Generalised pollination system of *Erythrina dominguezii* (Fabaceae : Papilionoideae) involving hummingbirds, passerines and bees

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Abstract. Plants in the genus *Erythrina* are pollinated by birds, such that passerine pollination is the plesiomorphic state, whereas hummingbird pollination is the derived character. Phylogenetic studies suggest that *Erythrina dominguezii* belongs to a basal clade characterised as pollinated by both passerines and hummingbirds. Here, we characterise the pollination system of *E. dominguezii*. Floral morphology, nectar traits, breeding system, visitation rates and pollen deposition by its floral visitors were studied. Floral morphology of *E. dominguezii* showed traits associated with both passerine and hummingbird pollination. Nectar sugar concentration showed an intermediate value but closer to the hummingbird type; however, it was rich in hexose, which is typical of the passerine type. Approximately 5% of the flowers set fruits under free pollination. Almost 80% of recorded flowers were visited by birds, with the rest visited by hymenopterans (bumblebees and honeybees). Among avian pollinators, five species of hummingbirds and three passerine species were identified as pollinators. The hummingbird *Chlorostilbon lucidus* was the most efficient visitor in terms of pollen deposition and was second in frequency of visits. The passerine *Icterus cayanensis* was second in efficiency at depositing pollen and was the most frequent pollinator. Our results show that *E. dominguezii* has a generalised pollination system. In addition, we report a new case of closed flowers and secondary nectar presentation. This is the first study that compares effectiveness among different pollinators in *Erythrina*.

Introduction

Erythrina L. (Fabaceae : Papilionoideae) is a pantropical genus of over 100 species that belongs to the primarily entomophilous tribe Phaseoleae (Doyle and Doyle 1993). Plants of *Erythrina* are pollinated by birds (Raven 1974; Morton 1979; Bruneau 1997; Ragusa-Netto 2002; Etcheverry and Alemán 2005; Rocca and Sazima 2010), although bees, mammals and lizards have also been reported as pollinators (Galetto *et al.* 2000; Rangaiah *et al.* 2004; Sazima *et al.* 2009). Several floral traits are associated with the ornithophilous syndrome within the genus, for example, the presence of odourless flowers, red or orange petals, diurnal anthesis, and the production of abundant nectar (Faegri and van der Pijl 1979). Old World *Erythrina* species and a few from the New World are pollinated by perching birds (passerines), whereas most species in the New World are hummingbird pollinated, indicating that passerine pollination is the plesiomorphic state in the genus,

whereas hummingbird pollination is a derived character (Bruneau 1997).

Toledo and Hernández (1979) and Neill (1987) described the characteristics of each pollination type. In the passerine pollination the inflorescence rachis is horizontally oriented, with flowers directed inwards (towards the central axis of the tree), allowing birds to perch as they reach the nectar. Flowers are widely open, with the vexillum (the upper broad petal of the corolla) ovate or obovate, the wings (the two lateral petals) and the keel (two lower fused petals) exerted from the calyx and elongated. Anthers and stigma are exposed and mostly adichogamous (i.e. male and female functions take place at the same time but spatially separated at anthesis (Neill 1988)). Pollen is sticky and often covered by abundant pollenkitt (Hemsley and Ferguson 1985). Nectar is abundant and hexose-dominant, with a high concentration of amino acids and a low sugar concentration (Baker and Baker 1982, 1983, 1990). In

contrast, species adapted for hummingbird pollination have vertical inflorescences and flowers oriented outwardly, thus being accessible to hovering birds. Flowers are pseudo-tubular, with a narrow, elongated and folded vexillum, and the wings and keel are reduced. The flowers are protandrous and the reproductive parts are concealed (Toledo and Hernández 1979; Neill 1987). Pollen is usually powdery and shows little pollenkitt (Hemsley and Ferguson 1985), whereas nectar production is low and sucrose-dominant, with high sugar concentration and low concentration of amino acids (Baker and Baker 1982, 1983, 1990).

It has been 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 nectar traits (Bruneau 1997). In this context, species that do not fit into one of the two groups, such as *Erythrina dominguezii* Hassl., are of special evolutionary interest because they might be experiencing a shift from passerine to hummingbird pollination (Toledo and Hernández 1979). Phylogenetic studies of the genus indicate that *E. dominguezii*, together with *Erythrina falcata* and *Erythrina crista-galli*, are included in a basal clade characterised as pollinated by both passerines and hummingbirds (Bruneau 1997). Indeed, the phylogenetic hypothesis of Bruneau (1997) has been confirmed for *E. falcata* in North Argentina by Etcheverry and Alemán (2005). However, for *E. crista-galli*, Galetto *et al.* (2000) described a generalised pollination system involving bees, hummingbirds and passerines.

For some angiosperms, generalised pollination systems have been described where unrelated species or groups of pollen vectors, typically associated with different floral syndromes, act as pollinators (e.g. Mayfield *et al.* 2001; Wilson *et al.* 2006; Fumero-Cabán and Meléndez-Ackerman 2007; Botes *et al.* 2009; Schmid *et al.* 2011). The role of animal visitors in the floral evolution of plant species where reproductive success is pollen-limited will ultimately depend on their pollination effectiveness (Herrera 1988; Gómez 2004). Pollination effectiveness refers to the total contribution to plant fitness by animals that pollinate flowers (Herrera 1987, 1989; Ivey *et al.* 2003). This fitness contribution can be estimated in terms of the animal's pollination efficiency and its frequency of flower visits (Herrera 1987, 1989; Gómez and Zamora 1999; Stone 1996; Mayfield *et al.* 2001; Fumero-Cabán and Meléndez-Ackerman 2007). Only experimental tests can reveal the pollination effectiveness of each visitor species and so far pollination effectiveness of visitors to *Erythrina* flowers has not been tested.

The main objective of this study was to test the hypothesis that members from the basal clade of the genus *Erythrina* are pollinated by both hummingbirds and passerines, and to analyse whether bees are also involved in fruit and seed production as has been shown for other species in the group (e.g. *E. crista-galli*, Galetto *et al.* 2000). The particular goals of the study were: (1) to characterise the inflorescence, floral morphology and nectar traits of *E. dominguezii* and relate them with passerine or hummingbird pollination; (2) to determine the dependence on pollinators to produce fruits; and (3) to identify the floral visitors of *E. dominguezii* and determine their pollination behaviour and effectiveness. The information obtained in this study will provide a better understanding of the evolution of *Erythrina* pollination biology as well as shifts in pollination mode within the genus.

Materials and methods

Study species

Individuals of *E. dominguezii* are deciduous trees, 10–20 m tall and up to 1 m in diameter. Their leaves are compound, with large, orange–pink flowers and brownish legumes with one to three seeds (Legname 1982). This species inhabits humid slopes (between 300–550 m.a.s.l.) and borders of rivers in eastern Bolivia, western Paraguay, northern Argentina (Jujuy, Salta, Formosa, Chaco and Corrientes), and western central Brazil (Mato Grosso, Goias) (Krukoff and Barneby 1974; Neill 1988). The flowering phenology of the species extends from late September to November (R. Neumann, pers. comm.). Voucher specimens were deposited in the Museo de Ciencias Naturales, Universidad Nacional de Salta.

Study site

Field observations were conducted in an Eastern Andes seasonal rainforest (Yungas, Cabrera 1976), in Ledesma Department, Jujuy Province, Argentina (23°54′S 64°48′W, 464 m.a.s.l.). There is a strong seasonality in this locality, with 80% of the rain concentrated in the period from November to May. Mean annual precipitation is 897 mm, and mean annual temperature is 21.5°C (Bianchi and Yáñez 1992).

Inflorescence and floral morphology

Inflorescence morphology was studied in September 2005. We collected 74 inflorescences of similar phenological stage from 22 trees between 10 and 12 m in height (three to four inflorescences per tree). From each inflorescence, rachis length was measured and total number of flowers was determined.

Floral morphology was studied in October 2005. Morphological measurements were conducted on 118 bagged flowers from 12 individuals (9–11 flowers per tree). All flowers chosen were right at the beginning of anthesis and untripped. Flowers were collected and preserved in 70% ethanol and brought to the laboratory for morphological measurements. We measured length and width of calyx, vexillum, wings and keel petals, as well as distance from both the longest antisepalous anther and the stigma to the tip of the keel petals. To estimate anther–stigma distance we measured the distance between both median antisepalous and antipetalous anthers to the stigma. Drawings were made with a camera lucida. Petal colour from fresh flowers was recorded using a Munsell chart (Munsell Color Charts 1977).

Pollen and ovule number were estimated from 10 randomly selected flower buds. All 10 anthers from each single flower were softened in a 1 N HCl solution for 12 h; then, all anthers were transferred to 0.5 mL of lactic acid : glycerin (3 : 1) solution and macerated with a glass rod. In order to homogenise the mixture, the macerated samples were vortexed for 60 s. Immediately after vortexing, a sample was placed in a haemocytometer and pollen grains were counted. This value was then used to estimate the total number of grains per flower according to Dafni (1992). Ovule number was directly

determined from dissections of ovaries under a stereoscopic microscope. The presence of pollenkitt was detected in 20 flowers from 12 individuals (one to two flowers per tree), following Genise *et al.* (1990).

Flower phenology was monitored in October 2005 on 10 individuals. On each plant, we marked and bagged with white voile bags 20 flower buds of similar length, which we subsequently observed twice a day (0800 and 1800 hours) up to flower senescence. At each observation we registered stigmatic receptivity and anther dehiscence of 10 randomly selected flowers. Stigmatic receptivity was estimated through peroxidase activity, using a 3% hydrogen peroxide solution (Kearns and Inouye 1993). Anther dehiscence was observed with a hand lens ($\times 20$).

Nectar and nectary

To determine the location of stomata on the nectary, dissected flowers that were previously fixed in FAA (formaldehyde-acetic acid-alcohol) solution, were cleared with NaOH (10%) and stained with a I_2-IK solution (Galetto *et al.* 2000) in October 2005.

Nectar was collected from 110 flowers from 12 individuals that were bagged with voile bags (nine to eleven flowers per tree) at 7 h after anthesis (i.e. when stigma and anthers are exserted, see Results). Nectar samples were collected around 1300 hours, right before the time in which the resorption phase in nectar secretion begins (A. Etcheverry, unpubl. data). Nectar was extracted with microcapillary tubes without removing the flowers from the tree and avoiding damage to nectaries. Immediately after nectar extraction, volume and sugar concentration were estimated. Volume (μL) was estimated using graduated micropipettes, whereas sugar concentration was determined with a pocket refractometer. The amount of sugar produced was expressed in milligrams (Dafni 1992). In order to determine nectar composition, nectar samples were stored on filter paper and brought to the laboratory for chemical analysis. Nectar sugars were determined by thin layer chromatography (Dafni 1992). Amino acids were quantified according to Baker and Baker (1975).

Breeding system

Assessment of the breeding system involved ten trees during September 2010. We performed the following treatments: (1) natural pollination (flowers were not manipulated, $n = 2214$); (2) autonomous self-pollination (buds were bagged throughout their flowering period, $n = 1225$); (3) hand self-pollination (bagged flowers were hand pollinated with their own pollen, $n = 123$); (4) geitonogamous crosses (emasculated hand-pollinated flowers with pollen from flowers of the same plant, $n = 145$); (5) apomixis (anthers of buds were clipped, $n = 124$); and (6) hand cross-pollination, in which emasculated flowers were pollinated with pollen from another tree located at least 20 m away from the recipient tree ($n = 54$). Mature fruits were collected in November 2010. An indirect measure of self-incompatibility was obtained by dividing the average fruit set after hand self-pollination by the average fruit set after hand cross-pollination (index of self-incompatibility, Lloyd

and Schoen 1992). A value of one indicates complete self-compatibility.

Floral visitors

Floral visitors were observed in October–November 2005, 2006 and 2010 during three non-consecutive days each year from 0700 to 1700 hours. Observations were conducted in 15-min periods (10 periods per day) using binoculars, totalling 1350 min. To count the visit frequency of each visitor species throughout the day, five focal trees with ~400 available flowers were selected. Identification of bird visitors was conducted *in situ* using a field guide for the birds of Argentina (Narosky and Yzurieta 2003). In the case of insects, they were collected after observations, identified and deposited at the Museo de Ciencias Naturales Bernardino Rivadavia (Buenos Aires, Argentina). During observation periods we recorded the number of open flowers per plant, the identity of each flower visitor, the number of visits made by each flower visitor, and whether visits were legitimate or involved nectar robbing. We calculated the relative frequency as the number of visits made by the species divided by the total number of visits observed during the period of study. In addition, we calculated the visitation rate by each animal species as the number of visits per flower per period and then averaged the value for each pollinator. When passerines were foraging in groups the behaviour of a single individual from the group was recorded. In the case of the passerine *Icterus cayanensis* only legitimate visits were recorded.

Pollinator efficiency

To compare pollinator efficiency among main visitors from each group (i.e. hummingbirds, passerines and bees) we followed the procedure described by Herrera (1987) and Etcheverry *et al.* (2008). Only those visitors with relative abundance greater than 5% were considered. Sixty inflorescences were bagged with voile bags after removing all the tripped flowers and emasculating the remaining flowers (untripped). These exclosures precluded access of all floral visitors, as demonstrated by frequent checks. Every few days from mid-September to October 2010, one of the bags was removed and the inflorescence was watched at close range. Each pollinator coming to flowers was allowed to visit one to two individual flowers. The visited flower was immediately removed and placed in a glass vial. Separate vials were used for each pollinator. Twenty stigmas were collected per visitor species, and 20 unvisited flowers were collected for the control. The collected flowers were dissected individually, the style removed and the number of pollen grains on stigmas counted under a microscope according to Kearns and Inouye (1993).

Data analyses

The results were analysed using InfoStat (2009). Chi-square tests were performed to compare the results from controlled pollinations and frequencies of visits. Non-parametric Kruskal–Wallis or Mann–Whitney tests were used when the assumptions required for parametric methods were not met (Sokal and Rohlf 1995).

Results

Inflorescence and flower morphology

The flowering season started in late September. At that time trees were generally aphyllous, and axillary buds from shoots produced the preceding year developed into lateral inflorescences ('pseudoracemes' *sensu* Tucker 1987; Fig. 1*b*). Inflorescences had triads of flowers (i.e. short-shoots with three flowers) with acropetal growth (Fig. 1*b*) and a rachis of 12.44 ± 6.5 cm ($\pm$s.e.) in length (range: 3.40–28.40 cm). The mean number of flowers produced per inflorescence was 26.55 ± 1.26 (range = 3–63, $n = 74$).

Initially, flowering shoots were erect but as the development of the inflorescence continued, and due to the weight of the new flowers produced, they became pendulous. Consequently, flowers became inverted, *sensu* Faegri and van der Pijl (1979).

Flowers were odourless and zygomorphic, with the wing and keel petals on the upper side and the vexillum at the lower side (Fig. 2*a*). The pedicel was flexible, 2.5 times longer than the calyx and represented 70% of the floral length (Fig. 2*b*). Thus, flowers hung down loosely from the inflorescence axis. The calyx was green–yellow (2.5 GY 8/10), fleshy and asymmetrically bowl shaped, with a blunt tooth in the carinal side (Fig. 2*a, b*).

The calyx was a strong structure that enclosed the base of all petals tightly, probably protecting the flower from disarticulation during visits (Fig. 2*c*). The vexillum was pale orange–pink (2.5 YR 7/8, internal face 7.5 YR 8/6), fleshy, conduplicately folded, forwardly arched (Fig. 2*b*), with an orbicular–elliptical form, as in Fig. 2*d*. The wings were pale green (2.5 GY 8/6), small and ovate to deltate (Fig. 2*e, f*). The wing length represented one-fifth of the keel length. The keel petals were pale red (5R 6/10, base 5R 4/10), falcate, acute at the apex, and stiff (Fig. 2*a*). The keel petals were joined along their lower margins (Fig. 2*c*). The length of the keel petals represented 80% of the vexillum length and was 2.8 times longer than that of the calyx.

Flowers of *E. dominguezii* lasted 3–4 days in anthesis. Given that the flowers remained closed during the entire floral cycle, i.e. the vexillum remained folded covering the other floral parts (Fig. 2*b, c*), the beginning of the anthesis was defined as the moment in which the stigma and the anthers extended beyond the keel by elongation of the filaments, ovary and style. Therefore, flowers were opened and the reproductive structures became exposed only through manipulation from floral visitors (Fig. 2*a*, see also the *Floral visitors* section). At this stage the median antisepalous anthers and the stigma were longer than the

Fig. 1. *Erythrina dominguezii* in Ledesma, Jujuy, Argentina. (*a*) Individual tree. (*b*) Inflorescence and flowers in an inverted position, as in nature. (*c*) Individual flower showing the reproductive structures covered by the vexillum.
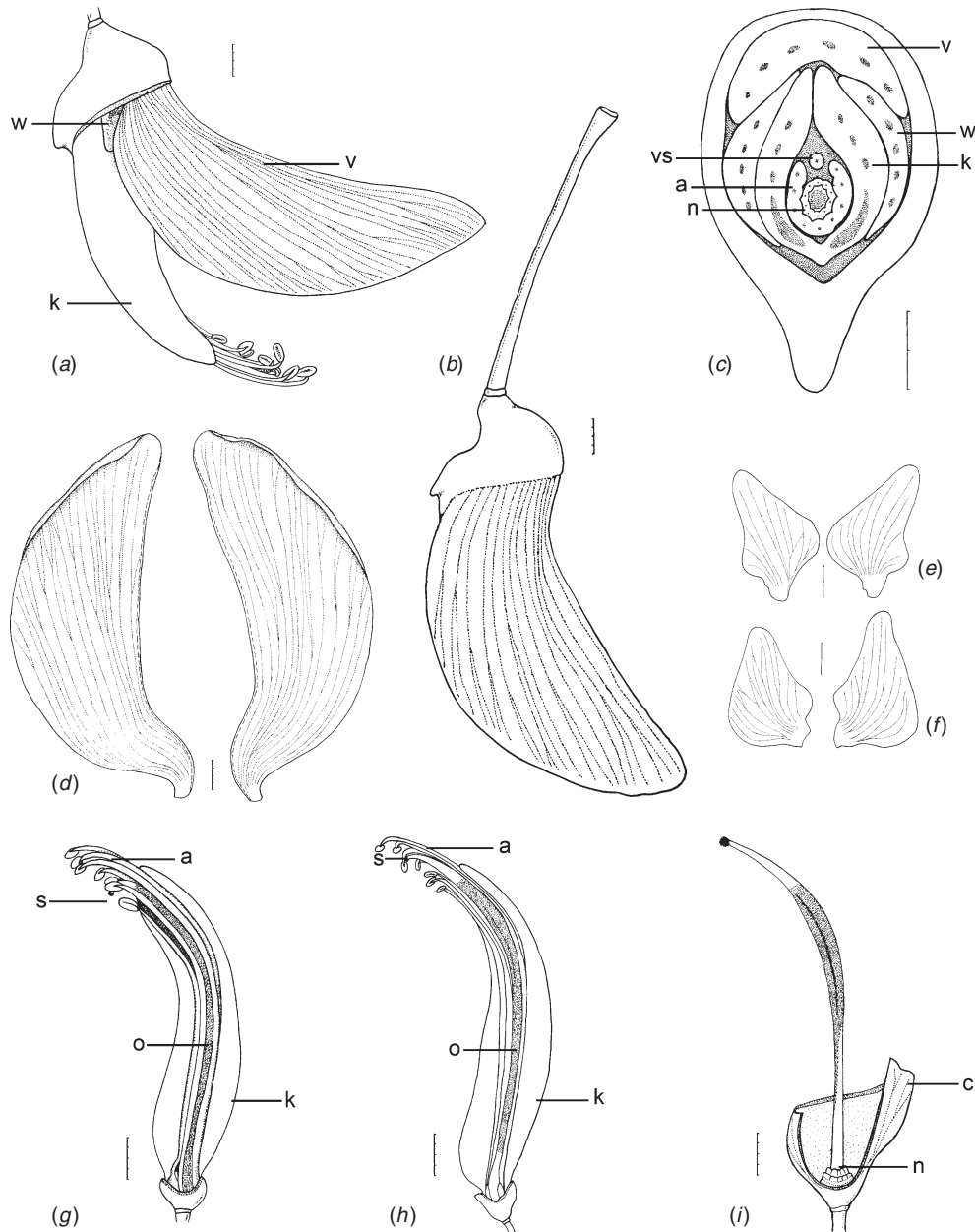
Fig. 2. Floral morphology of *Erythrina dominguezii*. (*a*) Open flower, shown inverted as in nature (Day 1). (*b*) Closed flower (Day 1). (*c*) Transverse section of a flower at the nectary level. (*d*) Vexillum (split in order to show its shape). (*e*) Ovate wing petals, outer surface (Day 1). (*f*) Deltate wing petals, outer surface. (*g*) Longitudinal section of a flower at the beginning of anthesis. Note that anthers (a) and stigma (s) project from the keel (k). The stigma (s) is shorter than anthers, except for one. (*h*) Longitudinal section of a flower at the end of anthesis (Day 4). (*i*) Gynoecium and nectary, the calyx was removed in part and the corolla and androecium were dissected away. References: (c) calyx, (v) vexillum, (w) wings, (k) keel, (a) androecium, (vs) vexilar stamen, (n) nectary, (o) ovary, (s) stigma. Scale bars = 4 mm, except for C, E and F = 2 mm.

keel by 9.72 and 3.34 mm, respectively (Fig. 2*g*). After visitation the vexillum became displaced while unvisited flowers remained closed.

The androecium, composed of ten stamens (Fig. 2*c, g*), had long (antisepalous) and short (antipetalous) filaments alternated with a separation of ~3 mm between whorl cycles. This feature resulted in a wider contact surface area with the body of the visitors. The anthers, dehiscent on the first day, face downwards, facilitating a nototribic deposition of pollen. Anthers opened

widely and presented pollen freely. We observed pollen grains on stigmas in mature closed (unvisited) flowers. Pollenkitt was absent in the examined flowers. Anther and pollen morphology was similar between the two whorls of stamens. The mean production of pollen grains per anther was 47343.75 ± 2412.20. Flowers produced a mean of 6.25 ± 0.30 ovules. Pollen–ovule ratio was 7871.90 ± 703.00.

The ovary was pubescent and, together with the style, curved along the keel (Fig. 2*g*). The stigma was terminal (Fig. 2*g*) and

'wet' (*sensu* Heslop-Harrison and Shivanna 1977). Stigmas of mature flower buds had a faint reaction to hydrogen peroxide, whereas flowers on the first day of anthesis showed strong reactivity, coinciding with anther dehiscence and indicating that *E. dominguezii* is adichogamous. On the first day of anthesis both whorls of anthers were above the stigma (-3.37 ± 0.22 and -1.68 ± 0.23 mm for the antisepalous and antipetalous stamens, respectively), thus there was herkogamy and outcross pollination could be favoured (Fig. 2g). However, by the fourth day of anthesis, the separation of anthers and stigma was reduced, so that the female and male reproductive structures contacted each other (Fig. 2h).

Nectary and nectar traits

The nectary was attached to the receptacle and surrounded the base of the ovary as a ring-shaped disk divided at the apex in ten short lobes (Fig. 2c, i). Open stomata were associated with such lobes. The nectar chamber was constituted by a staminal furrow, where nectar accumulated (Fig. 2c). It then overflowed into the keel, where most of the nectar produced was retained, and what was not stored there was drained towards the tip of the closed vexillum.

Mean nectar production per flower was $32.48 \pm 0.69\,\mu L$. Mean sugar concentration and mass were $22.08 \pm 0.39\%$ and 8.16 ± 0.24 mg respectively (Table 1). Chemically, the nectar of *E. dominguezii* was hexose dominated. Nectar had very low concentrations of amino acids, ranging from traces to $1.56\,\mu mol\,mL^{-1}$.

Breeding system

The results from the controlled pollinations are summarised in Table 2. Flowers used to test for apomixis did not set fruit. Fruit set differed significantly between: (1) hand-self-pollination and hand-crossed treatments ($\chi^2 = 17.75$, $P < 0.0001$); b) natural pollinated and hand-crossed treatments ($\chi^2 = 124.86$, $P < 0.0001$) and c) hand-self and spontaneous self-pollination treatments ($\chi^2 = 10.0$, $P = 0.0015$). We did not find significant

Table 1.   Nectar traits of *Erythrina dominguezii* in Ledesma population, Jujuy, Argentina
n = 113 flowers

Variable	Mean ± s.e.	Range
Volume (μL)	32.5 ± 0.69	20.0–48.0
Sugar concentration (%)	22.1 ± 0.39	10.0–31.0
Sugar mass (mg)	8.2 ± 0.24	2.0–15.3

Table 2.   Breeding system in *Erythrina dominguezii*
Fruit : flower ratio from controlled pollination treatments

Treatment	Fruit set (%)
Spontaneous self-pollination	2.36
Hand self-pollination	7.30
Natural pollination	4.56
Hand cross-pollination	28.0
Geitonogamy	4.13
Apomixis	–

differences between geitonogamy and natural pollination treatments ($\chi^2 = 0.06$, $P = 0.81$).

With an index of self-compatibility value of 0.26 (Lloyd and Schoen 1992), *E. dominguezii* is partially self-incompatible, with some fruits developed from self-pollination. 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

Flowers of *E. dominguezii* were visited by 10 animal species, distributed in three functional groups, i.e. hummingbirds, passerines and bees. Three species of passerines (Icteridae) were identified visiting the flowers of *E. dominguezii*: *I. cayanensis*, *Gnorimopsar chopi* and *Agelaioides badius*. When foraging on the flowers of *E. dominguezii* these birds used the rachis, the pedicel or a branch as a perch. Passerines usually foraged legitimately on the flowers of *E. dominguezii*. A typical behaviour of passerines visiting *E. dominguezii* was as follows: a bird placed its bill into the commissure formed by the lateral margins of the vexillum, then the bird opened its bill, causing a backward movement of the vexillum, rising and therefore exposing the stamens and the stigma. Then, the bird collected the nectar from the vexillum and the keel, contacting the reproductive parts with the bill. Passerine species also visit already opened flowers. However, *I. cayanensis* also presented an illegitimate behaviour while foraging, robbing nectar 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 structures.

Five species of hummingbirds were recorded visiting the flowers of *E. dominguezii*: *Adelomyia melanogenys*, *Chlorostilbon lucidus*, *Eriocnemis glaucopoides*, *Microstilbon burmeisteri* and *Thalurania furcata*. All of the hummingbirds had a legitimate foraging behaviour. Although hummingbirds commonly visited already opened flowers, they were able to trigger closed flowers while flying, through striking the vexillum with the bill repeatedly. Once the flower was opened, hummingbirds introduced their heads into the flower, accessing the nectar through the open margins of the keel and, at the same time, contacting the flower reproductive structures with the head.

Apis mellifera and *Bombus morio* workers (Apidae) contacted the reproductive structures during their visits to *E. dominguezii* flowers; however, they typically visited those flowers previously opened by birds. Occasionally, they forced pre-anthesal flowers. These insects introduced their bodies between keel and vexillum in order to take nectar and pollen. We observed both species grooming for short periods, often in pauses between flowers. The relative frequency of visits by passerines, hummingbirds and bees (40.8, 37.6 and 21.9% respectively) were significantly different from each other ($\chi^2 = 59.38$, df = 2, $P < 0.0001$). Regarding individual species, *I. cayanensis* (passerine) was the most frequent visitor and made 31.3% of total flower visits. The hummingbird *C. lucidus* was the second most frequent, making 22.9% of total flower visits. Bees were less frequent (*B. morio* = 11.6% and *A. mellifera* = 10.3%). The remaining species showed smaller values (less than 5%, see

Table 3). Considering the frequency of visits of the three groups throughout the day, we observed that passerines remained almost steady throughout the day. The hummingbird visits were more frequent during the morning (0700–1000 hours), whereas bee visits peaked at midday (1100–1300 hours) (Fig. 3).

Taking into account average visitation rates by the most frequent floral visitors, those of birds (*I. cayanensis* and *C. lucidus*) were significantly higher than those of bees (*B. morio* and *Apis mellifera*) ($H = 26.39$, df = 3, $P < 0.0001$; Fig. 4). When comparing the passerine *I. cayanensis* with the hummingbird *C. lucidus*, the former showed a higher visitation rate (0.09 ± 0.02 vs 0.06 ± 0.02; $U = 168.0$, $P = 0.0061$). In addition, average visitation rates by *B. morio* and *A. mellifera* were not significantly different from each other ($U = 123.5$, $P = 0.84$).

Pollination efficiency

We observed significant differences in the mean pollen load deposited on stigmas of flowers by the main pollinators of *E. dominguezii* ($H = 71.97$, df = 4, $P < 0.0001$; Fig. 5). The

Table 3. Visitors frequency to the flowers of *Erythrina dominguezii* in Ledesma, Jujuy, Argentina

Species	Frequency of visits (%)
Hymenoptera	
Apis mellifera	10.3
Bombus morio (workers)	11.6
Passeriformes	
Gnorimopsar chopi	4.9
Icterus cayanensis	31.4
Molothrus badius	4.0
Trochiliformes	
Adelomya melanogensis	2.4
Chlorostilbon lucidus	22.9
Eriocnemis glaucopoides	4.2
Microstilbon burmeisteri	3.6
Thalurania furcata	4.7

hummingbird *C. lucidus* was the most efficient pollinator in terms of pollen deposition on single flowers. The passerine *I. cayanensis* and the bumblebee *B. morio* (workers) showed an intermediate value. Pollen deposition on single flowers by *A. mellifera* was the lowest (Fig. 5).

Discussion

Floral traits

Considering previous schemes for categorising pollination biology in *Erythrina*, floral traits of *E. dominguezii* did not fall strictly within the passerine or the hummingbird type of pollination but were intermediate between them. Among the characters described for hummingbird-pollinated species (Toledo and Hernández 1979; Hemsley and Ferguson 1985; Neill 1987), *E. dominguezii* had vertical inflorescences, flowers oriented outwards, folded vexillum, reduced wings and absence of pollenkitt. Conversely, traits such as a broad vexillum, extended keel petals, reproductive structures exposed when the flowers open and adichogamy, are associated with pollination by passerines (Toledo and Hernández 1979; Neill 1987). Nectar traits were also intermediate between the passerine and hummingbird pollination types. Although nectar volume and amino acid content were typical of the hummingbird type

Fig. 4. Average (±s.e.) visitation rates of different visitors per flower per period (N = 22) to *Erythrina dominguezii*. Different letters indicate significant differences between means ($P < 0.05$).
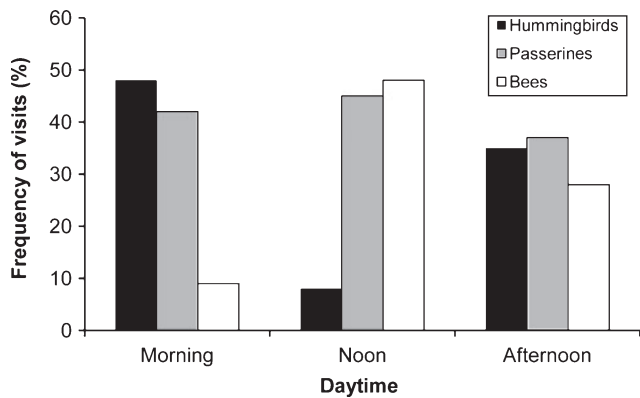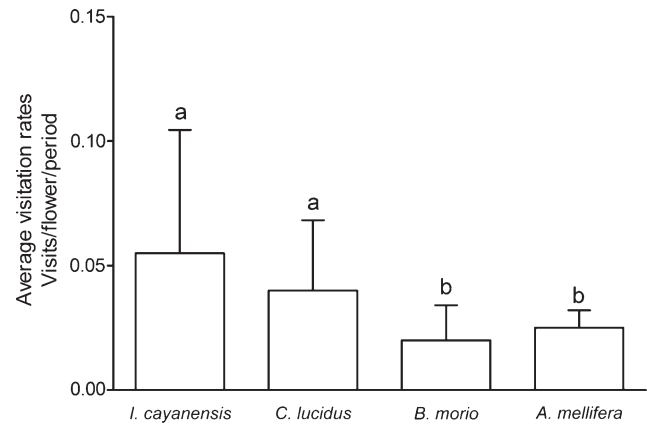

Fig. 3. Frequency of visits to *Erythrina dominguezii* flowers of the three functional groups throughout one day from 0700 to 1700. Morning = 0700 to 1000, Noon = 1100 to 1300, Afternoon = 1400 to 1700. Bars indicate the frequency of visits made by the floral visitors of each functional group with respect to the total number of visits at different times of the day.
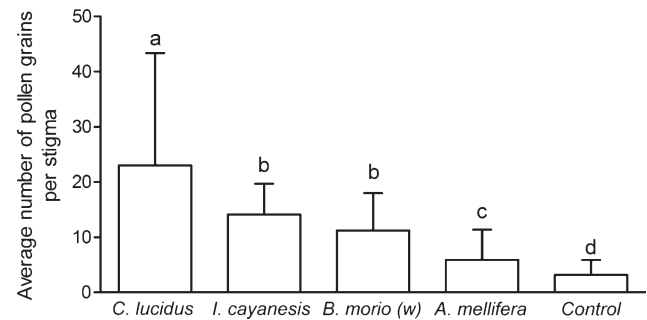

Fig. 5. Average (±s.e.) pollen deposition on stigmas of 20 flowers for the main pollinators visiting *Erythrina dominguezii* flowers. Different letters indicate significant differences between means ($P < 0.05$). (w): workers.

(Baker and Baker 1982), sugar concentration showed an intermediate value. However, nectar of *E. dominguezii* was rich in hexoses, which was described as typical of the passerine syndrome (Baker and Baker 1982, 1983, 1990) although sucrose preference has also been documented in passerines (Lotz and Nicolson 1996; Jackson *et al.* 1998; Schondube and Martínez del Rio 2003). Recently, Johnson and Nicolson (2008) suggested that the paradigm of nectar features should involve the difference between specialised and generalised bird pollination systems instead of the dichotomy between hummingbird and passerine flowers. According to these authors floral nectar of *E. dominguezii* fits with the traits described for generalised systems. Our findings suggest that the previous categorisation in passerine and hummingbird flowers in the *Erythrina* genus based on floral phenotypes (morphological and biochemical traits), is not useful to understand pollination biology in these species because it does not reflect the diversity of interactions that can occur in this kind of system, especially when, in addition to birds, bees visit the flowers and pollinate them efficiently. These results agree with the idea that the pollination syndrome hypothesis as usually articulated does not successfully describe the diversity of floral phenotypes (Ollerton *et al.* 2007).

Nectar production seems to vary among populations of *E. dominguezii*. Nectar produced by *E. dominguezii* in Ledesma, Argentina was $32.48 \pm 0.69\,\mu L$ per flower. In contrast, Ragusa-Netto (2002) recorded a production of $72 \pm 11\,\mu L$ of nectar per flower in one population of *E. dominguezii* in Mato Grosso, Brazil. This discrepancy may be attributed to different climatic factors experienced by each population. For example, the lower humidity in Ledesma may cause the evaporation of nectar and thus a decrease in its volume. Galetto *et al.* (2000) found a similar pattern for populations of *E. crista-galli* experiencing contrasting levels of humidity. Based on the existing data from species of subgen. *Micropteryx* (Feinsinger *et al.* 1979; Galetto *et al.* 2000; Etcheverry and Alemán 2005), *E. dominguezii* falls among the species with the lowest production of nectar.

A particular trait within the *Erythrina* genus is the presence of closed flowers *sensu* Faegri and van der Pijl (1979) (i.e. the vexillum remains folded, covering the remaining floral structures). This feature has been recorded in *E. dominguezii* in Argentina, as well as in populations of *E. crista-galli* (Galetto *et al.* 2000) and *E. falcata* (Etcheverry and Alemán 2005). Other legumes such as *Astragalus cymbicarpos* (Gallardo *et al.* 1993) and *Macroptilium panduratum* (Etcheverry *et al.* 2001) show cleistogamous flowers with an obligate autogamous breeding system, but this is not the case for *E. dominguezii*. In angiosperms, only a few cases of closed flowers have been reported: *Linaria vulgaris*, *Pedicularis sceptrum-carolinum* (both Scrophulariaceae) and *Trollius europaeus* L. (Ranunculaceae) (Faegri and van der Pijl 1979). This trait prevents many organisms from getting access to the nectar because closed flowers like these require 'both strength and ability from the visitors to open them' (Faegri and van der Pijl 1979). In addition, the flowers of *E. dominguezii* are inverted, so that the folded vexillum acts as a secondary nectar container and thus prevents the drainage of nectar out from the flower (secondary nectar presentation, Pacini *et al.* 2003). An

appropriated positioning of the nectar inside the flower ensures the efficiency of pollination; while collecting nectar, the visitor should inevitably contact the reproductive organs. In *E. dominguezii*, nectar is presented on the tip of the vexillum, that is, in close proximity to anthers and stigma. Thus, when floral visitors forage for nectar, they contact the reproductive parts of the flower. Other species within the genus (namely *E. fusca*, *E. falcata* and *E. oliviae*) have a similar morphology, with different floral structures acting as secondary nectar containers (Faegri and van der Pijl 1979; Toledo and Hernández 1979, Etcheverry and Alemán 2005). To our knowledge, these three species, together with *E. dominguezii*, are the only cases of secondary nectar presentation within Fabaceae. Because nectar is held within these concealed flowers, only floral visitors able to manipulate and open them will be able to get access to the reward and could act as legitimate pollinators.

Breeding system

Considering pollen : ovule ratio, *E. dominguezii* falls within the range that Cruden (1977) assigned as 'obligate xenogamy'. The controlled pollinations indicated that *E. dominguezii* had a mixed mating system, receiving mainly xenogamous and/or geitonogamous pollen at the beginning of anthesis and probably switching towards intra-floral autogamy by reduction in the stigma–anther separation distances late in floral development. A similar reproductive mechanism related with staminal growth was reported in other Papilionoideae, such as *Crotalaria micans* (Etcheverry *et al.* 2003) and *Crotalaria stipularia* (Etcheverry 2001). Considering the low fruit set obtained by hand self-pollination and geitonogamy, we suggest that this could be due to incomplete self-incompatibility. Besides, self-pollination would impose a high inbreeding depression. In general, the occurrence of geitonogamy may be affected by inflorescence size and pollinator behaviour (Harder and Barrett 1996). When a plant displays numerous flowers simultaneously, geitonogamy may occur if the pollinator moves between flowers within the same plant, as is the case for hummingbirds visiting *E. dominguezii* flowers. The fruit set obtained by natural pollination could be related to high pollen flow within a tree, mediated by flower visitors. The observed differences between hand self-pollination (geitonogamous or intra-flower) and spontaneous self-pollination treatments suggests that autonomous self-pollination is prevented in unvisited flowers by some device, such as the presence of stigmatic cuticle (or 'membrane') that needs to be ruptured to allow pollen germination, as was described for *E. crista-galli*, *Erythrina speciosa* and *Erythrina velutina* (Basso-Alves *et al.* 2011). The addition of xenogamous pollen to *E. dominguezii* flowers produced a significant positive effect on female fecundity, increasing the probability of flowers becoming mature fruits. This result suggests that the studied population was pollen limited.

Floral visitors

The floral visitors to *E. dominguezii* were birds and bees, although the former were the most frequent visitors. Overall, both groups of birds and bees were able to open the flowers

and had a legitimate behaviour, except for one passerine that also displayed an illegitimate behaviour by robbing nectar without contacting the reproductive structures. A dual behaviour of passerines has been previously described, e.g. *Pheucticus chrysopeplus* and *I. cayanensis* in *E. oliviae* and *E. falcata* respectively (Toledo and Hernández 1979; Etcheverry and Alemán 2005). The same three groups, namely hummingbirds, passerines and bees, have been recorded as visitors to other species within the genus. However, the relative frequencies of each species of floral visitor differed. For example, for *E. crista-galli*, bees were the main pollinators in populations from Argentina, Uruguay (Galetto *et al.* 2000) and Brazil (Costa and Morais 2008); among the birds, the hummingbird *C. lucidus* was one of the most frequently observed, whereas the passerine *I. cayanensis* was seen only occasionally. In *E. falcata*, bees were seen occasionally, whereas the passerine *I. cayanensis* seemed to be the main pollinator, given its high frequency of visits; however, it shows a dual behaviour, which could decrease its efficiency as a pollinator in comparison to the hummingbird, *Amazilia chionogaster* (Etcheverry and Alemán 2005).

Considering the efficiency of different pollinators, it was reported that hummingbirds have higher efficiency in relation to bees (Wilson and Thomson 1991; Larson and Barrett 1999; Thomson and Goodell 2001). Our results agree with this pattern, given that bees were less important pollinators than birds in terms of pollen deposition on the stigmas of *E. dominguezii*. However, taking into account the frequency of floral visitors throughout the day, we observed that hummingbirds were present in the patches earlier than bees. In this sense, Thomson *et al.* (2000) have predicted that ornithophilous *Penstemon* species would present rewards early in the day, when only hummingbirds are active, as was also observed for *Tecoma fulva* ssp. *garrocha* by Curti and Ortega-Baes (2011). As a consequence, when bees arrive, the service by hummingbirds is already done, minimising pollen wastage by bees.

Our results suggest that there is spatial variation in the floral visitors throughout the geographic range of *E. dominguezii*. For instance, in a Brazilian population (Mato Grosso do Sul), Ragusa-Netto (2002) recorded passerines from the Emberizidae family (*Psarocolius decumanus*, *Icterus icterus* and *I. cayanensis*) and parrots from the Psittacidae family as floral visitors. However, only the passerine *P. decumanus* was considered an effective pollinator, while *I. cayanensis* and *I. icterus* presented a dual behaviour.

In this study we have analysed floral traits and breeding system, as well as described the floral visitors and pollinator efficiency of *E. dominguezii*. Passerines and hummingbirds were the most frequent visitors, while bees were less abundant. Except for occasional illegitimate behaviour of one of the passerines, all groups of visitors were able to open and pollinate the flowers. Considering the pollinator efficiencies of the main pollinators, the hummingbird *C. lucidus* transferred the highest proportion of pollen grains, followed by the passerine *I. cayanensis* and workers of the bumblebee *B. morio*. These results confirm, in part, the hypothesis of a passerine−hummingbird pollination system in *E. dominguezii* proposed by Bruneau (1997), and demonstrate that bees play a

role as pollinators of the species, resulting in a generalised pollination system. As a consequence, *E. dominguezii* has a pollination system ecologically and functionally generalist, even when its flowers present phenotypic specialisation to bird pollination. Generalist pollination systems such as that described here are more common than one might expect if we only consider floral traits (Herrera 1996; Waser *et al.* 1996; Ollerton *et al.* 2007, 2009). Future studies should analyse, in a comparative way, how specialised or generalised are the pollination systems of the *Erythrina* genus throughout its pantropical distribution, analysing how they can vary over the geographic range of each species

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