

## HOW DOES SECONDARY POLLEN PRESENTATION AFFECT THE FITNESS OF *POLYGALA VAYREDAE* (POLYGALACEAE)?<sup>1</sup>

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Secondary pollen presentation is the relocation and presentation of pollen in floral structures (termed pollen presenters) other than the anthers. These pollen presenters are often found close to the stigma and have been hypothesized to increase the accuracy of pollen transfer, although no experimental studies have been done. We examined the function of the pollen presenter and its efficiency in pollen dispersal, female fitness, and the degree of interference created by self-pollen in the shrublet *Polygala vayredae*, an insect-pollinated species with secondary pollen presentation. Herkogamy, a mechanism generally involved in the reduction of self-interference, was also evaluated. Significant pollen was lost (49% of total pollen) during the secondary relocation in the pollen presenter. However, pollen was exported from the pollen presenter, and subsequent pollen losses were similar to those in species with primary pollen presentation. Despite the presence of a self-incompatibility system, the numbers of developed pollen tubes as well as fruit and seed production were significantly reduced by the self-pollen interference created at the stigmatic papillae level. The extent of herkogamy correlated positively with female fitness. The secondary pollen presentation mechanism may in fact be an accurate system for pollen transport, but it may also have its costs. Further comparative studies involving species with primary and secondary pollen presentation are needed to fully understand the advantages and disadvantages of secondary pollen presentation.

**Key words:** female fitness; herkogamy; pollen dispersal; pollen presenter; pollen relocation; Polygalaceae; self-incompatibility; self-interference.

The study of floral trait efficiency is important for identifying the role of adaptive evolution in floral diversification. To understand the evolution of floral traits, we must first identify the various elements involved in the process. In several plant species, pollen is presented in floral structures other than the anthers, either by simple deposition or by special expulsion mechanisms, causing the pollen to come into contact with other floral parts. This floral mechanism is known as secondary pollen presentation (hereafter called SPP), and the structure where pollen is presented to pollinators is referred to as the pollen presenter (hereafter called PP; following Faegri and van der Pijl, 1979; Yeo, 1993; Inouye et al., 1994; Ladd, 1994). The mechanisms through which pollen is secondarily presented are highly variable, with pollen being transferred to different areas of the style or stigma (e.g., Nilsson et al., 1990; Vaughton and Ramsey, 1991; Imbert and Richards, 1993; Nyman, 1993; Westerkamp and Weber, 1997), usually before flower opening. Pollen is then exposed in the PP during floral development or the mechanism is triggered by the pollination vectors (e.g., Brantjes, 1982, 1983; Nyman, 1993; Smith and Gross, 2002).

Secondary pollen presentation has traditionally been described as a mechanism that enhances the efficiency and accu-

racy of pollen exportation and/or pollen reception, thus increasing male and/or female fitness of the plant (Carolin, 1960; Lloyd and Yates, 1982; Ladd, 1994). Nevertheless, very few studies have experimentally assessed the effects of this mechanism on plant fitness (e.g., Lloyd and Yates, 1982; Imbert and Richards, 1993; Nyman, 1993). Moreover, a clash of interests may exist because the proximity of pollen-receiving and pollen-donating surfaces could result in self-interference, i.e., a conflict between male and female functions (Webb and Lloyd, 1986; Ladd, 1994; Barrett, 2002), with subsequent detrimental effects on plant fitness (e.g., Cesaro et al., 2004; Kawagoe and Suzuki, 2005; Waites and Ågren, 2006). Several functional or adaptive floral traits, such as self-incompatibility, dichogamy, and herkogamy, have evolved to avoid or minimize the effects of self-interference and thus improve outcrossing rates (Lloyd and Webb, 1986; Webb and Lloyd, 1986). Nonetheless, to date, self-interference has only been studied in species with primary pollen presentation, and all the functional and evolutionary assumptions made regarding SPP are based mainly on morphological descriptions.

The species belonging to *Polygala* L. (the largest genus of Polygalaceae comprising around 725 species; Paiva, 1998) have been described as presenting a SPP mechanism in which pollen is released before anthesis in a PP located on a sterile branch of the stigma (e.g., Ladd and Donaldson, 1993; Westerkamp and Weber, 1997; Fig. 1). The gynoecium, composed by two carpels, presents one sterile stigma functioning as PP and one fertile stigma with receptive papillae. However, there are some species in the genus that still present the ancestral gynoecia with two fertile stigmas, where the SPP mechanism is absent (*P. persicariaefolia* DC.; Venkatesh, 1956); in others the sterile stigma is reduced in size or has even disappeared (African *Polygala* sp.; Ladd and Donaldson, 1993); and in some derived species the sterile stigma has reevolved and

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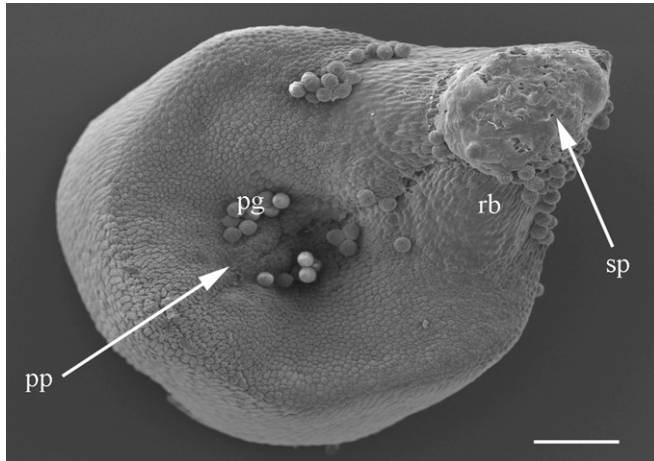


Fig. 1. Scanning electron micrograph of the stigma of *Polygala vayredae* showing the pollen presenter (pp), the stigmatic papillae (sp) in the apex of the receptive branch (rb) and several pollen grains (pg). Bar = 200  $\mu$ m.

functions as a pollen presenter (*P. vauthieri* Chodat; Ladd, 1994). Because the ancestral condition in Polygalaceae is probably the occurrence of two carpels, the PP most likely originated from the sterilization and specialization of one of the ancestral stigmas (Ladd, 1994), but comparative analyses are still needed to confirm these observations. The stigmatic region is highly diversified with structures in the shape of a basket, spoon, brush, or hair crown, and in several species of this family, the PP is slightly displaced in relation to the stigma (for illustrations, see Paiva, 1998; Castro, 2007). Ladd (1994) proposed that in species with SPP, herkogamy may minimize the effects of self-interference due to the proximity of the self-pollen and the stigma, but this theory has never been investigated experimentally. Moreover, the efficiency of pollen export/receipt by this mechanism has been questioned on theoretical grounds by Brantjes (1982) and Ladd and Donaldson (1993), who argued that stigma clogging and self-pollination would be difficult to avoid in several species of *Polygala*.

In this paper we selected the endemic *Polygala vayredae* Costa (Polygalaceae) to describe and evaluate experimentally how the SPP mechanism affects male and female components of plant fitness. The stigmatic region of this species is similar to the one observed in several other *Polygala* species, suggesting that results for *P. vayredae* could be broadly applicable in the genus. The following issues were addressed: (1) function of PP and associated pollen losses resulting from secondary pollen presentation, (2) the efficiency of the PP in pollen dispersal, and (3) female fitness and the degree of self-interference. Because the stigmatic papillae in *P. vayredae* are located on the receptive branch in a higher position than the PP (see Fig. 1; Castro, 2007), the function of herkogamy was also investigated for the first time in a species with SPP. To analyze the function of herkogamy, we assessed the variability in the distance between the PP and the stigmatic papillae and its consequences on female fitness.

## MATERIALS AND METHODS

**Plant and study area**—*Polygala vayredae* is an early-flowering perennial plant, endemic to the eastern Pre-Pyrenees (Alta Garrotxa, Girona, Spain). Be-

cause of its narrow distribution (approximately 12 km<sup>2</sup>), the species has been classified as vulnerable by IUCN categories (VV.AA, 2000). It is entomophilous, with large zygomorphic flowers and a self-incompatibility system at the stigmatic papillae level, strictly relying on pollinators to set seeds (Castro et al., 2008a). In the study area the main pollinator was *Bombus pascuorum* Scopoli (Apoidea, Hymenoptera), which visits intensively a large number of flowers in a patch and subsequently distances itself from the area (Castro, 2007).

As a result of the fusion of two monospermic carpels, the stigma is divided into two regions, a sterile one in shape of a basket (the PP) where SPP occurs, and a fertile region with the stigmatic papillae (see Fig. 1 and Castro, 2007). The curved style runs along the corolla tube and fits inside the keel. The anthers open introrsely toward the PP, which is recharged in subsequent pollinator visits through the downward movement of the keel activated by the pressure applied by the insect on the crest (this mechanism is illustrated in Fig. 2). Despite the deposition of the pollen close to the stigma, no spontaneous self-pollination occurs (Castro et al., 2008a).

The study was conducted during the spring of 2006 in the Colldecarrera population (Alta Garrotxa, Girona, Spain).

**Functioning of the PP as a self-pollen receptor**—Thirty-five flowers were bagged prior to anthesis. After anthesis, the number of pollen grains deposited in the PP was quantified in the first charge, i.e., after pollen dehiscence from the anthers to the pollen presenter (R1), and in subsequent recharges, i.e., after successive downward movements of the keel (R2, R3, R4), until pollen deposition was no longer observed (Fig. 2). The movement of the keel made by *B. pascuorum* was simulated by pressing on the crest. Pollen deposited in the PP after each movement of the keel was carefully removed with a needle to a microscope slide and mounted in a drop of 50% glycerine. This procedure was carried out under a stereobinocular microscope to guarantee that all the pollen grains were removed from the PP. Finally, pollen grains were counted under an Olympus CX31 light microscope (Olympus America, Center Valley, Pennsylvania, USA).

**Efficiency of the PP in pollen dispersal**—To evaluate the dispersal of the pollen deposited in the PP, fluorescent powdered dyes (Radiant Color, Richmond, California, USA) were used as pollen analogues (Waser and Price, 1982). Although dye and pollen dispersal properties differ (Thomson et al., 1986), dye transfer closely resembles pollen transfer when bumblebees, the main pollinators of *P. vayredae*, are the pollen vectors (e.g., Waser, 1988; Rademaker et al., 1997; Adler and Irwin, 2006). Fluorescent dyes were applied to the PP of 30 newly opened flowers after self-pollen had been removed. Flowers belonged to several individual plants arranged in clusters of roughly 0.25 m<sup>2</sup>. Three replicates clusters, each separated by at least 100 m, were selected along a single transect within the population. Because the sampling of the intermediate replica overlapped with the remaining two, a different colored dye was used to avoid contamination between replicas. After 8 d, 45–50 flower samples were collected at several distances from each cluster source (1, 2, 3, 4, 5, 10, 25–50, 50–100 m) and then preserved at –4°C. In the laboratory, flowers were examined under UV light using a stereobinocular microscope. The dye powder grains deposited in the stigma were classified into categories by number (class 0, zero; class 1,  $\leq 10$  grains; class 2, 11–100 grains; class 3, 101–500 grains; class 4, >500 grains). The proportion of flowers with fluorescent dye was calculated for each distance interval.

**SPP, degree of self-interference and female fitness**—The influence of SPP on female fitness and on the degree of self-interference were evaluated using the following treatments: (1) open pollinated flowers, i.e., with the possibility of both self- and out-crossed pollen reception; (2) flowers emasculated in bud and open pollinated, i.e., excluding the possibility of self-pollen reception; and (3) the control group, i.e., bagged and manually self-pollinated flowers. At the onset of fruit development, the corollas (with the style and stigma) were collected and preserved in 70% ethanol to assess the pollen load on the stigmas and the development of pollen tubes through the style. To prevent pollen loss during pistil treatment, pollen loads were evaluated after cutting and squashing the stigmatic papillae on a microscope slide. Styles were then softened with 8 M sodium hydroxide for 4 h, stained with 0.05% aniline blue (w/v, in 0.1 M potassium phosphate) overnight and squashed in a drop of 50% glycerine (v/v) (Dafni et al., 2005). Samples were observed through a Nikon Eclipse 80i epifluorescence microscope equipped with a UV-2A filter cube (330–380 nm excitation; Nikon Instruments, Kanagawa, Japan). The number of pollen tubes that successfully developed through the stigmatic papillae and style was recorded. Fruit and seed set were recorded when mature. In these experiments, only

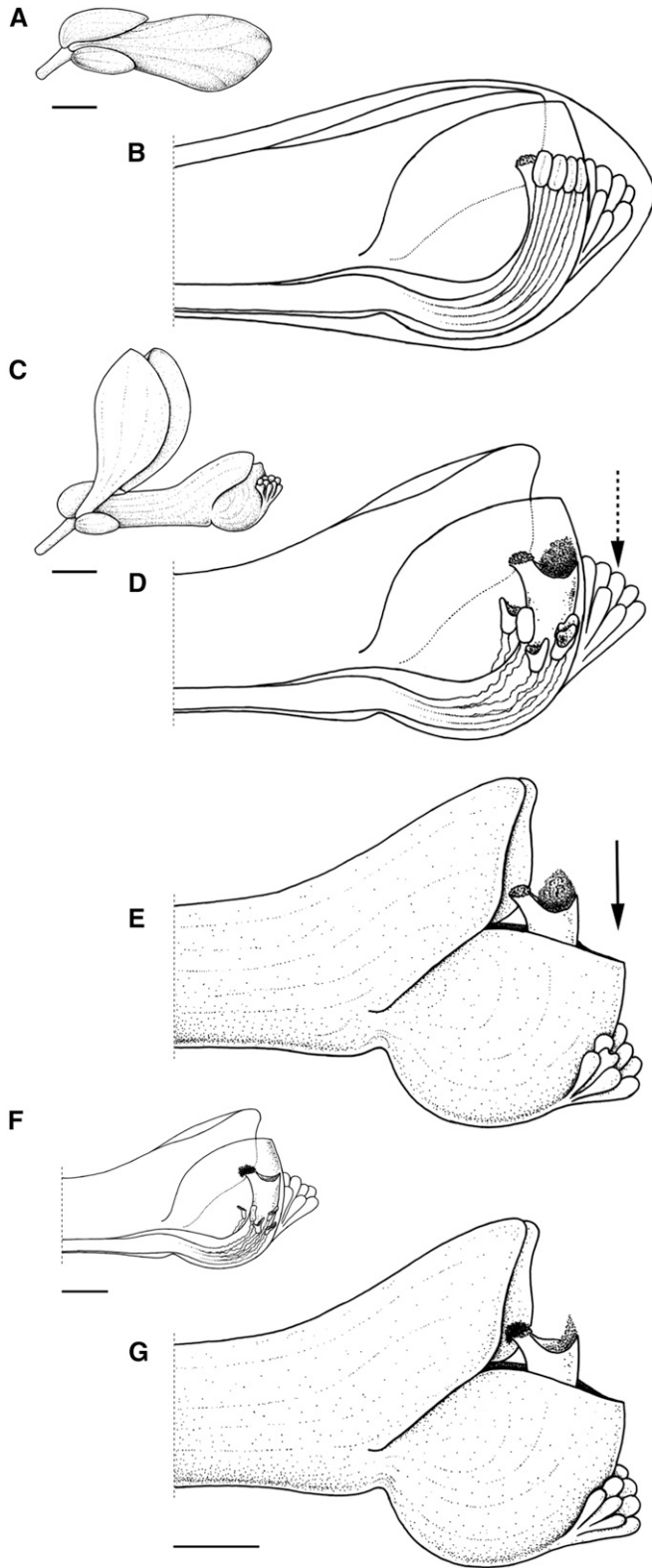


Fig. 2. Schematic diagram of the bud, flower and the functioning of the secondary pollen presentation mechanism in the stigmatic pollen presenter of *Polygala vayredae* when flowers are visited by a legitimate pollinator. (A) Flower bud. (B) Detail of the internal organization in a flower bud with anthers prepared to open introrsely toward the pollen presenter.

visited flowers were considered. Because no spontaneous self-pollination occurs in this species, the presence of pollen on the stigmatic papillae clearly indicates the visit of a pollinator (for details, see Castro et al., 2008a, 2008b).

**Distance between the PP and stigmatic papillae and effects on pollen tube development**—Flower samples were randomly collected throughout the population during the flowering peak and preserved in 70% ethanol. The length of the corolla, stigmatic papillae, and PP, as well as the distance between the last two structures were measured in 100 flowers. Measurements were taken on microphotographs using the program ImageTool 3.0 for Windows (University of Texas Health Science Center, San Antonio, Texas, USA). Pollen tube development was evaluated in all flowers using the procedure described.

**Statistical analysis**—Differences among the number of pollen grains deposited in the PP after each movement of the keel (groups with equal sample sizes) were evaluated with a Kruskal–Wallis one-way ANOVA on ranks followed by a Tukey test for all multiple pairwise comparisons. A previous estimate of total pollen production per flower in the same population ( $5001 \pm 90.1$ , mean  $\pm$  SE; Castro et al., 2008a) was used to calculate the cumulative proportions of the pollen deposited in the PP after successive movements of the keel.

The proportions of flowers with fluorescent dye among distances (categorical data adjusted to a binomial distribution) were analyzed with generalized linear/nonlinear models using a logit link function; the type 3 likelihood-ratio test was run (McCullagh and Nelder, 1989; Dobson, 1990). The proportion of total delivered pollen was also estimated by inferring the total amount of pollen potentially available in the three clusters (90 flowers with ~5000 pollen grains per flower) and estimating the delivered pollen using the proportion of flowers with fluorescent dye and its amount.

To evaluate the influence of SPP on female fitness, we analyzed differences among treatments in the number of pollen grains on the stigmas and pollen tubes in the styles with a Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's method (which takes into account the different sample sizes of the treatments). The effect of self-interference on fruit and seed production was evaluated with a  $\chi^2$  test for the comparison of more than two proportions and multiple comparison tests for proportions according to Zar (1984).

A Spearman's rank order correlation coefficient was calculated to evaluate the relationship between female fitness (number of developed pollen tubes below the stigmatic papillae) and the distance between the PP and stigmatic papillae. Because no correlation was found between the PP–stigmatic papillae distance and corolla length ( $R^2 = 0.161$ ,  $P = 0.109$ ), the values were not corrected with this parameter. The mean, standard deviation of the mean (SD), and coefficient of variation (CV) of the PP–stigmatic papillae distance were also calculated. All statistical analyses were performed using the program STATISTICA (StatSoft., Tulsa, Oklahoma, USA).

## RESULTS

**Functioning of the PP as a self-pollen receptor**—Pollen grains were deposited in higher numbers on the pollen presenter during the first charge of the PP than in subsequent movements of the keel ( $H = 101.03$ ,  $P \leq 0.001$ ; Fig. 3A). When the results are analyzed using cumulative proportions of the total pollen produced, 48.6% of the pollen grains remained inside the keel until flower senescence (Fig. 3B).

**Efficiency of the PP in pollen dispersal**—In *P. vayredae*, the pollen dehisces inside the keel. Without the pollen presenter,

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(C) Open flower with androecium and gynoecium enclosed in the corolla. (D) Detail of the internal organization of an unvisited open flower with the pollen presenter charged with pollen. (E) First legitimate visit to the flower, where the downward movement of the keel activated by the pressure applied on the crest, exposed the pollen in the pollen presenter and the stigmatic papillae. (F) Flower after the visit, returning to the initial state and wilting anthers. (G) Flower subjected to a second legitimate visit. Bars = A, C: 3 mm; B, D–G: 2 mm. Illustrations by S. Castro.

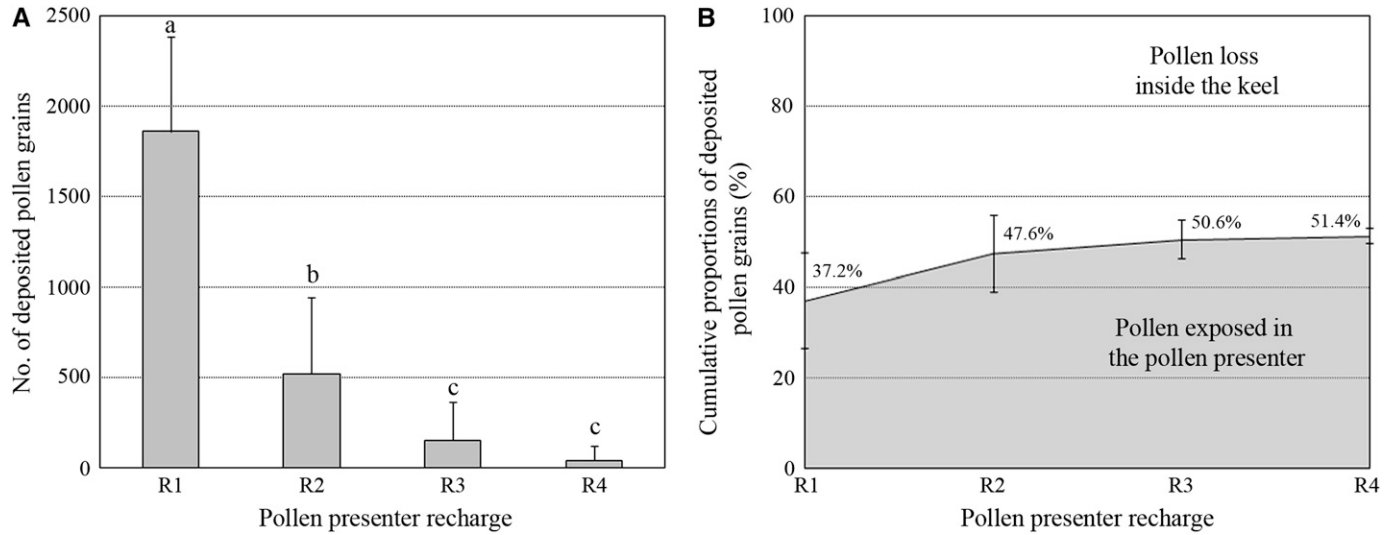


Fig. 3. Pollen presenter functioning in *Polygala vayredae* after four successive downward movements of the keel (R1–R4). (A) Mean and standard deviation of the mean number of pollen grains deposited at the style basket. (B) Mean estimated cumulative proportion of pollen grains that were exposed in the pollen presenter.

pollen exportation is null because the anthers remain enclosed in the keel during the pollinator’s visit and are virtually inaccessible to them (Figs. 2C-E). Thus, the PP allowed the pollen to come into contact with the pollination vector and permitted export of pollen to the stigmas of other flowers (Fig. 4). After an exposure of 8 d, 16 focal flowers (53.3%) were visited, and the fluorescent dye removed. Fluorescent dye was encountered in the stigmatic papillae of 7.8% of the target flowers that were analyzed. Fluorescent dye was also observed in small amounts (<10 grains) on the crest, keel and/or petal tips of 3.0% of the flowers analyzed. Although diminished, the pollen flow was higher in the first few meters, dropping significantly as the distance to the focal plants increased ( $\chi^2 = 87.34$ ;  $P < 0.001$ ). A particularly low proportion of delivered pollen was obtained (0.44%) as estimated from the transfer of dye particles (see Materials and Methods).

**SPP, degree of self-interference and female fitness**—An analysis of the control flowers (i.e., bagged and manually self-pollinated flowers) confirmed the presence of a self-incompatibility system at the stigmatic level, with the near absence of pollen tube development and fruit and seed production (Table 1). Self-interference and reduced female fitness resulting from the SPP mechanism were evident through comparison of pollen grain number, number of pollen tubes, and seed production of open-pollinated, unmanipulated flowers and the open-pollinated, emasculated flowers. Significant differences for all the variables were found between these treatments, which are differentiated by the presence vs. absence of self-pollen. The open-pollinated flowers (unmanipulated) received a significantly higher number of pollen grains (around 117 pollen grains more on average) than the emasculated, open-pollinated flowers (Table 1). Nonetheless, they developed a significantly lower number of pollen tubes through the style at all levels, and lower proportions of fruits and seeds than the group where self-pollen was removed (Table 1). The self-pollen deposited during the insect visits was approximately half the amount of the total pollen deposited in the stigmatic papillae.

**Distance between the PP and stigmatic papillae and the effects on pollen tube development**—The distance between the PP and the stigmatic papillae ( $1.24 \pm 0.110$  mm, CV = 8.86%) had a significant and positive correlation with the number of pollen tubes developed in the style ( $R^2 = 0.274$ ,  $P < 0.01$ ). Thus, the number of pollen tubes increased as the distance between the PP and the receptive area grew.

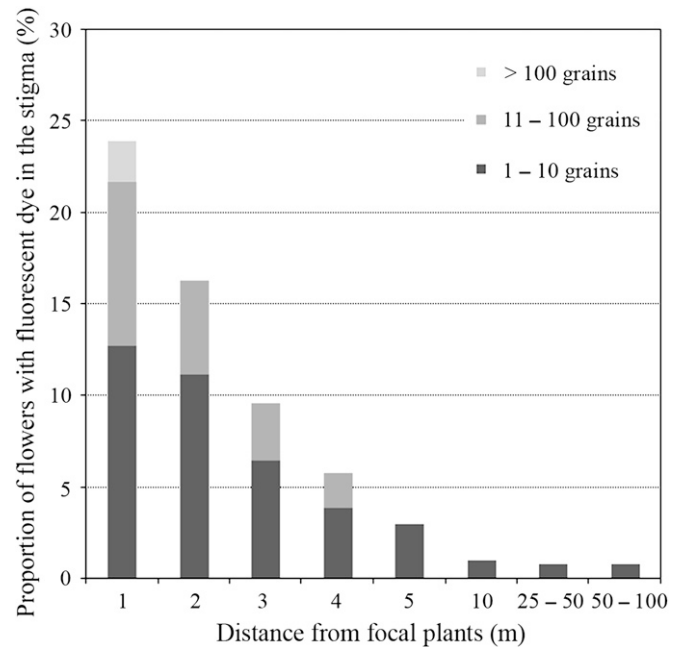


Fig. 4. Dispersal of fluorescent dyes among flowers of *Polygala vayredae*. Proportions of flowers with fluorescent dye in the stigma in terms of distance to the focal plant cluster. The amount of dye observed in each stigma is shown in categories.

TABLE 1. Results of treatments designed to examine the effect of possible self-interference on female fitness resulting from the secondary pollen presentation mechanism of *Polygala vayredae*.

Treatment	N	No. pollen grains	No. pollen tubes along the style			No. fruits (fruit set)	No. seeds (seed ovule ratio)
			Beginning	Middle	End		
Open-pollinated flowers (unmanipulated)	86	230 ± 16.3 <sup>a</sup>	5.3 ± 0.56 <sup>a</sup>	4.8 ± 0.54 <sup>a</sup>	4.1 ± 0.48 <sup>a</sup>	56 (65.1%) <sup>a</sup>	84 (48.8%) <sup>a</sup>
Emasculated and open-pollinated flowers	30	113 ± 18.3 <sup>b</sup>	8.9 ± 0.91 <sup>b</sup>	8.1 ± 0.85 <sup>b</sup>	6.9 ± 0.77 <sup>b</sup>	26 (86.7%) <sup>b</sup>	39 (65.0%) <sup>b</sup>
Bagged and self-pollinated flowers	35	29 ± 3.5 <sup>c</sup>	0.6 ± 0.17 <sup>c</sup>	0.4 ± 0.15 <sup>c</sup>	0.3 ± 0.15 <sup>c</sup>	2 (5.7%) <sup>c</sup>	3 (4.3%) <sup>c</sup>
Comparison test		$H = 72.59^{***}$	$H = 46.79^{***}$	$H = 47.61^{***}$	$H = 45.71^{***}$	$\chi^2 = 50.17^{***}$	$\chi^2 = 80.11^{***}$

Notes: Values are means ± SE. Differences in the number of pollen grains and tubes among treatments were evaluated with a Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn's method; differences in the fruit and seed production among treatments were evaluated with a  $\chi^2$  test for the comparison of more than two proportions and multiple comparison tests for proportions following Zar (1984). Values followed by different letters are significantly different. \*\*\* $P \leq 0.001$ .

## DISCUSSION

In outcrossing species, a conflict often arises between selection to present pollen and stigmas in similar positions to improve pollination success and selection to keep them apart to minimize or avoid interference between pollen export and pollen receipt on the stigma (Lloyd and Webb, 1986; Webb and Lloyd, 1986). Secondary pollen presentation, which has evolved in several groups of angiosperms, allows the pollen and stigmas to be presented in similar positions within a blossom (Carolin, 1960; Yeo, 1993; Ladd, 1994). Nevertheless, even though this is a widespread, highly diversified and morphologically studied feature (for a review, see Yeo, 1993), the functional aspects and consequences of such a mechanism on plant fitness are still largely unknown. The current study is the first evaluation of the consequences of SPP in both pollen exportation and pollen reception, in addition to the possible detrimental effects of the proximity of the self-pollen and stigmatic area.

Flowering plants that rely on animal vectors to transport pollen grains to conspecific stigmas of other flowers are exposed to great uncertainty and are frequently subjected to high rates of pollen loss (e.g., Inouye et al., 1994; Morris et al., 1994). Several different pollen fates may occur in this pathway, ranging from non-exportable pollen to pollen deposition on their own stigmas during removal and transport or at the presentation site (Inouye et al., 1994; Harder and Wilson, 1998). Our results showed that in *P. vayredae* the SPP mechanism results in considerable pollen loss within the flowers. The flowers produced roughly 5000 pollen grains, but only around 51% became available on the PP for export during flower's life span. The remaining pollen (non-exportable pollen) remained inside the corolla without the possibility of being exposed and dispersed. Compared with the total amount of pollen produced by the flower, this pollen loss due to the SPP mechanism reduced the opportunities for mating by half before the pollen had the chance to be presented to the vector. Pollen losses before collection by pollinators (pre-collection losses, following Inouye et al. 1994) are generally overlooked in the studies performed so far, yet have been found in both species with primary (e.g., Harder and Thomson, 1989; Rademaker et al., 1997) and secondary pollen presentation (e.g., Vaughton and Ramsey, 1991; and results in the present work). Nonetheless, pre-collection pollen losses must be carefully analyzed when assessing the efficiency of the SPP mechanism because secondary relocation of the pollen constitutes an additional step in the process of pollination. Consequently, more studies on pre-collection pollen losses involv-

ing species with primary and secondary pollen presentation are needed to fully understand the degree of pollen loss due to the mechanism of SPP.

In *P. vayredae* the downward movement of the keel activated by a legitimate visitor of some mass (such as the long-tongued bumblebee *B. pascuorum*) exposes the PP and allows for the dispersal of pollen among flowers. The results of this study show that in the Coldecarrera population pollen flow and the proportion of pollen received were low in 2006. This reduced pollen flow is in accordance with pollinator activity reported for this year in this population. Although *P. vayredae* was the main flowering plant in the study area and *B. pascuorum* was its main pollinator, *B. pascuorum* interacted infrequently (0.088 for 15 min using the methodology in Herrera [1989]) with *P. vayredae* flowers (Castro, 2007). Thus, the low pollen flow was due to pollinator limitation and, in the focal flowers that were visited, to high pollen losses by the pollen vector during pickup, transport, and delivery. Intrinsic pollen losses due to the SPP mechanism, pollinator limitation (both cases of pre-collection pollen losses) and subsequent losses during transport on the pollen vector (pre-deposition pollen loss) were observed in *P. vayredae*. Despite this, the observed efficiency of pollen transport (estimated with fluorescent dyes dispersal) was similar to what has been reported for species with similar granular pollen (percentage of removed pollen delivered to stigmas below 0.5%; Thomson and Thomson, 1989; Galen, 1992; Rademaker et al., 1997). If only the pollen available for transportation is considered, pollen transfer efficiency was slightly higher than what was observed in species with primary pollen presentation (e.g., Harder and Thomson, 1989; Thomson and Thomson, 1989; Galen, 1992; Rademaker et al., 1997). It is important to note that the use of powdered fluorescent dyes as pollen analogues provide only a good qualitative prediction of pollen movement (Waser and Price, 1982; Thomson et al., 1986; Waser, 1988; Campbell et al., 1991; Rademaker et al., 1997; Adler and Irwin, 2006), and the pattern of dye and pollen movement may vary among the study species (Waser and Price, 1982; Thomson et al., 1986; Waser, 1988).

Self-interference has been described as the conflict between male and female functions due to their close proximity (Webb and Lloyd, 1986; Ladd, 1994; Barrett, 2002). In *P. vayredae* we observed that the presence of self-pollen in the stigmatic papillae reduced the number of pollen tubes in the style and the number of fruits and seeds as a result of interference with outcross pollen, although a self-incompatibility system possibly limits the negative effects of self-pollen interference. As suggested by

Webb and Lloyd (1986) and recently shown empirically by Koelling and Karoly (2007), the presence of a self-incompatibility system may minimize or eliminate this conflict of interests. Additionally, temporal separation of male and female functions may also limit interference (Lloyd and Yates, 1982; Lloyd and Webb, 1986; Routley and Husband, 2006). In the few available studies involving species with SPP, these species have floral features similar to the ones described here. For example, in *Cephalanthus occidentalis* L. (Rubiaceae) a higher growth rate of outcrossed pollen tubes and an inhibition of self-pollen tubes at the base of the style largely prevented selfing (Imbert and Richards, 1993), and in *Rauvolfia grandiflora* Mart. ex A. DC. (Apocynaceae), a late acting self-incompatibility mechanism was also found (Lopes and Machado, 1999). In several species of *Campanula* L. (Campanulaceae), on the other hand, the tactile stimulation of the style hairs, where SPP occurs, controlled the male and female phases, reducing the maturation of the male phase while accelerating that of the female (Nyman, 1993). In *P. vayredae* a mechanism of self-incompatibility prevents self-fertilization, with rejection occurring at the stigmatic papillae level (results herein and Castro et al., 2008a). This mechanism does not avoid self-interference, but could mitigate the potential negative effects by limiting pollen tube growth in the style.

Somewhat paradoxically, considering the proximate female and male functions in species with SPP, herkogamy has also been suggested as another mechanism that may emerge to avoid self-interference (Ladd, 1994; Lopes and Machado, 1999). In *P. vayredae* it was previously observed that stigmatic papillae are located somewhat in a higher position on their stigmatic branch in relation to the PP (Castro, 2007). In this study, the relationship between the PP–stigmatic papillae distance and female fitness (measured by the number of pollen tubes growing in the style) revealed a slight increase in fitness with increased distance. Thus, this separation appears to be slightly advantageous for plant fitness. In experiments with *Narcissus assoanus* Dufour (Amaryllidaceae), a species lacking SPP, floral traits like herkogamy limited the cost of self-interference, which exerted a detrimental effect on seed set (Cesaro et al., 2004). Because self-pollination can reduce opportunities for outcrossing, selection will favor floral traits that reduce self-interference and improve outcrossing (Webb and Lloyd, 1986). This selection could be of special importance in xenogamous species with SPP mechanisms, where self-interference has significant detrimental consequences.

Although the current study has provided new insights into the function of SPP in *P. vayredae*, further comparative studies on the efficiency of pollen transfer, female fitness and presence of herkogamy involving related taxa with primary and secondary pollen presentation would be useful to assess the selective advantages and disadvantages of the SPP mechanism. As a result of the high diversity in SPP structures, Polygalaceae are very interesting and valuable for evaluating differences in the efficiency of pollen transfer among species with distinct pollen presentation structures and provide new insights into the adaptive significance of secondary pollen presentation.

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