance, reddish streaks along the main nerves of the petal resemble the female chamber. While the entrance region of the male booth is comparatively

dark, the chamber becomes more and more well-lit, when entering it. Until the other end of the chamber, the relative importance of the transparent intercostal fields increases, and thus the illumination of the room. When following the path to the light, the visitor will undoubtedly touch the pollen-offering area of the three anthers that is some 0.5 mm above the running path towards the light (Fig. 2g, arrow 2).

Presumed pollinators

There are certain characters in favour of myiophily: the strong (even nauseating) honey-like scent (or even stench); the translucent windows that guide the visitors into the right position to passively take up pollen (similar to other well-known examples like commercial fly traps or those of certain carnivorous plants – Troll and Höhn, 1973; flowers of *Apios americana* – Westerkamp and Paul, 1993; Westerkamp, 1993 – and *Aristolochia*, Endress, 1994).

Several insects (all still unidentified) have been caught in or near *Guazuma* flowers: flies (lengths 1.3–2.9 mm), beetles (2.2 mm, height ≥ 1 mm) and ants (1.5–2.6 mm). Some of them carried pollen, but only the flies entered the male and female chambers.

Integrated functional scenario

At the beginning of anthesis, the petal hoods are already stuck with their knees in position between the different levels of the outward curved free androecial tips, thus forming a connected system of five male chambers. The free parts of the petals unfold (uncurl) to a degree slightly dissimilar in different specimens and give way to the central female chamber. At least in certain plants, the ligules spread into a horizontal position, at right angles to the floral axes. They thus assume a star-shaped form with a reddish centre, the female chamber. Attracted by the expectancy of nectar and guided by a far-reaching cloud of honey-like scent, certain micro-dipterans land on one of the ligules and walk downwards in direction of the flower centre, lured by a different bouquet. With the female chamber widely open and led along the reddish guides of the staminodes, they enter the flower. To reach its depth, they squeeze themselves into the narrow spaces left between the style and the staminodes that bulge out from the androphore (Fig. 2g, arrow 1). Maybe, they use the larger spaces left in front of the fertile stamens between the staminodes (Fig. 2f) as parallel walking paths for the legs of either side of the body, and thus as guide rails. Short stiff hairs make that the upper side of their body is pressed against the stigmatic areas along the style. When already carrying a pollen load from another flower visited earlier, they passively deliver at least part of it to the stigmas. The anthers of this particular flower are still closed at this time. On their approach to the base, their way in is finally impeded by the gynoeceum that totally

fills the interior of the androphore tube. Frustrated by the lack of nectar in this entrance, the visitor might try others of this dry revolver blossom – and is frustrated again and finally leaves this flower.

In the meantime, the wilting process makes the ligules to move upward and inward. In one instance, the angle from horizontal (i.e., at right angles with the axis) to 45° was performed within 45 min. The upward-inward movement of the ligules finally comes to an end, when they encounter each other and intermingle in the centre of the flower, thus hermetically closing the female chamber (Fig. 2c). On the other end of the flower, the two calyx complexes also wither. The compound of two fused sepals moves outward-downward and approaches the pedicel. Thus, it opens some of the access holes present between the petal bases (Fig. 2c, e), offering even its smooth interior as a walking path towards these entrances. The complex of three by way of the narrow opening is mechanically impeded to make the same outward curvature and instead curls inward. At least initially, it blocks some of the entrances above.

In a new approach to the flower at a later time, a visitor – again guided by the strong honey scent – will land on the ligules another time. In this instance, walking along them, however, will not lead into the female chamber (that is closed, Fig. 2c) but to the outer side of the petals and finally towards one of the openings between the petal bases. Lured again by the different scent of the androecium, the visitor finally enters a male chamber, using the inner side of the two-sepal complex as a stepping-stone. Sometimes, a glistening drop of nectar (?) (Fig. 1) intensifies attraction. To entirely consume the nectar, the fly enters deeper into the chamber. From the entrance onwards, reddish guide lines (similar to those of the female chamber) on the petal (Fig. 1) and androecium accompany the inwards movement. Having drunk, the insect searches for an exit and instead is lured by the illumination at the end of the chamber. Thus, it only gets deeper into it and finally is stopped by the petal curvature in front of it and the staminal complex above (Fig. 2g, arrow 2). As further movement is impossible, the fly has to withdraw in reverse gear and either leaves via the entrance hole used earlier or enter the next chamber via the connecting corridor (Fig. 2g). Here, it again follows the lure of the well-lit window, thus receiving more pollen on its dorsum. After visiting some of the male chambers, the visitor finally leaves via one of the open entrance/exit holes, i.e. those ones not blocked by the incurved three-sepal-complex.

Conclusions

In spite of its minute dimensions, *Guazuma ulmifolia* displays a surprisingly complex flower with six separate chambers and female and male entrances on

opposed – upper and lower – sides. The attractant “nectar” is only available in certain male chambers. It is still unknown, which is the baited chamber and why the others remain bait-less. To our knowledge, the female chamber is always dry. Attracted from afar by a strong honey-like scent of the ligules and lured into the depth of whatever entrance by the different scent of the androecium, visitors enter indiscriminately, additionally confused by the optical similarity (red guides) of the interiors. With some male chambers offering attracting nectar and the female chamber always dry, we observe a system similar to that described by Baker (1976) in *Carica* as “mistake” pollination. While in *Carica*, however, male and female flowers are involved in a cheating system, male and female chambers within the same flower are concerned in *Guazuma*. In addition to the overall herkogamy, there is also a certain degree of dichogamy, the degree of which is still unknown.

Variability, phenology, movements and structure and function of nectar and scent glands of this tiny flower are so perplexing that every new result confuses more than resolves. Much more work is badly needed – in this special case as in floral biology in general.

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