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Reproductive Biology of *Erythrina falcata* (Fabaceae: Papilionoideae)[1]

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ABSTRACT

Reproductive phenology, floral biology, degree of self-incompatibility, and floral visitors of *Erythrina falcata* were studied in an Argentinean population. Flowering occurs during the dry season from late August to late October. Flower lifetime is 5–6 d. Phylogenetic studies indicate that *E. falcata*, together with *E. fusca* and *E. crista-galli*, are included in a basal clade within *Erythrina*. Its phylogenetic position, floral morphology, and nectar characteristics suggest a hummingbird–passerine mixed pollination system. The flowers are nontubular, and the vexillum (the upper petal of the corolla) covers the other remaining floral parts until displaced by a visiting passerine (*Icterus cayanensis*) or a hummingbird (*Amazilia chionogaster*). Both birds act as pollen vectors. Bees were observed as occasional pollinators. Nectar production begins at anther dehiscence and coincides with maximum stigmatic receptivity. The base of the keel forms a secondary nectar reservoir. Controlled pollinations showed that this species is self-incompatible, although a few fruits develop from selfing. Pollen:ovule ratio (43,200:7) is as expected for a xenogamous plant. Only 1 percent of the flowers set seeds under natural conditions. Possible explanations for the low reproductive success are discussed.

RESUMEN

La fenología reproductiva, la biología floral, el grado de auto-incompatibilidad y los visitantes florales de *Erythrina falcata* fueron estudiados en una población argentina. La floración ocurre durante la estación seca desde fines de Agosto hasta fines de Octubre. El tiempo de vida de las flores es de 5 a 6 días. Estudios filogenéticos indican que *E. falcata*, junto con *E. fusca* y *E. crista-galli*, están incluidos en un clado basal del género *Erythrina*. Su posición filogenética, la morfología floral y las características del nectar, sugieren un sistema de polinización mixto "colibrí-paseriforme." Las flores son no-tubulares y el vexilo (el pétalo superior de la corola) cubre las partes florales restantes hasta que es desplazado por un paserino (*Icterus cayanensis*) o un colibrí (*Amazilia chionogaster*). Ambas aves actúan como vectores de polen. También las abejas fueron observadas como polinizadores ocasionales. La producción de nectar comienza con la dehiscencia de las anteras y coincide con la máxima receptividad estigmática. La base de la quilla forma un reservorio secundario de néctar. Polinizaciones controladas mostraron que esta especie es auto-incompatible, aunque pocos frutos se desarrollan autógamicamente. La proporción Polen: óvulos (43.200:7) es la esperada para plantas xenógamas. Solo el 1 por ciento de las flores produjo semillas bajo condiciones naturales. Se discuten las posibles explicaciones del bajo éxito reproductivo observado.

Key words: Argentina; Erythrina falcata; *Fabaceae; floral visitors; phenology; phylogenetic hypothesis; pollination; reproductive biology; secondary nectar presentation; self-incompatibility.*

ALMOST ALL *ERYTHRINA* SPECIES (FABACEAE: PAPILIONOIDEAE, PHASEOLEAE) ARE BIRD POLLINATED (Raven 1974, 1977; Toledo 1974), although the genus belongs to the primarily entomophilous tribe Phaseoleae (Doyle & Doyle 1993). The plants of *Erythrina* species present typical features of the ornithophilous syndrome: odorless flowers, red or orange petals, copious nectar production, and diurnal anthesis (*e.g.,* Faegri & van der Pijl 1971). Cruden and Toledo (1977) recognized two main pollination syndromes in the genus: one involving perching birds and the other hovering birds. All of the 42 Old World species and 15 of the 70 New World species are pollinated by perching birds, whereas the remaining 55 species are hummingbird pollinated (Neill 1987). Toledo and Hernández (1979) and Neill (1987) described the characteristics of each pollination type. In the case of perching bird pollination, the inflorescence rachis is oriented horizontally, with flowers directed inward (toward the central axis of the tree), allowing birds to perch as they reach the nectar. The flowers are widely open, with the vexillum (or standard petal: the upper broad petal of the corolla) ovate or obovate, the wings (the two lateral petals of the corolla) and keel (or carine: the two lower fused petals) exserted from the calyx, the reproductive parts exposed, and mostly homogamous (*i.e.,* male and female functions take place at the same time, Faegri & van der Pijl 1971). Nectar is hexose-dominated, with high concentration of amino acids, copious, but low in sugar concentration (range: 5–16.5% sucrose equivalents; Baker & Baker 1982, 1990). In contrast, species adapted to hummingbird pollination have vertical inflorescences, with the flowers directed outward providing easy access for a hovering bird. These flowers are pseudo-tubular, with a narrow, folded vexillum, concealed reproductive parts, and are protandrous (Toledo & Hernández 1979, Neill 1987). Less nectar is produced than in species pollinated by passerines, but nectar concentration is higher (range: 25–38% sucrose equivalents); with respect to chemical composition, it is "sucrose-rich or -dominated" and has less concentrated amino acids (Baker & Baker 1982, 1990).

Bruneau (1997) suggested that shifts from passerine to hummingbird pollination have occurred several times within the genus, each event implying a switch not only in floral morphology, but also in floral nectar composition. In this context, species that do not fit into one of the two

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groups in every respect are of special evolutionary interest (Toledo & Hernández 1979). *Erythrina falcata* Benth is one such species. Phylogenetic studies of the genus indicate that *E. falcata*, together with *E. fusca* and *E. crista-galli*, is included in a basal clade characterized as pollinated by both passerines and hummingbirds (Bruneau 1996, 1997), based on field observations (for *E. fusca*, Feinsinger *et al.* 1979) or on nectar traits (Baker & Baker 1982, 1983, 1990). Thus, this species was considered from its nectar sugar concentration and sucrose/hexose ratio to be pollinated by passerines (Baker & Baker 1983, 1990), but the amino acid concentration of its nectar follows the pattern generally obtained for hummingbird-pollinated *Erythrina* species (Baker & Baker 1982). A recent study reports bees (*Apis mellifera* and *Xylocopa* sp.) as the main pollinators of *E. crista-galli* in Argentina and Uruguay (Galetto *et al.* 2000). These authors suggested that this basal clade of *Erythrina* experienced a shift from entomophily, prevalent in Phaseoleae, to ornithophily, more typical of *Erythrina*. They also note the necessity of further studies of pollination in this clade to test that hypothesis.

Erythrina falcata is a semi-deciduous tree, 10–20 m tall, and up to 1 m in diameter, with large, red flowers and brownish legumes (Digilio & Legname 1966). In cultivated trees, Burkart (1972) observed that flowering occurred 11 yr after sowing, with trees reaching 10 m height after 14 yr. It occurs naturally on humid slopes and borders of rivers and streams in Bolivia, Paraguay, northern Argentina, southern Brazil, and Peru (Burkart 1987). This species is of ornamental and medicinal interest (Martínez-Crovetto 1964, Burkart 1972) and its wood is used for the manufacture of handicrafts (Novara 1984).

As part of an ongoing study of the reproductive ecology of *E. falcata*, we (1) analyze its floral functional morphology and its floral biology, (2) estimate the degree of self-incompatibility, (3) describe its reproductive phenology, and (4) report floral visitors, their behavior, and their pollination role. The information obtained will be useful for a better understanding of the evolution of pollination biology of the genus, and of shifts in pollination mode within genera.

METHODS

Our field observations were made in a semi-deciduous forest near Vaqueros town (24°42′20″S, 65°25′10″W; 1288 m elevation), La Caldera Department, Salta Province, Argentina, during the flowering seasons of 1997 and 1998. The regional climate is seasonal, with 80 percent of rains (total: 1300 mm) concentrated in the period November–March (Bianchi & Yáñez 1992). Voucher specimens have been deposited in the Museo de Ciencias Naturales de la Universidad Nacional de Salta (MCNS). For the purposes of this paper, the first day after the flower opens is considered as D 1, and so on.

Flower and fruit production was estimated on ten selected trees. In order to estimate flower production we counted the total branch number per tree, and the inflorescences number per branch and flower number per inflorescence for a random sample of 20 branches. Then, the total number of flowers per plant was calculated. The same procedure was carried out to quantify production of fruits. Seeds per fruit were estimated on ten selected fruits per tree ($N = 10$ trees).

Flower development and phenology were monitored in September 1997 on ten other individuals, each 10–15 m height. On each plant we marked 20 flower buds of similar length, which we subsequently observed twice a day (0800 and 1800 h) until the flowers senesced, monitoring pollen presentation and stigmatic receptivity. Stigmatic receptivity was estimated through peroxidase activity, using a solution of 3 percent hydrogen peroxide (Kearns & Inouye 1993). For morphometric analysis of the entire floral cycle, we marked 20 flower buds of similar length on the same trees. Ten flowers from different trees (one from each tree) were removed in each sample, twice a day (0800 and 1800 h). We measured length and width of corolla segments (vexilum, wings, and keel petals), filaments and anthers of abaxial antisepalous (outer) and antipetalous (inner) stamens, distance from both series of anthers to the stigma, and distance from both the longest anther and the stigma to the tip of the keel petals.

Nectar availability (standing crop) was evaluated by measuring the amount of accumulated nectar from individual flowers that have been exposed to visitors (*i.e.,* unprotected open flowers). Data were collected from 10–15 randomly sampled flowers of the same age (anthesis stage: D 1); 2–3 flowers were sampled from five trees every 2 h (from 0800 to 2000 h). Each flower was measured only once. We recorded volume of nectar using graduated microcapillary tubes and sugar concentration using a hand-refractometer (Atago, Japan). Nectar was extracted without removing the flowers from the tree and avoiding damage to the nectaries. Nectar volume data were compared among hours using one-way ANOVA (Zar 1999). Homogeneity of variances was verified by Levene's test. Kruskal–Wallis test was used to compare sugar concentration among hours (Zar 1999) because of heterogeneity of variances even after data transformations. Subsequent to a Kruskal–Wallis test, we made nonparametric multiple comparisons for tied ranks and unequal sample sizes (Zar 1999).

Pollen and ovule numbers were estimated from ten randomly selected flower buds. All ten anthers from a single flower were softened in 1 N HCl for 12 h, transferred to a known volume of lactic acid:glycerin (3:1) in a test tube, and macerated with a glass rod. In order to homogenize the mixture we used a vortex, and a sample of known volume was placed in a hemocytometer where pollen grains were counted; this value was used to estimate the total number of grains per flower. Ovule number was directly obtained from dissections of ovaries under a stereoscopic microscope.

To study flower anatomy, flowers were fixed in FAA, dehydrated through an alcohol/xylol series, and embedded in paraffin. Sections were stained with safranin-fast green. To determine the location of stomata, nectary tissue was cleared with NaOH (10%) and stained with I_2–IK solution. Drawings were made with a camera lucida.

The assessment of the breeding system involved ten trees in the Vaqueros population, during September 1998. We performed the following treatments: (a) natural pollination, in which flowers were not manipulated; (b) autogamous self-pollination, in which buds were bagged throughout their flowering period; (c) hand self-pollination, in which bagged flowers were hand pollinated with their own pollen; (d) apomixis, in which anthers and stigma of buds were clipped; and (e) hand cross-pollination, in which emasculated flowers were pollinated with pollen from another tree, at least 20 m away from the recipient tree. To compare fruit set among hand-selfed and hand-crossed treatments, and among open pollinated and hand-crossed treatments, we used chi-squared analysis (Zar 1999). An indirect measure of self-incompatibility was obtained

by dividing the average fruit set after self-pollination by the average fruit set after cross-pollination (index of self-incompatibility, ISI, Lloyd & Schoen 1992). A value of one indicates complete self-compatibility.

Observations of flower visitors were made at 20 trees in both the 1997 and 1998 flowering seasons. Total observation time was 62 h, which included 15 min observation periods at all hours of the day, from dawn through dusk (0700 to 2000 h). Before each observation, we counted the number of available flowers on the tree. For each visit, we recorded the number of visited flowers, the duration of each visit, direction of ingress and egress, contact with the reproductive parts, and interactions with other visitors. We compared the number of probed flowers per foraging trip among birds using a non-parametric Mann Whitney *U* test (Zar 1999). The duration of flower visits among legitimate visits by *Amazilia chionogaster*, and legitimate and illegitimate visits by *Icterus cayanensis* was analyzed using Kruskal–Wallis non-parametric multiple comparisons (Zar 1999) because of the high heterogeneity of variances even after data transformations. A visitor was considered to be territorial if it remained in the immediate vicinity of a tree during the observation period and attempted to prevent other visitors of the same species from visiting by threatening or attacking them (Stiles & Wolf 1970). In case of species that forage in groups, we observed one individual to record its activity. As it had been suggested that *Erythrina fusca* was bat-pollinated (Helversen, cited in Raven 1977), we spent one evening (from 2000 to 0100 h) watching for possible nocturnal visitors. Additionally, we placed two mist nets (12.5 m long × 2.8 m high, 36 mm mesh) near the most visited trees.

The phenology of *E. falcata* was observed weekly for 20 plants in the Vaqueros population from August 1997 to January 1998. Additional data were collected until May 1998. In general terms, we followed the methods proposed by Newstrom *et al.* (1994). Phenological events were registered on four quadrants (north, south, east, and west) using binoculars. The amplitude scale had four classes: none, light, medium, and heavy flowering (fruiting or seed dispersal) with respect to the typical crop of flowers for each tree (based on field observations carried out the previous year). For each observation date we obtained the median from the four quadrants for each tree.

FIGURE 1. Inflorescences of *E. falcata*. (a) Note the pendulous flowering shoots and the inverted position of the flowers. Arrow: One triad (short-shoot with three flowers, see text). (b) Detail of the flowers in one inflorescence; they were opened manually by separating the margins of the vexillum to show the inner parts.

RESULTS

FLOWER AND INFLORESCENCE MORPHOLOGY.—At the time of flowering, axillary buds on shoots produced the preceding year develop into short branches that produce two lateral, leafless inflorescences ("pseudoracemes" *sensu* Tucker 1987), (Figs. 1a and b) from the axils of the prophylls. The terminal meristems of the short branches produce vegetative shoots after flowering. The inflorescences bear triads (*i.e.,* short-shoots with three flowers, see Fig. 1a, arrow) that grow acropetally. The plants produce 7894 ± 1777 (range: 1058–18,072, $N = 10$) inflorescences. Mean number of flowers produced per inflorescence is 26.96 ± 0.08, range = 2–84, $N = 1157$).

Initially the flowering shoots are erect, but over the course of the inflorescence's growth, they become pendulous due to the increasing weight of the flowers; as a consequence, the flowers become inverted in the sense of Faegri and van der Pijl (1971) (see Fig. 1a). Plants produce a mean of 219,680 ± 48,464 flowers (range: 30,935–450,479, $N = 10$), with a mean density of 2436 flowers/m^2 of the plant's surface over the entire flowering period.

Flowers of *E. falcata* last 5–6 d. They are odorless, zygomorphic, with the wing and keel petals on the upper side and the vexillum below (Fig. 2a). The calyx is chartaceous and campanulate (Figs. 2a and b). It constitutes a strong structure that encloses the base of all the petals tightly, perhaps protecting against disarticulation during visits. The vexillum is fleshy, red, with an orbicular-elliptic form (Fig. 2c). The width and length of this petal are almost equivalent (35.51 ± 0.37 mm, and 36.00 ± 0.60 mm, respectively, $N = 30$). The wings (Fig. 2d, arrow) are pale-green colored, small (1/3 the size of the vexillum) and ovate, 10.43 ± 0.36 mm long and 5.60 ± 0.24 mm broad. The keel petals (Figs. 2d and k) are broadly falcate, reddish, stiff, 31.49 ± 0.53 mm long and

10.23 ± 0.23 mm broad. There is a swelling in the base of the keel that forms a secondary nectar reservoir.

Over the course of this study, the flowers remained closed during the entire floral cycle, that is, the vexillum remains folded, covering the other floral parts (Fig. 2b). Only by action of the visitors (see below) can the flowers be opened. Anthesis was defined as beginning when stamens and stigma extend beyond the keel (by elongation of the filaments, ovary, and style during floral development) to become exposed to visitors that are able to open the flowers, as in Figure 2d.

The androecium, composed of nine fused and one free stamen (Figs. 2g and f), have alternating long (outer) and short (inner) filaments with a separation of *ca* 4 mm between the cycles during the entire floral lifetime. This feature results in a larger contact surface with the visitor. The anthers, dehiscent on D 1, face downward, facilitating a nototribic deposition of pollen. The filaments of both cycles grow from 4.17 ± 0.38 and 7.41 ± 0.22 mm (4 d before anthesis) to 9.27 ± 0.30 and 12.59 ± 0.35 mm, respectively, 2 d after anthesis. The inner anthers reach the level of the stigma 1 d before anthesis and remain in this position until floral senescence (D 6). We did not find any morphological differences between the anthers and the pollen of the two whorls of stamens. Anthers produced 43,199.97 ± 6366.45 pollen grains. Flowers produced a mean of 7.40 ± 0.22 ovules, giving a pollen-ovule ratio of 5909.02 ± 1123.00.

The ovary is pubescent and, together with the style, forms a curve that follows the keel shape (Fig. 2f, arrow). The stigma is terminal, and "wet" (*sensu* Heslop Harrison & Shivanna 1977), that is, with a free-flowing secretion. Stigmas of mature flower buds react faintly with hydrogen peroxide, whereas activity reaches a maximum on D 1, coinciding with anther dehiscence and indicating that *E. falcata* is homogamous.

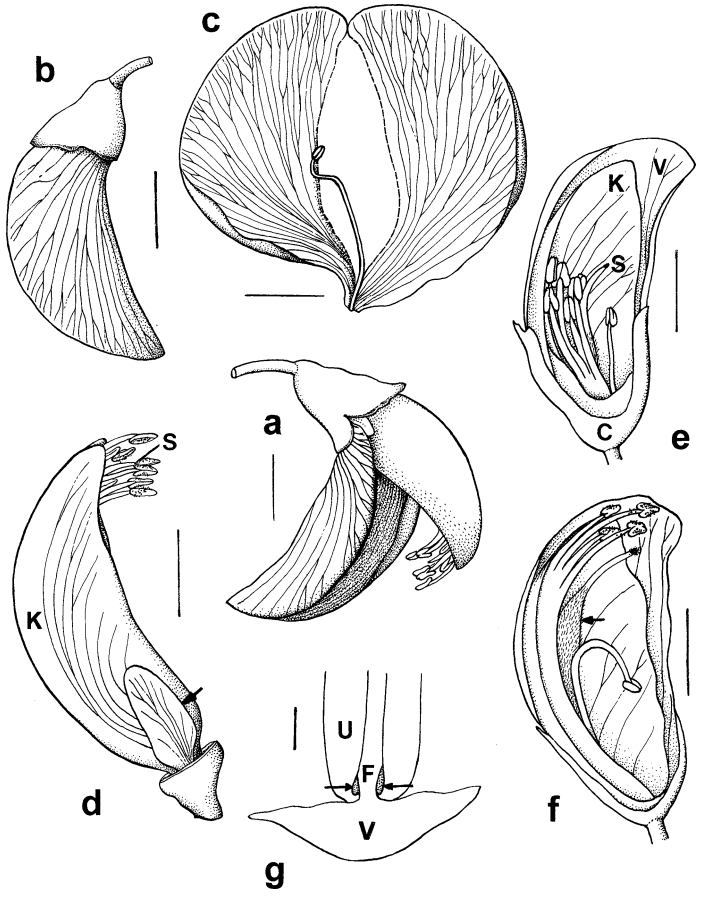

FIGURE 2. Floral morphology of *E. falcata*. (a) Open flower, shown inverted as in nature (D 1). (b) Closed flower (D 1). (c) Vexillum (split in order to show its shape) and free stamen that is adnate to its base. (d) D 1 flower, vexillum removed. Note that anthers and stigma (s) project from the keel (k). Note the reduced size of the wings (arrow). (e) Bud at pre-anthesis in longisection (96 h before anthesis) showing androecium and gynoecium enclosed by the keel (k). Note that the stigma (s) is longer than the stamens. The vexillum (v) covers all other floral parts; c: calyx. (f) Bud initiating anthesis (D 1) in longisection, showing anther dehiscence. The ovary is shown by an arrow. Note that the anthers and stigma project from the keel. (g) Base of androecium from above; the two openings to the nectar chamber between the free stamen (f) are indicated by arrows; u = united stamens; v = base of vexillum. Bar = 1 cm, except for d (Bar = 5 mm) and g (Bar = 1 mm).

NECTAR AND NECTARY.—Nectar is secreted from the nectary situated around the base of the ovary. The nectary is attached to the receptacle and has the form of a ring, divided at the apex into ten short lobes. We observed open stomata related to these lobes. Anatomically, the external part is composed of 10–13 layers of densely staining secretory cells, whereas the internal part is composed of 20–25 parenchymatous layers with larger and less stained cells. In longitudinal sections, vascular bundles were evident, having both phloem and xylem.

Nectar accumulates within the nectar chamber formed by the staminal furrow, and overflows from this chamber into the keel. Nectar is retained by the keel, but part of it is drained to the tip of the closed vexillum.

Analysis of standing crop data (Table 1) shows that D 1 flowers offer copious volumes of nectar with low sugar concentration. Nectar volumes obtained throughout the day were similar (ANOVA: $F_{6,84} = 0.085$, $P = 0.100$). Sugar concentration differed among samples taken throughout the day (Kruskal–Wallis test: $H = 22.09$, df = 6, $N = 91$, $P = 0.001$). Samples taken in the early morning (0800 h) and late afternoon (2000 h) had significantly higher sugar concentration than those taken in the early afternoon (1400 h) ($P < 0.05$).

TABLE 1. *Standing crop of nectar in D 1 flowers of* Erythrina falcata *exposed to foraging birds at Vaqueros (Salta Province, Argentina).*

Time	No. of flowers	Nectar volume (μl)	Concentration (percent of sucrose equivalents w/w)
0800	13	81.54 ± 15.64[a]	17.23 ± 1.16[a]
1000	14	80.00 ± 17.42[a]	14.21 ± 11.97[ab]
1200	14	85.71 ± 17.47[a]	14.50 ± 7.93[ab]
1400	15	83.33 ± 16.14[a]	10.73 ± 7.98[bc]
1600	15	80.00 ± 14.61[a]	12.53 ± 9.55[ab]
1800	10	81.50 ± 16.98[a]	16.30 ± 3.11[ab]
2000	10	95.00 ± 17.42[a]	17.61 ± 0.50[a]

Numbers with different letters differ significantly at the 5 percent level. Values are means ± 1 *SE*.

TABLE 2. *Reproductive mechanism in* Erythrina falcata. *Flowers from ten trees were used in all treatments.*

Treatment	Number of flowers	Number of fruits	% Fruit set
Apomixis	187	0	0
Autogamous self-pollination	1450	2	0.1
Natural pollination	2196	26	1.2
Hand self-pollination	178	6	3.4
Hand cross-pollination	60	12	20.0

FRUIT AND SEED PRODUCTION.—The fruits of *E. falcata* are coriaceous brown pods that dehisce along their two margins at maturity, although some trees at our study site presented indehiscent pods. They have a mean of 1.12 brownish seeds (range 1–7 seeds). Under natural conditions, only 20 percent of the available ovules produced seeds and the observed fruit/flower ratio was also low (mean = 1.26 fruits/100 flowers, range: 0–7, $N = 10$ trees), that is, the most productive tree had seven fruits per 100 flowers.

Controlled pollinations.—The results from the controlled pollinations are summarized in Table 2. Flowers used to test for apomixis did not set fruit. Fruit set differed significantly between the hand-selfed and the hand-crossed treatments ($\chi^2 = 17.75$, $P < 0.0001$), and between the open pollinated and the hand-crossed treatments ($\chi^2 = 124.86$, $P < 0.0001$). With an ISI value of 0.15 (Lloyd & Schoen 1992), *E. falcata* is basically self-incompatible, although a few fruits developed from selfing. Such fruits were smaller than the fruits produced by open pollinated flowers and from hand-crossed flowers, and most aborted early in development.

FLORAL VISITORS.—Most visits (80%) occurred in the morning and early afternoon. Birds of two species were observed as follows.

Icterus cayanensis (epaulet oriole, Icteridae): These birds approach the unopened mature flower of *E. falcata* using the rachis of the inflorescence or a proximate branch as a perch. From the rachis, the bird inclines its head downward, performing acrobatic movements. At this point, the oriole may visit legitimately by placing its bill into the commisure formed by the lateral margins of the vexillum and opening the bill when it is fully inserted. This has been referred to as the "bill scissoring" technique and is reported for several oriole species including *Icterus spurius* on *E. fusca* (Morton 1979). This causes the vexillum to rise backward (see Fig. 1b), exposing the stamens and stigma. The bird collects nectar from the tip of the vexillum and keel, contacting the reproductive parts with its bill. Orioles may also rob flowers of *E. falcata* by cutting the vexillum (through the central vein) of closed flowers and taking the nectar from between the free margins of the keel, without contacting the reproductive parts.

Amazilia chionogaster (white-bellied hummingbird, Trochilidae): These birds exploit the nectar in a legitimate way, foraging from flowers already opened by *I. cayanensis*. We also noted that this hummingbird was able to open the flowers while flying, by means of reiterated strikes on the vexillum with the bill, which is 22 mm in length. After opening the flower, the bird introduces its head between the vexillum and the reproductive parts, accessing nectar through the open margins of the keel. During these movements, the head contacts the reproductive parts of the flower (Fig. 3). One netted individual of *A. chionogaster* confirmed our observations, revealing that it carried *ca* 100 *E. falcata* pollen grains on its forehead and crown (picked up with transparent tape, Dafni 1992). *Amazilia chionogaster* also forages on the other co-flowering species of the community (*e.g., Tillandsia pulchella*, Bromeliaceae; *Tecoma stans*, Bignoniaceae; *Cestrum parqui*, Solanaceae). *Chlorostilbon auroventris* (Glittering-bellied emerald, Trochilidae) was also a frequent visitor to flowers of these other species, but was never seen drinking nectar from *E. falcata*.

FIGURE 3. A white-bellied hummingbird (*Amazilia chionogaster*, Trochilidae) visiting a flower of *E. falcata*. Note that this bird is visiting legitimately, contacting the reproductive parts with its head.

The social behavior of the observed birds was different. Whereas *I. cayanensis* always travel and forage in groups, *A. chionogaster* was always solitary, showing territoriality. We did not observe aggressive interactions between the orioles and the hummingbirds.

Based on pooled 1997 and 1998 data, the most frequent visitor was *I. cayanensis* (Table 3). Considering the number of probed flowers per foraging trip, *A. chionogaster* visited more than twice as many flowers as *I. cayanensis* (Table 3), although this difference was not statistically significant (Mann Whitney test: $U = 1.56$, $N = 43$, $P = 0.12$). Analyzing the data for individual visits, we found that *A. chionogaster* probed a wide range of flowers in each trip, from # 1 to 76. Both birds visited less than 1 percent of the available flowers per plant per bout. The duration of flower visits differed among legitimate visits by *A. chionogaster*, and legitimate and illegitimate visits by *I. cayanensis* (see Table 3) (Kruskal–Wallis test: $H = 6.20$, df = 2, $N = 23$, $P = 0.045$). In the case of *I. cayanensis*, robbing visits lasted twice as long as legitimate visits.

TABLE 3. *Visits to the* Erythrina falcata *population (total of 62 h of field observations) by bird species and manner of visits.*

Variable	Icterus cayanensis	Amazilia chionogaster
Number of recorded visits	134 (77%)	40 (23%)
Mean number of probed flowers per foraging trip (*N*)	8.03 ± 1.10 (22)	19.86 ± 4.61 (21)
Mean duration of flower visits in seconds (*N*)	L = 8.00 ± 1.71 (8) R = 16.45 ± 4.04 (6)	L = 5.42 ± 1.13 (9)
Percent of available flowers visited	0.21%	0.53%

L: legitimate; R: robbing.

Apis mellifera and *Xylocopa* sp. were occasionally seen foraging for nectar on two of the 20 observed trees. When these insects visited the flowers, they touched the anthers and the stigma on their way toward the nectary. However, bees were unable to open the flowers, restricting their action to those already opened by birds.

In the studied population, predation by three parrot species (Psittacidae) was observed on flowers or fruits of *E. falcata. Aratinga mitrata* (mitred conure) (more than 2 individuals/h) and *A. acuticaudata* (blue-crowned parakeet) (1–2 individuals/h) plucked the flowers with their bill or feet, then extracted nectar and dropped the flowers without consuming any floral part. *Amazona aestiva* (turquoise fronted parrot) (<1 individual/h) plucked the fruits (immature) with its bill and bit the pericarp to extract unripe seeds. Nocturnal observations provided no evidence for any visitors.

PHENOLOGY.—Individuals of *E. falcata* flowered synchronously. Nevertheless, we observed some differences among trees in onset of flowering, fruiting, and seed dispersal (Fig. 4). As a consequence, plants may have flowers and/or fruits in different phenological stages at the same time. Trees were leafless when blooming, as is typical for many *Erythrina* species (Standley 1922, cited in Morton 1979). Flowering starts in late winter (end of August) and finishes in late spring (end of October), with a peak in September (Fig. 4). This period coincides with the dry season in the study area. Mean duration of an individual's flowering period was 50 ± 6 d (range: 43–59 d, $N = 13$). Fruits begin to develop in September and reached maturity in November, when dispersal begins. Three trees did not reproduce and five others lost all their fruits before seed dispersal (Fig. 4). Following Newstrom *et al.* (1994), the phenology of *E. falcata* is described as annual (only one major flowering episode per year), and of intermediate duration (*ca* 2 mo).

DISCUSSION

Erythrina falcata has reproductive characters intermediate between the hummingbird and passerine type, as described by Cruden and Toledo (1977), Toledo and Hérnandez (1979), and Neill (1987). Typical of the hummingbird type, the flowers are directed outward. However, as expected for passerine pollinated flowers, the vexillum is broad and reproductive parts are exposed when the flower is open. With respect to nectar characteristics, the amounts secreted and the observed concentrations (14.73% ± 0.95) are typical of the passerine type (Baker & Baker 1982), although data on sucrose:hexose ratio and amino acid concentration are lacking. Our results show that this prediction is partially true, but also indicate hummingbird visitation of *E. falcata* flowers. Two other New World species, *E. fusca* and *E. poeppigiana* (Feinsinger *et al.* 1979), both visited by passerines and hummingbirds, showed the same pattern, with concentration values that correspond to the passerine type (13.4% and 15.5%, respectively). Bruneau (1997) suggested that whereas predicting pollination type from nectar chemistry seems accurate for most species in the genus, it may not be adequate for species that show intermediacy in pollination-related characters, such as *E. falcata.*

As was presented in the introduction, Galeto *et al.* (2000) hypothesized that the basal clade of the genus that includes *E. falcata* experienced a shift from entomophily to ornithophily, given that *E. crista-galli* is pollinated mainly by bees. Our results support this idea in part: even though *E. falcata* is pollinated by the hummingbird *A. chionogaster* and the passerine *I. cayanenesis*, bees (*A. mellifera* and *Xylocopa* sp.) were also observed visiting the flowers in a legitimate way.

Studies on pollination biology have led to the assumption that interactions between plants and animal pollinators are specialized as implied by suites of associated characters that delimit pollination syndromes (van der Pijl 1961). In a broad review, using comparisons across large floras, Waser *et al.* (1996) showed that there is no strong association with pollinator type as syndromes would predict, concluding that pollination systems are generalized. In the case of *Erythrina*, with red, odorless flowers and copius nectar, generally considered to be bird adapted (Proctor *et al.* 1996, Bruneau 1997), one species of its basal clade (*E. crista-galli*) is pollinated by birds and also by bees. Other evidence indicates that pollinators in the tropics tend to visit diverse genera and families (Feinsinger 1983, 1987; Waser 1983). *Amazilia chionogaster* was observed regularly visiting many species in different families, some of which do not have the characteristics of bird pollinated flowers (*e.g., Bahuinia candicans*, Fabaceae: Caesalpinoideae; *Cestrum parqui*, Solanaceae; Etcheverry, pers. obs.).

Our results showed that *E. falcata* is a predominantly self-incompatible species, with very low fruit and seed production under natural conditions. A number of factors may be responsible for low seed set in this species. Analyzing the floral features, the temporal and spatial overlap of receptive stigmas and dehiscent anthers on D 1 could lead to clogging of stigmas with incompatible self-pollen, reducing female fitness. Galen *et al.* (1989) observed that in self-incompatible *Polemonium viscosum* self-pollen deposition prior to outcrossing reduces compatible pollen germination by 32 percent and seed set by 40 percent. At the inflorescence level, the large display observed in this species represents a conflict, depicted by Harder and Barrett (1996); although attractiveness likely increases with the number of flowers open at one time, it could be costly in terms of lost mating opportunities if geitonogamy decreases outcrossed siring success by reducing pollen flow between plants. Additionally, the high nectar production might lead visitors to remain on the same tree for extended time periods (Stiles 1975). In *E. falcata*, the observed high number of flowers per tree and the large nectar production per flower could lead to the regular occurrence of pollen flow within trees. The foraging behaviors of *I. cayanensis* and *A. chionogaster* suggest that they have different consequences for the breeding system of *E. falcata*. Stiles (1981) suggested that, in the case of passerines that forage in

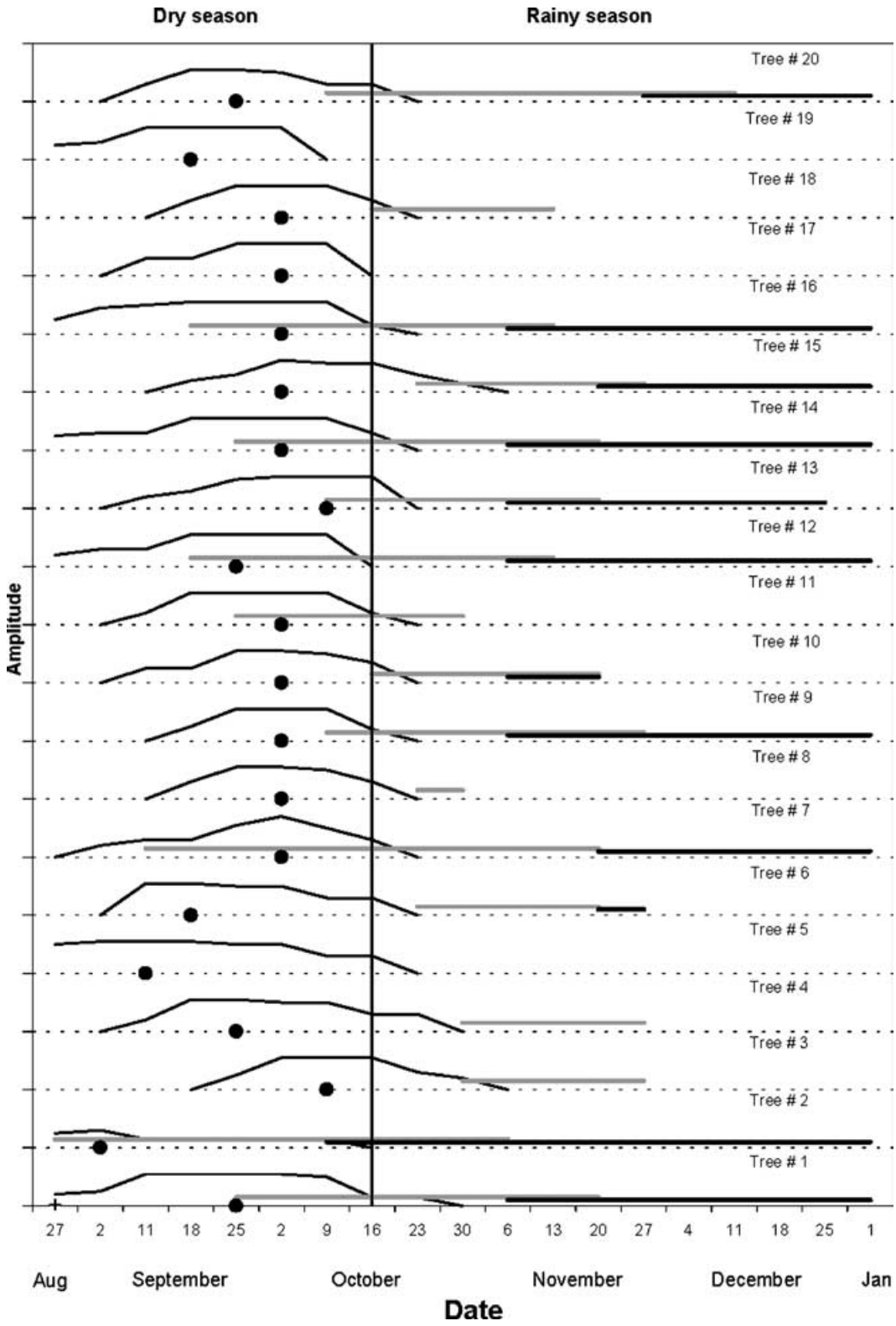

FIGURE 4. Flowering, fruiting, and seed dispersal periods for 20 trees of *E. falcata.* The phenological events were registered on four quadrants (north, south, east, and west) for each tree using binoculars. The amplitude scale had four classes: none, light, medium, and heavy flowering, fruiting or seed dispersal with respect to the typical crop of flowers for each tree. For each date, we obtained the median from the four quadrants. Dots indicate qualitative flowering peak. Narrow black lines indicate flowering; gray lines, developing fruits present; and thick black lines, seed dispersal (*i.e.,* fruits dehiscing). Dates of observation (each 7 d) are displayed on the X axis.

groups, nectar could be exhausted rapidly, and thus the group would have to move to another tree, thereby favoring cross-pollination. In contrast, because hummingbirds parcel the flowers of large trees into individual feeding territories, cross-pollination would be limited. Our observations would support this hypothesis for *E. falcata*, with *I. cayanensis* likely the more effective pollinator given that these birds probed fewer flowers per bout than *A. chionogaster*, although the observed difference was not statistically significant. This apparent advantage is, however, obscured by its dual behavior (legitimate vs. robbing). When *I. cayanensis* visits the flowers legitimately we suggest that contact between its bill (and/or head) and the reproductive parts is inevitable, although we have not captured orioles to show that they carried pollen. Many *Erythrina* species from the Americas are pollinated by passerines. For example, *I. spurius* is considered the primary pollinator of *E. fusca* (Morton 1979) and *E. breviflora* (Cruden & Toledo 1977); *E. olivae* is also visited by orioles (Toledo & Hernández 1979). Further studies concerning pollination effectiveness will be necessary to compare the efficacy of *I. cayanensis* and *A. chionogaster* as pollinators.

Floral and seed predation could be another reason for the observed low fruit and seed set of *E. falcata*. Parrots have been reported as consumers of immature seeds and also as nectar robbers from many species of *Erythrina.* For example, Skutch (1971) observed *Brotogeris jugularis* to be an important nectar robber of *Erythrina berteroana* in Costa Rica, with similar behavior to that observed for *A. mitrata* and *A. acuticaudata* in the present study. However, Cotton (2001) suggested that *Aratinga weddellii* and *Brotogeris cyanoptera* acted as pollinators of *E. fusca*.

At the Vaqueros population, we observed that the flowers of *E. falcata* are closed, that is, the vexillum remains folded, covering the remaining floral parts. Many other populations from Northern Argentina showed the same characteristic (Etcheverry & Trucco, pers. obs.). A similar situation was reported by Galetto *et al.* (2000) in some trees from two populations of *E. crista-galli*. Faegri and van der Pijl (1971: 28) noted that closed flowers are complicated structures that require both strength and agility by visitors to open them. The flowers of *E. falcata* are also inverted; in this position, the closed flower prevents nectar from draining out because the folded vexillum acts as a secondary nectar container. Schrottky (1908) observed that the inverted flowers of *E. crista-galli* may be alternatively viewed as a strategy to allow hummingbirds easy access to the nectar. A similar situation was described for *E. oliviae*, but in that species the keel and wing petals act as the secondary nectar container (Toledo & Hernández 1979). To our knowledge, *E. oliviae* and *E. falcata* are the only cases of secondary nectar presentation in Papilionoideae (*cf*., Faegri & van der Pijl 1971). The passerine bird *I. cayanensis* and the hummingbird *A. chionogaster* are both capable of opening *E. falcata* flowers in order to forage on nectar. In the case of *E. fusca*, which also presents closed flowers, the oriole *Icterus jamacaii* (and also some parrots) was able to open flowers, whereas hummingbirds relied on other visitors to open the flowers (Cotton 2001).

The floral nectary of *E. falcata* has the typical form of Papilionoideae (Waddle & Lersten 1973), with modified stomata through which nectar is secreted. Similar observations were reported for *E. crista-galli* (Galetto *et al.* 2000). Stomata commonly occur in floral nectaries of Fabaceae (Davis *et al.* 1988 and references therein).

Standing crop of nectar is mainly a function of two variables: nectar production and nectar removal by flower visitors (Pleasants & Zimmerman 1983). In the case of *E. falcata*, the large volumes observed throughout the day can be explained, at least in part, by the observed low visitation rate. Among American species of *Erythrina*, *E. falcata* appears to be among the highest in nectar production, together with *E. oliviae* (280 μl; pollinated by passerines, Toledo & Hernández 1979), and *E. fusca* and *E. poeppigiana* (135 μl and 80 μl, respectively; both pollinated by passerines and hummingbirds, Feinsinger *et al.* 1979). As Toledo and Hernández (1979) suggested for *E. oliviae*, this feature may be a consequence of the size of the flower, but may also be adaptive: flowering of *E. falcata* and *E. oliviae* occurs during the dry season, when other food sources are probably scarce for most flower-visiting birds. The large quantity of nectar provided by these flowers likely constitutes an important source of water for the birds, in addition to providing nutritional value. However, the flowering period of *E. falcata* overlaps with those of other species with which it shares visitors, suggesting that production of large volumes of nectar may be related to competition for pollinators (Feinsinger *et al.* 1991).

Like many species of *Erythrina* (*e.g., E. fusca*, Morton 1979; *E. oliviae*, Toledo & Hernández 1979), the flowering period of *E. falcata* is synchronous among individuals and coincident with the dry season. In contrast, some woody species of *Erythrina* flower during the rainy season (*e.g., E. crista-galli*, Galetto *et al.* 2000, and *E. breviflora*, Cruden & Toledo 1977), and others have asynchronous flowering (*E. poeppigiana*, Borchert 1980). These differences among species are not surprising given the extensive distributional range of the genus. Pollination of an individual's flowers may be influenced by its own pattern of flower production and/or by its synchrony with conspecifics (Augspurger 1983). The length of the flowering period may be a response to competition for pollinators (Bawa 1983). The long flowering period of individual plants of *E. falcata* may increase the chance of cross-pollination. Synchrony among individuals could also increase cross-pollination via attraction of pollinators (Augspurger 1983).

The pollen/ovule ratio of *E. falcata* is within the range that Cruden (1977) assigned to "xenogamy," and this is in agreement with the results of our controlled pollinations. Xenogamous species require a pollinator, and are dichogamous or self-incompatible (Cruden 1977). According to the ISI value, *E. falcata* can be considered as a mainly self-incompatible species. This is not the case with *E. crista-galli*, which seems to be facultatively xenogamous, although seeds produced from autogamy had the lowest mass and germination percentage (Galetto *et al.* 2000). Outbreeding is prevalent among woody perennials (Sutherland 1986) and, specifically, few neotropical trees are selfing (*e.g.,* Bawa 1974, Bawa *et al.* 1985). Kalin Arroyo (1981) and Neill (1988) documented both self-compatibility and self-incompatibility in *Erythrina*. In the present study, some fruits developed from autogamy (the inner anthers and stigma are adjacent to one another from the day before anthesis), and from hand self-pollination, but most of them aborted early.

CONCLUDING REMARKS.—Fabaceae are a morphologically variable group that exhibits a diversity of pollination systems. Knowledge of directional changes in pollination mode and breeding systems can be a valuable clue for reconstructing phylogenies (Kalin Arroyo 1981). The present study confirms predictions about the reproductive biology of *E. falcata* based on its phylogenetic position in the genus (Bruneau 1997), reinforcing the predictive value of the cladistic approach. The phylogenetic position and,

to some degree, floral morphology and nectar characteristics suggested a passerine–hummingbird pollination system (Bruneau 1997). Our results showed that both passerines and hummingbirds visit *E. falcata* flowers legitimately, confirming that the plesiomorphic state in the genus is a general ornithophilous system, from which more specialized passerine and hummingbird pollination modes have evolved. More detailed field studies and careful analysis of characters potentially associated with pollination systems in *Erythrina* species will be necessary for a complete understanding of these evolutionary shifts in pollination mode.

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