

***Tetrastylis ovalis*: a second case of bat-pollinated passionflower (*Passifloraceae*)**

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Abstract: The floral biology of *Tetrastylis ovalis* and its pollination by bats was studied in southeastern Brazil. The flowers of *T. ovalis* seem more fitted to bat-pollination than those of *Passiflora mucronata*, the other known chiropterophilous passionflower. Two species of bats were observed visiting flowers of *T. ovalis*: the phyllostomids *Glossophaga soricina* and *Rhinophylla pumilio*. Due to the attributes of *Tetrastylis*, its presumed primitiveness and a better defined bat-pollination syndrome we suggest that chiropterophily evolved independently in the former genus and in *Passiflora*.

Pollination biology studies in the *Passifloraceae* indicate that bees and hummingbirds are the main pollinators of most species of passion flowers (KNUTH 1904, JANZEN 1968). In his revisionary studies, VOGEL (1969) suggested that the number of known bat-pollinated taxa would increase as more field studies on chiropterophily were carried out in South America and predicted bat-visited flowers amongst the *Passifloraceae*. Until now bat-pollination in the passion flower family was described only for the Brazilian *Passiflora mucronata* by SAZIMA & SAZIMA (1978).

In the present paper, a second bat-pollinated passion flower from southeastern Brazil is described, supporting VOGEL's suggestion. We present here observations on floral biology and bat-pollination of *Tetrastylis ovalis*, compare our results to those described for *Passiflora mucronata* by SAZIMA & SAZIMA (1978), and discuss the possibility that chiropterophily evolved independently in *Tetrastylis* and in *Passiflora*.

Material and methods

Field observations were made in the Forest Reserve of the Companhia Vale do Rio Doce, at Linhares, Espírito Santo, southeastern Brazil (19° 06' S, 39° 45' W). This forest, named "Mata de Tabuleiro", is regarded to be complex due to its species richness and high density of tall trees and lianas. Even though this forest shows strong phytogeographic and physiognomic affinities to the Amazonian "terra firme" forest, its floristic composition is quite different (PEIXOTO & GENTRY 1990).

The individuals of *T. ovalis* were studied in situ; we observed the number of open flowers per night, phases of anthesis, and the movements of the reproductive organs. Sugar con-

centration in the nectar was measured with a pocket refractometer. Pollen viability and the presence of viscin threads were tested using the aceto-carmin and sudan IV techniques, respectively (RADFORD & al. 1974). The receptivity of stigmata was tested using the H_2O_2 reaction described by ZEISLER (1938). Morphological observations were made on material fixed in 70% alcohol. Plant voucher specimens are deposited at the herbarium of the Universidade Estadual de Campinas (UEC 21967).

Direct visual records of bat visits to flowers were made during July and August 1989 in natural illumination. Observations were carried out in seven inconsecutive nights during intermittent sessions from 17:00 to 5:00 h, totalling about 30 man-hours. Photographs were taken in order to analyse the foraging behaviour of bats in greater detail. The main data recorded were the behaviour of the bats at the flowers and the duration and frequency of bat visits. One bat was caught in a nylon mistnet set in front of a flowering *T. ovalis* and its fur was examined for presence of pollen. This bat was deposited as a voucher in the Museu de História Natural, Universidade Estadual de Campinas (ZUEC 1375). Species of bats visiting flowers of *T. ovalis* were identified by catching individuals in mistnets or from photographs taken during their visits to flowers.

Results

Tetrastylis ovalis (VELL.) KILLIP is a woody vine growing at the edge of the forest, bearing stalks placed outside the foliage; the flowers are grouped in racemose pendent inflorescences (flagelliflory) without leaves. The flowers of an inflorescence open from its base to the tip (Fig. 2 b). Some inflorescences bore young buds, open flowers, and almost mature fruits at the same time. At the beginning of our observations, few individuals were flowering, most of them being in vegetative state or already fruiting.

In each inflorescence one to two flowers open nightly. The flowers start opening at dusk from 17:30–19:15 h, and wilt at about 7:00 h in the following day. The flowers open asynchronously, with an interval of thirty minutes between two openings. Anthesis can be characterized by six distinct phases (Fig. 1 a–f): phase 1: The bud in pre-anthesis is swollen and the sepals are slightly apart, the petals can already be seen (Fig. 1 a, arrow). Phase 2: anthesis starts with a very fast movement of one lower sepal, and subsequently sepals and petals move slowly curving backward (Fig. 1 b). Phase 3: the sepals and petals are fully expanded and the flower now exposes the androgynophore. The filaments and the five introrse anthers are arranged together in a semicircle with its inner side facing the stalk (Fig. 1 c). This position of the stamens is already present in the bud. The stigmata are clustered and placed slightly above the anthers and the androgynophore is bowed eccentrically. In this phase, the nectar is already available to the visitors, but pollen grains are not released. Phase one to three habitually last nine minutes. Phase 4: sepals and petals are slightly bent backwards. Anthers start drawing an arc of almost 180° thus becoming extrorse (Fig. 1 d) and pollen grains begin to be released. This phase is thus characterized by a brief period of protandry. Phase 5: sepals and petals are reflected (Fig. 1 e). Up to this phase the stigmatic surface appears opaque and without any visible secretion. The phases 4 to 5 may be regarded as the “protandrous” condition (see Fig. 2 a). At the beginning of the style movements in phase 5, the stigmata are covered with a sticky secretion, becoming receptive and anthers are completely extrorse with a large amount of pollen available. Phase 6: due to style movements the stigmata begin to bend apart by curving downwards,

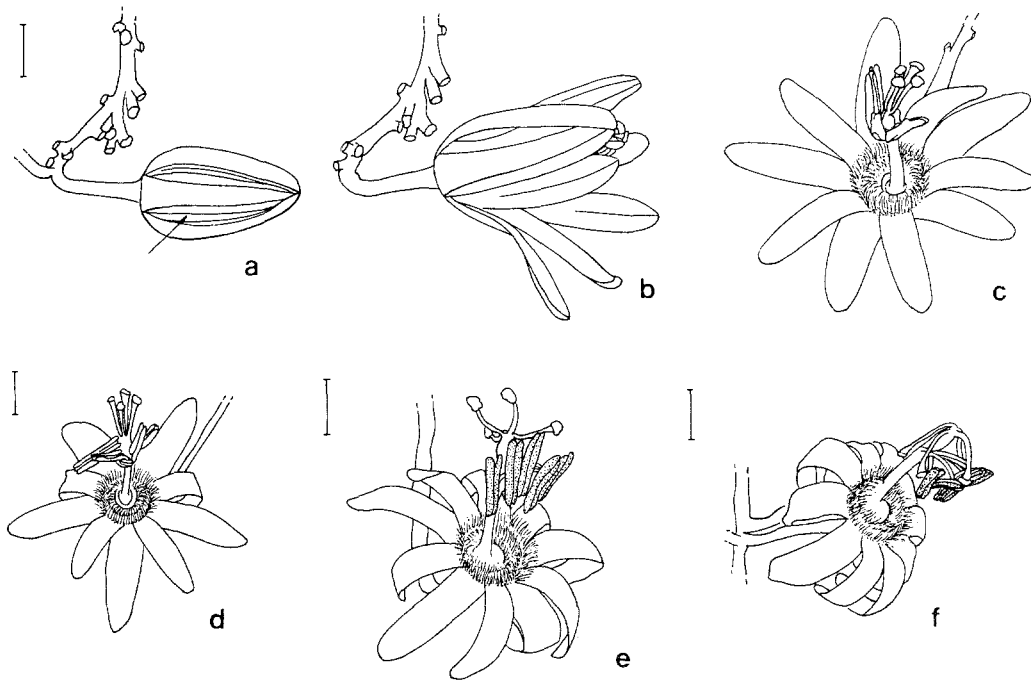


Fig. 1. Phases of anthesis in *Tetrastylis ovalis*. *a* Bud in pre-anthesis (phase 1). *b* First fast displacement of lower sepal. *c* Flower with androgynophore exposed. *d* Anthers begin to reflex. *e* Anthers are extrorsed with pollen grains available, and style begins movements ("protandrous" condition). *f* Petals and sepals reflected and stigmata radially arranged ("hermaphroditic" condition). Bars: 10 mm

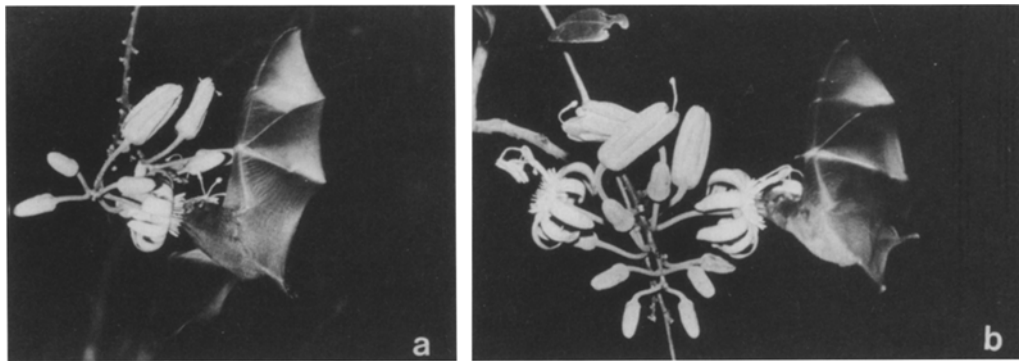


Fig. 2. The bat *Glossophaga soricina* while visiting the flower of *T. ovalis*. *a* At phase 5 the stigmata do not touch the bat's head (protandrous condition). *b* At phase 6 the anthers and stigmata now touch the top of the bat's head (hermaphroditic condition). Photo: João VASCONCELLOS-NETO

and become arranged radially. Three stigmata end up at the same side of the anthers, one at the opposite side (Fig. 1 f); this may be regarded as the "hermaphroditic" condition (see Fig. 2 b). The whole anthesis from phase one to six lasts about 150 min.

The flowers have vinaceous sepals, and the petals are creamy and ornated with vinaceous lines. The nectar chamber, operculum, androgynophore, ovary, and styles, as well as the stigmata, are greenish, and the hair-like projections of the corona (fringe) are vinaceous. Pollen grains are vivid yellow and have viscin threads. The corona filaments, the reflected position of sepals and petals, and the oblique position of the flowers in the inflorescences give the flowers of *T. ovalis* the appearance of a large gullet. The flower emits a very strong fragrance which resembles that of garlic. Nectar is produced in large amounts until about 4:00 h, then its production stops. The sugar concentration in the nectar is about 20% ($s = 3.11$; $n = 11$). During wilting the petals and sepals return to their original position, as seen in the bud, thus enclosing the ovary.

The flowers of *T. ovalis* were visited by two phyllostomid bat species: *Glossophaga soricina* (PALLAS) of the *Glossophaginae* and *Rhinophylla pumilio* (PETERS) of the *Carollinae*. Visits by bats were more frequent between 19:00–23:00 h; the last visit was recorded at $\varnothing$ 1:30 h. The bat may visit the flowers directly or fly over the inflorescence once or twice before the actual visit. A bat approaches a flower from one direction only, due to the presence of the stalk and the bent position of the androgynophore. During the visits, the bat hovers briefly in front of the flower and, while thrusting its snout into the flower to lap the nectar, the top of its head touches the anthers and stigmata (Fig. 2).

Visits of bats begin when flowers are at phase 3, when little nectar is available although the fragrance emitted is very strong. At phase 4, when the flowers are still in the protandrous condition, the bats touch only the anthers and, as a consequence, pollen is deposited on the fur between and behind their ears (Fig. 2 a). Later, when the flowers are in the hermaphroditic condition, the bat's head also touches three of the stigmata (Fig. 2 b). The remaining, fourth stigma is not touched by the bat due to its position (Fig. 2 b). The visits by bats last tenths of a second, and occur at intervals of 2 to 90 min.

Discussion

Tetrastylis ovalis shows all the needed features to fit the “chiropterophilous syndrome” as defined by several authors (VOGEL 1958, BAKER 1961, FAEGRI & VAN DER PUJL 1980). As may be expected, some of the chiropterophilous features of *T. ovalis* are similar to those of *Passiflora mucronata*, e.g., whitish colour, zygomorphic flowers, sugar concentration in the nectar, large amount of pollen (on large anthers), and pollen grains being placed on a particular site of the bat's head due to the position of the anthers (SAZIMA & SAZIMA 1978). However, some attributes found in *T. ovalis*, such as flagelliflory, anthesis at dusk, very strong garlic fragrance, and position of the anthers in the bud seem to approach this species closer to the “classical” chiropterophilous syndrome than *P. mucronata*, which does not show some of the features expected for bat-pollinated plants (SAZIMA & SAZIMA 1978).

The flagelliflory in *T. ovalis*, like in *Mucuna andreana* (BAKER 1973), is a condition adequate for the bat visitors, relatively large flying animals, because the pendent flowers allow them to fly freely around the inflorescences (BAKER 1961, DOBAT & PEIKERT-HOLLE 1985). The opening of the flowers of *T. ovalis* at dusk is similar to other typical chiropterophilous flowers such as *Lafoensia pacari* of the *Lythraceae*, *Marcgravia myriostigma* of the *Marcgraviaceae* (SAZIMA & SAZIMA

1975, 1980), and *Luehea speciosa* of the *Tiliaceae* (SAZIMA & al. 1982), matching well the beginning of the activity of flower-visiting phyllostomid bats (HOWELL 1977). Thus the flowers of *T. ovalis* are available to the bats for a longer time (about ten hours) than the flowers of *P. mucronata*, which open late at night and remain available to the bats for only five hours nightly (SAZIMA & SAZIMA 1978).

The strong fragrance, present during the first minute of the production of the nectar, in the moment of opening of the flowers of *T. ovalis*, seems to be an important cue for the bat. VOGEL (1958) stated that odour is probably a more important feature for attraction of bats than colour. We believe that the strong fragrance produced by the flowers of *T. ovalis* is an important long-distance attractant for the bats, whereas the reflection of sepals and petals and the position of the flower in the inflorescence seem to be important at a closer range, probably being used as visual cues to guide the bat to the flowers. The large and evident gullet-like appearance of the flowers of *T. ovalis* may indicate where food resource (nectar) is placed, a feature not evident in *P. mucronata* (SAZIMA & SAZIMA 1978).

The position of the stamens of *T. ovalis* in a semi-circle is already present in the bud, and thus may be regarded as a morphological feature. In the flowers of *P. mucronata* such positioning in semi-circle occurs after anthesis and is due to movement of the filaments (SAZIMA & SAZIMA 1978) and thus may rather be regarded as a physiological feature.

The presence of viscin threads among the pollen grains of *T. ovalis* likely increases its adherence to the bat's fur. Viscin threads also were observed in flowers of *Bauhinia pauletia* (CRUDEN & JENSEN 1979) which is pollinated by bats (HEITHAUS & al. 1974). ARROYO (1981) stated that sticky pollen in species of *Elizabetha*, *Eperua*, and *Jacqueshuberia* may be regarded as highly adaptive to pollen transfer by bats.

The asynchronous opening of one to two flowers of *T. ovalis* in each inflorescence per night, the slight dichogamy due to the delay of the stigma deflection in relation to open anthers (JANZEN 1968), and the flowering over a long period, probably, decrease the chance of self-pollination.

Among the bat visitors of flowers of *T. ovalis*, the long-tongued *Glossophaga soricina* is one of the best known anthophilous bat, and its behaviour in this passionflower is similar to that recorded from several other neotropical chiropterophilous plants (VOGEL 1969, SAZIMA & SAZIMA 1978, SAZIMA & al. 1982, DOBAT & PEIKERT-HOLLE 1985). The *Carollinae* *Rhinophylla pumilio* is regarded as a frugivorous bat (REIS 1984) but is able to seek nectar in flowers (present study) in a way similar to another carollinae bat, *Carollia perspicillata* (SAZIMA & SAZIMA 1975, 1978; FLEMING 1982). The visits of *R. pumilio* to the flowers of *T. ovalis* may be due to a decrease of fruit availability at the studied area during the dry period, the nectar thus being a supplementary food source (SAZIMA & SAZIMA 1975).

In agreement with the current concepts of angiosperm evolution, KILLIP (1938) and BENSON & al. (1976) regard *Tetrastylis* as a genus more primitive than *Passiflora* within the *Passifloraceae*. Due to some attributes presented in the flowers of *T. ovalis*, which seem more adjusted to the chiropterophilous syndrome than those found in the flowers of *P. mucronata*, we regard the former as more specialized to bat-pollination than the latter. These floral differences between *T. ovalis* and *P. mucronata*, and the supposed primitiveness of *Tetrastylis* let us suggest that chiropterophily probably evolved independently in these two genera of passionflowers.

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References

- ARROYO, M. T. K., 1981: Breeding systems and pollination biology in *Leguminosae*. — In POLHILL, R. M., RAVEN, P. H., (Eds.): *Advances in legumes systematics*, 2, pp. 723–769. — Kew, England: Royal Botanic Gardens.
- BAKER, H. G., 1961: The adaptation of flowering plants to nocturnal and crepuscular pollinators. — *Quart. Rev. Biol.* **16**: 64–73.
- 1973: Evolutionary relationships between flowering plants and animals in American and tropical forests. — In MEGGERS, B. J., AYENSU, E. S., DUCKWORTH, W. D., (Eds.): *Tropical forest ecosystems in Africa and South America: a comparative review*, pp. 145–159. — Washington: Smithsonian Institution Press.
- BENSON, W. W., BROWN, JR., K. S., GILBERT, L. E., 1976: Coevolution of plants and herbivores: passion flower butterflies. — *Evolution* **29**: 659–680.
- CRUDEN, R. W., JENSEN, K. G., 1979: Viscin threads, pollination efficiency and low pollen-ovule ratios. — *Amer. J. Bot.* **66**: 875–879.
- DOBAT, K., PEIKERT-HOLLE, T., 1985: Blüten und Fledermäuse (Chiropterophilie). — Frankfurt am Main: W. Kramer.
- FÆGRI, K., VAN DER PIJL, L., 1980: *The principles of pollination ecology*. 3rd edn. — New York: Pergamon Press.
- FLEMING, T. H., 1982: Foraging strategies of plant-visiting bats. — In KUNZ, T. H., (Ed.): *Ecology of bats*, pp. 287–325. — New York, London: Plenum Press.
- HEITHAUS, E. R., OPLER, P. A., BAKER, H. G., 1974: Bat activity and pollination of *Bauhinia pauletia*: plant-pollinator coevolution. — *Ecology* **55**: 412–419.
- HOWELL, D. J., 1977: Time sharing and body partitioning in bat-plant pollination systems. — *Nature* **270**: 509–510.
- JANZEN, D. H., 1968: Reproductive behavior in the *Passifloraceae* and some of its pollinators in Central America. — *Behaviour* **32**: 33–48.
- KILLIP, E. P., 1938: The American species of *Passifloraceae*. — *Field. Mus. Nat. Hist.* **19**: 335–613.
- KNUTH, P., 1904: *Handbuch der Blütenbiologie*. III — Leipzig: W. Engelmann.
- PEIXOTO, A. L., GENTRY, A., 1990: Diversidade e composição florística da mata de tabuleiro na Reserva Florestal de Linhares (Espírito Santo, Brasil). — *Revta. Brasil. Bot.* **13**: 19–25.
- RADFORD, A. E., DICKSON, W. C., MASSEY, J. R., BELL, C. R., 1974: *Vascular plant systematics*. — New York: Harber & Row.
- REIS, N. R., 1984: Estrutura de comunidade de morcegos na região de Manaus, Amazonas. — *Rev. Brasil. Biol.* **44**: 247–254.
- SAZIMA, M., SAZIMA, I., 1975: Quiropterofilia em *Lafloensia pacari* St. HIL. (*Lythraceae*), na Serra do Cipó, Minas Gerais. — *Ci. Cul.* **27**: 405–416.
- — 1978: Bat pollination of the passion flower, *Passiflora mucronata*, in southeastern Brazil. — *Biotropica* **10**: 100–109.

- 1980: Bat visits to *Marcgravia myriostigma* TR. et PLANCH. (*Marcgraviaceae*) in south-eastern Brazil. – *Flora* **169**: 84–88.
- FABIAN, M. E., SAZIMA, I., 1982: Polinização de *Luehea speciosa* (*Tiliaceae*) por *Glossophaga soricina* (*Chiroptera, Phyllostomidae*). – *Rev. Brasil. Biol.* **42**: 505–513.
- VOGEL, S., 1958: Fledermausblumen in Südamerika. – *Österr. Bot. Z.* **104**: 491–530.
- 1969: Chiropterophilie in der neotropischen Flora. Neue Mitteilungen III. – *Flora Abt. B.* **158**: 289–323.
- ZEISLER, M., 1938: Über die Abgrenzung der eigentlichen Narbenfläche mit Hilfe von Reaktionen. – *Beih. Bot. Zbl.* **58**: 308–318.

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