

**POLLINATION BIOLOGY OF A COLOMBIAN AMAZON LILY,
Eucharis caucana (AMARYLLIDACEAE)**

The Cauca Valley comprises 400,000 ha of mostly flat terrain lying between the Western and Central Cordilleras of the Andes in western Colombia. It formerly was covered by forests (partly seasonally inundated), bamboo groves, marshes, and lagoons. The original vegetation has now mostly been replaced by extensive sugar cane plantations. Only a small number of forest patches remains; none of these covers more than 25 ha. The original forest-adapted fauna and flora of the Cauca Valley now survive only in these forest fragments.

Since 1986, personnel of the Universidad del Valle have carried out studies of the biota of these tropical dry forests. In 1987, the author and colleagues discovered a new species of Amaryllidaceae in one of these forests (El Medio); this species, *Eucharis caucana*, was described by Alan Meerow (1989). This species now is known from only four populations and probably is endemic to the Cauca Valley and adjacent piedmont of the Andes. The cause of its rarity is the destruction of its habitat.

The wild population of *Eucharis caucana* at the type locality of El Medio flowers twice a year, but individual plants usually flower no more than once a year. Peak anthesis of the population coincides with peak rainfall (see Chapter 2 of this book).

Very little is known about the pollination ecology of the genus *Eucharis*, except a few reports of visitation by euglossine bees (Vogel, 1963; Zucchi et al., 1969).

Pollinators reported in genera of Amaryllidaceae other than *Eucharis* are birds (Nectariniidae, Trochilidae), bats, bees (*Andrena*, *Anthophora*, *Apis*, *Proxycopa*), butterflies, hawkmoths, and syrphid flies (Ford et al., 1979;

Dafni & Werker, 1982; Grant, 1983; Howell & Prakash, 1990; Johnson & Bond, 1994; Arroyo & Dafni, 1995; Herrera, 1995; Alan Meerow, pers. comm.). Most of these studies were done in extratropical regions.

The purpose of this study was to expand knowledge of the pollination biology of tropical species of the family Amaryllidaceae and to elucidate one facet of plant-pollinator relationships of native forests of the Cauca Valley before this remnant ecosystem disappears entirely.

This study was carried out at the type locality of *E. caucana*, in a remnant secondary forest of 12.5 ha at the Hacienda El Medio in the Municipio de Zarzal, Departamento del Valle del Cauca, Colombia (4°20'07"N, 76°04'52"W), at 950 m elevation. Mean annual precipitation is 1316 mm and mean annual temperature is about 23° C. There are two rainy seasons (March to May and September to November) and two dry seasons. In the Holdridge (1967) system, this zone would be placed approximately in the Tropical Dry Forest formation, but proximity to the Río La Paila (120 m distant) causes a high water table, so that the forest floristically and physiognomically resembles Tropical Moist Forest, with a very low percentage of deciduous trees. This forest formerly was a cacao grove which was abandoned in the 1930's. It is dominated by giant *caracolí* trees, *Anacardium excelsum* (Kunth) Skeels, Anacardiaceae, which attain 40 m height and 2 m dbh.

Field and laboratory observations of *E. caucana* were made from 1989 to 1998; 324 wild plants at El Medio were labeled. Insect visitors to wild plants were observed for 276 h at El Medio from 16 September 1989 to 21 September 1991. Voucher specimens of insect visitors were deposited in the collection of the Departamento de Biología of the Universidad del Valle; additional voucher specimens of Syrphidae were deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D. C., U. S. A.

Breeding experiments were carried out with wild plants at El Medio and with cultivated plants in Cali. In all breeding experiments, flowers were individually labeled and inflorescences were enclosed before anthesis with fine-mesh bags. In flowers tested for agamospermy, anthers were removed shortly before onset of anthesis. Anthers also were removed from flowers used for hand-pollination in cross- and self-pollination tests; some of these flowers were pollinated before stigma enlargement and others after stigma enlargement. Anthers were not removed from flowers tested for autogamy without intervention. In three experiments to test whether *Plebeia* sp. nov. (Hymenoptera: Apidae: Meliponinae) can pollinate *E. caucana*, the inflorescence of a wild plant was bagged before anthesis. At the onset of anthesis, the bag was removed, and only *Plebeia* sp. nov. were allowed to visit the flower, for 80-103 min; all other visitors were driven away. The flower was then emasculated, the other flowers in bud were removed, and the inflorescence was rebagged.

To measure duration of pollen viability, six anthers from a single flower were removed and placed in a dry sterile vial. Each day during the following six days, one of these anthers was rubbed on a slide; a few drops of a solution of boric acid, glucose, magnesium sulfate, and potassium nitrate were added, and a cover slip was applied. The slide was placed in a covered Petri dish lined with wet filter paper and was examined for pollen germination under the microscope after 24 h.

To test stigma receptivity, stigmas were excised 24, 48, and 72 h after pollination, placed in Alexander's stain (Alexander, 1969; Snow & Roubik, 1987) 75-90 min, and observed with a dissecting microscope.

Nectar samples were measured with Drummond micropipettes and immediately spotted onto Whatman #1 filter paper; analysis of sugar content was carried out by Ben-Erik van Wyk at Rand Afrikaans University in South Africa, using high pressure liquid chromatography.

Significance of differences in fruit set was tested by a Z test comparing two proportions (Zar, 1999).

Eucharis caucana is a herbaceous bulbiferous perennial that lacks an aerial stem. At the onset of the flowering season, it produces a single erect peduncle 21-79 cm tall, which is covered with a whitish, waxy bloom (not present on petioles); this may be a mechanism to protect nectar against ants, which slip when they attempt to climb the peduncles.

The white perianth is 6.5-8 cm long, with a curved tube and a patent limb. In wild plants, two to six flowers (mean four) are produced per plant. Flowers open sequentially, often with a one-day overlap between the last day of anthesis of the older flower and the first day of anthesis of the younger flower. Usually there are no more than two flowers open simultaneously, and when this occurs, one flower always is several days older than the other. The period of anthesis of each flower is five to six days. Flowers are protandrous; anthers usually dehisce shortly before anthesis, but at the beginning of anthesis, the stigma is small (ca. 1.5 mm diameter) and unreceptive. During anthesis, the stigma enlarges (to 4 mm diameter) and becomes receptive.

E. caucana is capable of reproducing asexually by offsets (short basal subterranean shoots); this and poor seed dispersal explain its clumped distribution at the type locality.

Some pollen of *E. caucana* remains viable for at least six days after anther dehiscence (germination in medium on slides from a one-day-old anther was 15%, and from a six-day-old anther from the same flower, 10%). Thus at least some of the pollen placed by an insect on an immature unreceptive stigma on the first day of anthesis can remain viable until the stigma becomes receptive, and then germinate.

Pollen on stigmas excised one and two days after pollination had not yet germinated, and the ovaries of the flowers from which these stigmas had

been excised aborted. Pollen on a stigma excised three days after pollination showed 25% germination, and the ovary of the flower from which this stigma was removed developed into a fruit. Thus the stigma becomes receptive on the third day of anthesis (that is, at about the midpoint of the five- to six-day period of anthesis); this coincides with noticeable enlargement of the stigma.

E. caucana, unlike most species of *Eucharis* (Meerow, 1989), is self-compatible (Table 3.1). (Another amaryllidaceous genus, *Zephyranthes*, also includes both self-compatible and self-incompatible species [Ghosh & Shivanna, 1984; Broyles & Wyatt, 1991].) Exclusion experiments in both cultivated and wild plants of *E. caucana* showed that this species is capable of reproducing, without hand-pollination and without pollinator visits, by autogamy (but with greatly reduced success compared with pollination by a vector). Agamospermy probably does not occur in this species (Table 3.1).

In experiments with cultivated plants (Table 3.1), there was no significant difference [Z test, Zar (1999)] in percentage of fruit-set between small- and large-stigma self-pollinations, small- and large-stigma cross-pollinations, total self- and cross-pollinations, and total small- and large-stigma pollinations. Offspring (F1 and F2) resulting from self-pollination are viable and fertile.

In cultivated plants, nectar was produced only during the first day of anthesis (during daylight or at night); volume per flower was 9-63.5 μ l (mean = 29 μ l, n = 21 flowers). Nectar sugar composition was analyzed from four samples from cultivated plants and two samples from wild plants. Ratios of fructose: glucose: sucrose were, in cultivated plants: 6:6:88, 7:6:87, 7:7:86, 8:8:84; in wild plants: 9:8:83, 14:13:73. Thus the ratio of fructose to glucose in all samples was about 1:1, and the mean sucrose: hexose ratio was 6.35 in cultivated plants and 3.79 in wild plants.

The perianth limb of *E. caucana* is open, and pollen is available to all visitors. Nectar, however, which is produced in septal nectaries (Meerow, 1989), is hidden within the perianth tube and is available only to long-tongued insects or to small insects able to crawl down to the base of the staminal cup and ingest nectar from the mouth of the perianth tube.

Flowers of *E. caucana* do not emit any perceptible odor, neither by day nor at night.

Flowers begin to open either in daylight or at night. Once a flower opens, it remains open continuously, day and night, until the end of the five- to six-day period of anthesis.

The only visitors observed during 18 h of nocturnal observation, including one entire night, were mosquitoes (Culicidae), which use the flowers as a perch. All other visitors observed are diurnal. Apart from predators and nectar thieves, 43 species of insects visited the flowers of *E. caucana* during 258 h of diurnal observation at El Medio (Table 3.2). The most common visitors were *Plebeia* sp. nov. (Hymenoptera: Apidae:

Meliponinae), *Copestylum chalybescens*, *C. tympanitis*, and *C. vagum* (Diptera: Syrphidae), and *Cyrsulus* sp. (Coleoptera: Chrysomelidae) (Fig. 3.1). *Apis mellifera* was seen only once and is not an important visitor. There were no vertebrate visitors.

Bees and flies visited flowers of *E. caucana* much more frequently than did beetles and butterflies (Table 3.3). There is a marked difference in peak visiting hours between syrphid flies and meliponine and halictid bees (Fig. 3.2). Syrphid flies sought only pollen; they never descended the staminal cup to seek nectar, although many species of syrphids (particularly males) are known to feed on nectar (Gilbert, 1986). In contrast, bees sought both pollen and nectar and often descended to the base of the staminal cup. Bees touched the stigmas with greater frequency than other insects except butterflies (but butterflies were infrequent visitors). When seeking nectar, *Plebeia* sp. nov. often descended a filament and followed the green longitudinal line that is aligned with each filament, along the staminal cup, until reaching the mouth of the perianth tube. This behavior suggests that these green lines function as nectar guides. Selective exclusion experiments showed that *Plebeia* sp. nov. can pollinate *E. caucana* (Table 3.1).

Hesperiid butterflies usually passed through two behavioral stages in their visits (Fig. 3.3). First, they perched on the border of the perianth limb, inclined their body forward, and inserted their tongue into the perianth. After (apparently) failing to reach the nectar by this method, they inserted their entire body into the perianth, with only the posterior portion of the wings protruding. While entering the perianth, they touched the anthers and stigma with their feet, folded wings, and abdomen.

Although *Eucharis caucana* is completely self-compatible, under natural conditions it probably frequently practices xenogamy. It possesses two mechanisms to block self-pollination: first, the flowers are protandrous, and by the time the stigma becomes enlarged and receptive, insect visitors probably will have removed most pollen from the anthers; second, the flowers open sequentially, with usually no more than a one-day overlap in anthesis between two successive flowers. In these cases of overlap, the older flower probably already will have been pollinated before pollen from the younger flower can be carried by an insect to the stigma of the older flower.

Self-compatibility in this species may act primarily as a “back-up mechanism,” in case xenogamy does not occur. Self-pollination, by means of insect vectors, could occur either by geitonogamy (deposition of pollen from the recently opened flower upon the enlarged stigma of the older flower, on the same plant, in its final day of anthesis) or by deposition on the small, immature stigma of pollen from anthers of the same flower, with subsequent stigma maturation and pollen germination. Experiments described above (pollen viability, stigma receptivity, small-stigma self-pollination [Table

3.1]) show that the latter is possible. *Plebeia* sp. nov., in its passage from one anther to another of the same flower, frequently touches the stigma. Specimens of *Plebeia* sp. nov. and of halictid bees collected while visiting flowers of *E. caucana* bore the distinctive large pollen grains (Meerow, 1989) of that species on the ventral surface of their abdomens; thus they could self-pollinate flowers at the small-stigma stage.

The fructose: glucose ratio of 1:1 is typical of Amaryllidaceae, but the very high sucrose: hexose ratio is not typical; it is known in Amaryllidaceae only in the genus *Hymenocallis* (Ben-Erik van Wyk, pers. comm.). *Hymenocallis* is pollinated by sphingid moths, and the strongly fragrant species of *Eucharis* subgenus *Heterocharis* probably also are pollinated by sphingids (Alan Meerow, pers. comm.).

Baker & Baker (1983) classified nectar according to the sucrose: hexose ratio (<0.1 = hexose-dominant, 0.1-0.499 = hexose-rich, 0.5-0.999 = sucrose-rich, ≥ 1.0 = sucrose-dominant). They found that flowers pollinated by short-tongued bees, flies, and bats tend to be hexose-rich or hexose-dominant, whereas flowers pollinated by long-tongued bees and hummingbirds, and long-tubed flowers pollinated by lepidopterans (including sphingids and skippers) tend to be sucrose-rich or sucrose-dominant.

The nectar of *Eucharis caucana* is extremely sucrose-dominant. Field observations indicate that at the type locality its flowers are not, at present, visited by hummingbirds nor nocturnal lepidopterans; they are visited by diurnal lepidopterans (mostly hesperiids [skippers]), but these are not common visitors. Flowers of *E. caucana* (which belongs to subgenus *Eucharis*) lack the strong fragrance typical of sphingid-pollinated species of *Eucharis* of the subgenus *Heterocharis*. Meerow (1989) noted that subgenus *Heterocharis* is the more primitive of the two subgenera.

I propose the following hypothesis for future investigation: *E. caucana* is descended from an ancestor whose flowers were strongly fragrant and sphingid-pollinated; it has lost strong fragrance and changed pollinators, but has retained nectar content typical of sphingid-pollinated flowers. Lack of strong fragrance (during the entire period of anthesis) suggests that sphingids are not now pollinators of *E. caucana*.

At present, *Eucharis caucana* employs what Baker (1961) called a "combined pollination system." No single species nor order acts as the sole pollinator. Instead, pollination may be carried out by species of all four orders of insects that visit the flowers; many species of all these orders contact both anthers and stigma during their visits.

Nevertheless, some species of insects (particularly the most common visitors) probably pollinate more frequently than do other visitors. I believe that *Plebeia* sp. nov. and the three most common species of *Copestylum* are the most important pollinators at the study site. Fly pollination in tropical forests is known in certain other understory species of plants and (at least

in some places) may be restricted to understory species (Henderson, 1986; Kress & Beach, 1994; Ervik & Feil, 1997).

In some species of other plant families, certain species of *Trigona*, s.l., have been shown to be nectar thieves rather than pollinators (Roubik, 1982; Mori & Boeke, 1987), although some species of *Trigona*, s.l., apparently do pollinate (Mori & Boeke, 1987). For *Eucharis caucana*, *Plebeia* sp. nov. (previously placed in *Trigona*) is a pollinator (Table 3.1).

To reach the nectar, butterflies must insert their entire bodies into the perianth. This behavior, which has been photographed in Californian *Datura* (Solanaceae) by Baker (1961), results from a plant strategy to force the insect to pollinate; the plant maintains its stigma-nectar distance longer than the tongue length of the pollinator, so that the insect may not obtain nectar from a distance while avoiding contact with the anthers and stigma (Nilsson, 1988; Goldblatt et al., 1995).

The noteworthy difference between peak visiting hours of syrphid flies and bees (Fig. 3.2) may be caused by selection for avoidance of agonistic encounters, to maximize pollen-gathering activity during visits. This temporal separation may serve the same function as the spatial separation of syrphids and bees found in the flowers of an Old World species of Amaryllidaceae, *Sternbergia clusiana* (Dafni & Werker, 1982). It also is possible that peak visiting hours are not linked to interspecific interactions, but are a response to some environmental parameter (such as optimal temperature for activity).

Several of the insect visitors to *E. caucana* are very rare or undescribed species. These pollinators, the plant they pollinate, and the whole ecosystem, with its complex biotic interactions, of which they form a part, are in grave danger of extinction. These species, persisting in small fragments of their original habitat, are doomed because of loss of dispersers, inadequate food resources, and reduced gene pools. Once they disappear, our entire knowledge of them will be based on a few studies such as this one.