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## Floral traits variation, legitimate pollination, and nectar robbing in *Polygala vayredae* (Polygalaceae)

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**Abstract** The variation of floral morphology and its effect on the flower visitors of *Polygala vayredae* Costa (Polygalaceae), a narrow endemic species from the Oriental pre-Pyrenees, were examined. First, to account for the main floral reward (i.e., nectar), the relationship between the dimensions of the nectar gland and nectar production was investigated. Second, floral traits variation was assessed within and between the three most representative populations of the species. Finally, the role of several floral traits in the female fitness was evaluated. Furthermore, as nectar robbing was highly frequent, preferences of robbers for specific floral traits and their impact on legitimate pollinations were also evaluated. The flowers of this species are characterized by significant variations in floral characteristics and nectar rewards. A significant and positive correlation between the nectar gland dimensions and nectar production per flower was observed, with the gland dimensions being a good measure to infer the rewards offered by the flowers of *P. vayredae*. In general, corolla traits were significant and positively correlated with each other. Nectar was revealed to be an important trait in flower–visitor interactions, with legitimate pollinations being primarily influenced by this floral reward. Negative correlations between robbing frequency and legitimate pollinations were observed in two of the studied

populations, and positive correlations between flower size and robbing frequency were observed in one population. An indirect negative selection over phenotypic floral traits mediated by nectar robbers is proposed.

**Keywords** Floral morphology · Endemic species · Nectar rewards · Nectar robbers · Phenotypic selection

### Introduction

In animal-pollinated plants, floral morphology has tightly evolved with its pollinators (Stebbins 1970; Galen 1999). Thus, it is possible to interpret several floral traits as adaptations to certain pollination vectors that result from effective selective pressures exerted by them over floral morphology. For this, it is essential to have phenotypic and genetic variation in the floral traits directly involved with pollinator attraction and efficiency, which can result in different reproductive outputs and further selection (Herrera 1990, 1996). Facing a variable assemblage of floral displays, pollinators have shown to prefer plants with certain floral traits, such as a high number of flowers (Mitchell et al. 2004; Benitez-Vieyra et al. 2006), large flowers (Ishii and Harder 2006; Celedón-Neghme et al. 2007), large nectar rewards (Thomson 1986; Fenster et al. 2006), specific corolla color or shape (Sutherland and Vickery 1993; Gómez et al. 2006), or particular flowering time (Hall and Willis 2006).

Nevertheless, recent works have shown that the selection of flower traits not only results from pollinator-mediated selection, but is, rather, a dynamic process, where other biotic and abiotic factors can also exert strong selective pressures (e.g., Herrera 1993; Herrera et al. 2002; Gómez 2003; Giles et al. 2006; Sánchez-Lafuente 2007). Among the biotic factors, nectar robbing was shown to be an important feature affecting the plant fitness (e.g., Maloof and Inouye 2000; Irwin et al. 2001). Understanding the effects of nectar robbers on the visited plants and on the remaining floral visitors is especially important, considering the high rates of

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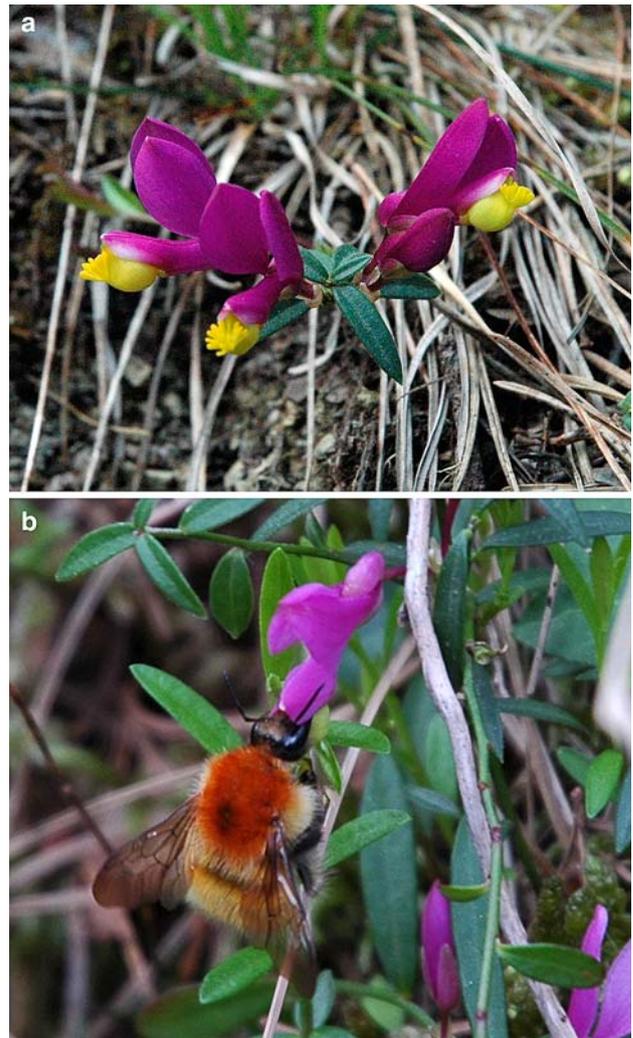
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robbing and the high proportion of robbers' visitations observed in several species (Malooof and Inouye 2000). In some of these works, a diverse array of effects of nectar robbing on plant reproductive success have already been observed. Negative interactions of nectar robbers, such as changes in the patterns of the available nectar, the reduction of flower attractiveness, and/or the damaging of floral structures, have been frequently described to affect further floral interactions, visitor's behavior, and plant fitness in several species (e.g., Zimmerman and Cook 1985; Irwin and Brody 1999; Traveset et al. 1998; Zhang et al. 2007). On the other hand, positive interactions, such as the accidental involvement of nectar robbers in pollination when accessing nectar (e.g., Higashi et al. 1988; Navarro et al. 1993, 2000) and neutral interactions (e.g., Arizmendi et al. 1996; Morris 1996), have also been observed. In the particular case of tubular flowers, nectar robbing may act as an opposite selective force that balances the selection of floral traits by specialist pollinators (Lara and Ornelas 2001). Nonetheless, to date, only a few studies have shown the direct or indirect effect of nectar robbers on the selection of plant traits (but see Irwin 2006).

The elaborated floral morphology of Polygalaceae (keel flowers) and its superficial resemblance to Fabaceae raises questions on the function of floral components and their role in the attraction of pollinators (Eriksen 1993; Westerkamp 1997; Westerkamp and Weber 1999). Keel flowers are generally described as bee pollinated (Faegri and van der Pijl 1979; Westerkamp 1997) and are composed of two elementary functional structures: a flag for visual advertisement/attraction and a keel protecting the sexual organs (Westerkamp 1997). In *Polygala* (and in Polygalaceae in general), the two lateral petaloid sepals are described as the advertisement structure, while the keel, besides protecting the organs, presents an appendage (crest) used by visitors to land (Fig. 1a). Furthermore, the fusion and imbrication of the upper petals produce a tubular-like flower that can influence the assemblage of visitors, including nectar robbers (Navarro 2001; Lara and Ornelas 2001). Despite these observations, in Polygalaceae, no studies have been developed to evaluate the direct correlation between floral traits and legitimate pollination, and its consequences on floral traits selection.

The present work focuses and explores how the quantitative variation in floral traits influences the interactions between the flowers of *Polygala vayredae* and its visitors. *P. vayredae* is a narrow endemic species from the Oriental pre-Pyrenees and is an entomophilous plant that strictly relies on pollen vectors to set fruit (Castro et al. 2008). The shape of its flowers requires the visit of specialized pollinators, such as long-tongued insects (namely, *Bombus pascuorum* and *Anthophora* sp.), which, during nectar exploitation, move down the keel and come into contact with the concealed reproductive organs, accomplishing pollination (Fig. 1b). Furthermore, nectar robbing has been described as a steady feature in this species, with high robbing fre-



**Fig. 1a, b** Flowers of *Polygala vayredae* (a) and *Bombus pascuorum* visiting the flowers (b)

quencies and high visitation rates being observed in its main populations (Castro 2007). Our objectives were to investigate the variation in floral characteristics within and among populations in order to assess how the variation in floral traits influences legitimate pollinations, and to evaluate the extent of nectar robbing as a factor of selective pressures. For this, morphometric analyses of floral characteristics were performed on flowers from the three main populations of *P. vayredae*, with the data on nectar reward and nectar robbing being included in the analysis. Standard regression methods were applied for detecting patterns of natural selection on floral traits.

## Materials and methods

### Plant and study area

*P. vayredae* Costa (Polygalaceae) is an early flowering, perennial and self-incompatible species, endemic from

**Table 1** Studied populations of *Polygala vayredae*

Population	Habitat	Altitude (m)	Main pollinators	Main nectar robbers	Nectar robbing (%) <sup>a</sup>
Montmajor	Rocky place ( <i>Saxifragion mediae</i> )	1,070	<i>B. pascuorum</i>	<i>Macroglossum stellatarum</i> <i>B. terrestris</i>	94.9
Serrat dels Boixos	Mesophytic meadow ( <i>Mesobromion, Brometalia erecti</i> )	750	<i>B. pascuorum</i> <i>Anthophora</i> sp.	<i>B. terrestris</i> <i>Gonepteryx rhamni</i>	92.1
Coldecarrera		630	<i>B. pascuorum</i> <i>Anthophora</i> sp.	<i>B. terrestris</i> <i>B. pratorum</i>	92.1

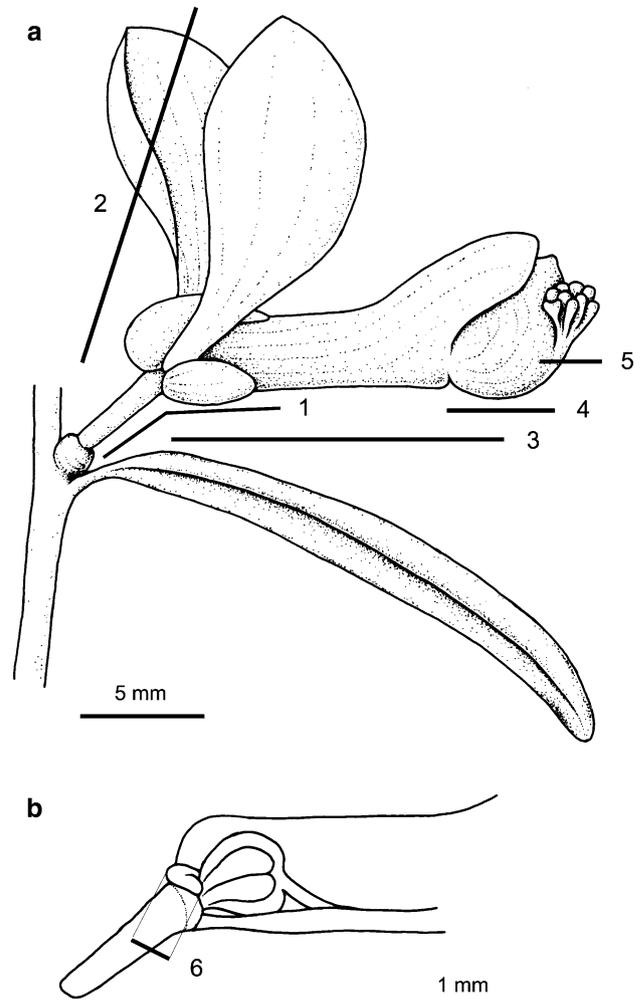
<sup>a</sup> Nectar robbing was assessed by observing up to 1,000 randomly selected flowers from distinct plants in 2005

the Oriental pre-Pyrenees, where it occurs in a few dense populations distributed in a restricted area of approximately 12 km<sup>2</sup> in Alta Garrotxa, Girona (Catalunya, Spain). This species presents large zygomorphic flowers, arranged in small axilar inflorescences of 1–3 flowers. The two lateral petaloid sepals and the corolla tube are pink, while the keel, where androecia and gynoecia are concealed, is sulphur yellow (Fig. 1a). Flowers present a large life span and nectar rewards are produced in a gland at the base of the corolla tube. *P. vayredae* strictly relies on pollinators to set fruit and is frequently subjected to pollen/pollinator limitation and nectar robbing (Castro et al. 2008).

The study was carried during the Spring of 2005 by collecting flowers from distinct individuals in three populations: Montmajor ( $n=131$ ), Serrat dels Boixos ( $n=76$ ), and Coldecarrera ( $n=112$ ). Flower samples were preserved in ethanol 70% until laboratory analysis. Information about the main floral visitors and the frequency of nectar robbing for each population is given in Table 1.

#### Relationship between the nectar gland dimensions and nectar production

To include a measure of the general reward offered, the correlation between the nectar gland dimensions and nectar production was assessed. For this, the nectar production was quantified in 30 flowers which were 1–2-days old, randomly selected among the Coldecarrera population. Flowers of this age were selected because the production of nectar in this species occurs mainly during the first three days of the flower life span (Castro et al. 2008). The flowers were identified and bagged. In each flower, after 24 and 48 h, nectar was extracted and quantified with a capillary micropipette, and the sugar concentration was determined (in w/w, %) with a portable refractometer. The amount of sugar produced by each flower was calculated according to Prys-Jones and Corbet (1987). After nectar quantification, flowers were collected for morphometric analysis of the nectar gland dimensions (Fig. 2).



**Fig. 2a, b** Illustration of the *P. vayredae* flower (a) and detail on the location of the nectar gland (b). Morphometric measures under study: angle of flower presentation (1), and length of wings (2), corolla tube (3), keel (4) crest (5), and nectar gland (6)

#### Variation of floral morphology and its effect on flower visitors

Morphometric analyses were performed on *P. vayredae* flowers by collecting the following measurements: angle

of flower presentation (directly involved with flower presentation to visitors), length of the wings (involved in advertisement), length of the corolla tube (a limiting factor in nectar access), length of the keel (involved in advertisement and organ protection), length of the crest (a foot holding for visitors), and length of the nectar gland (an indirect measurement of floral reward) (Fig. 2). The measurements were taken from photographs using Analysis 5.0 software. Furthermore, in each flower, signs of nectar robbing were also recorded. Nectar robbing (Inouye 1980) was easily evaluated, due to the visible and repeated incisions made in the corolla by robbers, and allowed an approximation to the nectar robbing intensity. To some extent, this measure could be underestimated, as nectar robbers may use the existing holes, and behave as secondary nectar robbers. Nonetheless, it should be noticed that the frequency of interactions obtained for the main nectar robber (*B. terrestris*) during the year of study (Castro 2007) was consistent with the robbing intensity observed in the present work. Pistils were treated with sodium hydroxide 8 M for 4 h, stained overnight with aniline blue 0.05%, and squashed with a drop of glycerine 50% (Dafni et al. 2005). Finally, they were observed through an epifluorescence microscope with a UV-2A filter cube. The number of pollen tubes able to germinate and grow throughout the stigmatic papillae and style was recorded. As this species is self-incompatible, blocking the pollen tube development mainly at the stigmatic papillae (Castro et al. 2008), the presence/absence and the number of pollen tubes in the style were used as measures of legitimate visits and successful pollination.

### Statistical analysis

The relationship between the nectar gland dimensions and the nectar production (nectar volume and the amount of sugar) was assessed by applying the Spearman's rank order correlation coefficients.

Descriptive statistics of flower traits and nectar robbing intensity (i.e., the number of holes in the corolla) were calculated for each population. Variation in these traits within and among the populations was investigated with one-way analysis of variance (ANOVA) or with a Kruskal-Wallis one-way ANOVA on ranks, followed by Dunn's test for pairwise multiple comparisons. For each studied population, relationships among morphometric variables were investigated through Spearman's rank order correlation coefficients. The relative variability of each morphometric characteristic was assessed by the coefficient of variation (CV).

Descriptive statistics of each flower trait and nectar robbing intensity were also calculated for successful (with pollen tube development below stigmatic papillae) and unsuccessful flowers (without pollen tube development below stigmatic papillae). Variation in floral morphology and robbing intensity between these two groups was investigated with a *t*-test.

For each population, linear multiple regression analyses were applied for detecting the relationship between the occurrence of legitimate pollinations (given by the number of developed pollen tubes) and morphometric characteristics. This approach allowed, to some extent, to perceive pollinators' preferences and to investigate the directional selection of floral traits (Lande and Arnold 1983). Correlated variables (corolla tube, keel, crest, and wings) were integrated using principal component analysis (PCA). The PCA factor coordinates provided indices of flower size (for further detail on the contribution of each variable, see the Appendix). The two first factors explained more than 80% of the variability and were both used in the regression analysis. The intensity of nectar robbing was also included. The same procedures were applied for detecting the relationship between the occurrence of nectar robbing and morphometric characteristics. In all of the regression analyses, tolerance was higher than 0.75 and the variance-inflated factors were lower than 1.35.

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## Results

### Relationship between the nectar gland dimensions and nectar production

The nectar gland dimensions were significantly and positively correlated with both nectar volume ( $R^2 = 0.688$ ,  $P < 0.0001$ ) and the amount of sugar ( $R^2 = 0.716$ ,  $P < 0.0001$ ) produced per flower. Thus, the flowers of *P. vayredae* with larger nectar glands produced greater nectar rewards.

### Variation on floral morphology and its effect on flower visitors

The results of the morphometric analysis for each floral trait are described in Table 2. Multivariate ANOVA revealed significant differences among populations ( $F = 6.86$ ,  $P < 0.0001$ ). With exception of the nectar gland dimensions, the mean values for each floral characteristic varied significantly among populations (Table 2), with the Montmajor individuals presenting slightly larger flowers. Coldecarrera was the most heterogeneous population, showing the highest values of CV for all of the parameters and the highest intensity of nectar robbing. The corolla tube and the keel were the less variable characteristics (overall CV of 5.2% and 6.6%, respectively), while the nectar gland size and the crest were the most heterogeneous (overall CV of 13.9% and 23.2%, respectively).

Correlations between floral characteristics within each population are presented in Table 3. In general, the corolla traits, i.e., corolla tube, keel, crest, and wing, were significantly and positively correlated with each other. The exceptions were the crest versus wing in the

**Table 2** Intensity of nectar robbing and mean and standard deviation of the mean and coefficient of variation (CV, given in parentheses; %) of the floral characteristics studied in three populations of *P. vayredae*

Population	Floral traits						
	Robbing intensity	Angle (°)	Corolla tube (mm)	Keel (mm)	Crest (mm)	Wing (mm)	Nectar gland (mm)
Montmajor	1.3 ± 0.85 <sup>a</sup>	152.8 ± 10.97 <sup>a</sup> (7.2)	14.8 ± 0.74 <sup>a</sup> (5.0)	6.6 ± 0.38 <sup>a</sup> (5.8)	2.4 ± 0.45 <sup>a</sup> (18.8)	16.0 ± 1.02 <sup>a</sup> (6.4)	0.88 ± 0.130 (14.8)
Serrat dels Boixos	1.0 ± 0.73 <sup>b</sup>	154.5 ± 11.64 <sup>a</sup> (7.5)	14.6 ± 0.73 <sup>a, b</sup> (5.0)	6.3 ± 0.37 <sup>b</sup> (5.9)	2.1 ± 0.46 <sup>b</sup> (21.9)	15.4 ± 1.10 <sup>b</sup> (7.1)	0.89 ± 0.102 (11.5)
Coldecarrera	1.7 ± 0.88 <sup>c</sup>	149.1 ± 12.34 <sup>b</sup> (8.3)	14.5 ± 0.78 <sup>b</sup> (5.4)	6.3 ± 0.45 <sup>b</sup> (7.1)	2.1 ± 0.52 <sup>b</sup> (24.8)	16.1 ± 1.26 <sup>a</sup> (7.8)	0.89 ± 0.132 (14.8)
Comparison test	<i>H</i> = 59.54***	<i>F</i> = 5.46**	<i>F</i> = 3.61*	<i>H</i> = 24.48***	<i>F</i> = 18.97***	<i>H</i> = 12.26**	<i>F</i> = 0.18 n.s.
All populations		151.9 ± 11.79	14.6 ± 0.76	6.4 ± 0.42	2.2 ± 0.51	15.9 ± 1.15	0.89 ± 0.124
Range		111.7–191.0	11.6–16.8	5.4–7.7	1.1–3.8	12.8–19.2	0.48–1.32
CV (%)		7.8	5.2	6.6	23.2	7.2	13.9

Robbing intensity is given as mean and standard deviation of the mean of the number of holes in the corolla. The different letters reveal significant differences

n.s. = not significant

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001

**Table 3** Spearman correlation coefficients between all of the analyzed floral characteristics for each studied population

Population	Floral traits	Angle	Corolla tube	Keel	Crest	Wing
Montmajor	Corolla tube	0.171 n.s.	–	–	–	–
	Keel	0.065 n.s.	<b>0.442***</b>	–	–	–
	Crest	0.007 n.s.	<b>0.228**</b>	<b>0.685***</b>	–	–
	Wing	–0.120 n.s.	<b>0.584***</b>	<b>0.462***</b>	<b>0.415***</b>	–
	Nectar gland	0.219*	<b>0.394***</b>	0.125 n.s.	–0.014 n.s.	<b>0.339***</b>
Serrat dels Boixos	Corolla tube	0.171 n.s.	–	–	–	–
	Keel	–0.016 n.s.	<b>0.434***</b>	–	–	–
	Crest	0.042 n.s.	<b>0.245*</b>	<b>0.530***</b>	–	–
	Wing	–0.112 n.s.	<b>0.600***</b>	<b>0.511***</b>	0.178 n.s.	–
	Nectar gland	0.110 n.s.	0.104 n.s.	0.148 n.s.	–0.026 n.s.	0.154 n.s.
Coldecarrera	Corolla tube	0.025 n.s.	–	–	–	–
	Keel	–0.118 n.s.	<b>0.297**</b>	–	–	–
	Crest	–0.181 n.s.	0.056 n.s.	<b>0.635***</b>	–	–
	Wing	–0.065 n.s.	<b>0.491***</b>	<b>0.509***</b>	<b>0.349***</b>	–
	Nectar gland	0.317***	0.141 n.s.	0.041 n.s.	0.022 n.s.	0.076 n.s.

The significant coefficients are highlighted in **bold**

n.s. = not significant

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001

Serrat dels Boixos population, and the crest versus corolla tube in the Coldecarrera population, both of which had low positive and not significant correlation coefficients. The relationships between the nectar gland size and corolla traits were only significantly (and positively) correlated for the corolla tube and the wing in the Montmajor population, being not significantly correlated for the remaining traits in the other populations. No correlations were expected between the angle of flower presentation and the remaining variables, but significant and positive correlations were found between this floral trait and the nectar gland size in the Montmajor and Coldecarrera populations.

*P. vayredae* flowers received visits from legitimate pollinators and/or nectar robbers. From the 319 flowers collected in the three populations under study, 153 (48.0%) were successfully pollinated, while the remaining 166 (52.0%) did not receive any efficient visits. Floral

traits for successful and unsuccessful flowers are described in Table 4. Successful flowers presented significantly smaller keels ( $t = 2.88$ ,  $P < 0.01$ ) and crests ( $t = 3.82$ ,  $P < 0.001$ ), and significantly larger nectar glands ( $t = -3.33$ ,  $P < 0.001$ ). Furthermore, the intensity of nectar robbing was significantly lower in successful flowers in comparison with unsuccessful ones ( $t = 4.60$ ,  $P < 0.001$ ). No significant differences were found between successful and unsuccessful flowers in the remaining characteristics (Table 4).

The results of the multiple linear regression analysis of the effect of floral traits in legitimate pollinations are presented in Table 5. Several differences among the studied populations were found on the contribution of each characteristic. In the Montmajor and Coldecarrera populations, the variables nectar gland dimensions and nectar robbing intensity were significantly correlated with the occurrence of an efficient visit from a legitimate

**Table 4** Intensity of nectar robbing and mean and standard deviation of the mean of floral traits for successful and unsuccessful flowers of *P. vayredae*

Floral traits	<i>n</i>	Robbing intensity	Angle (°)	Corolla tube (mm)	Keel (mm)	Crest (mm)	Wing (mm)	Nectar gland (mm)
Successful flowers	153	1.1 ± 0.67	151.4 ± 11.87	14.6 ± 0.83	6.4 ± 0.43	2.1 ± 0.50	15.9 ± 1.27	0.91 ± 0.130
Unsuccessful flowers	166	2.0 ± 0.88	152.4 ± 11.73	14.7 ± 0.69	6.5 ± 0.40	2.4 ± 0.49	15.9 ± 1.03	0.86 ± 0.115
<i>t</i> -test		<i>t</i> = 4.60***	<i>t</i> = 0.76 n.s.	<i>t</i> = 1.47 n.s.	<i>t</i> = 2.88**	<i>t</i> = 3.82***	<i>t</i> = 0.15 n.s.	<i>t</i> = -3.33***

Successful flowers refer to an efficient pollination performed by a legitimate pollinator. As this species is self-incompatible, blocking the development of pollen tubes at the stigmatic papillae, successful and unsuccessful flowers were easily determined by the presence or absence of pollen tubes in the style

*n.s.* = not significant

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001

**Table 5** Results of the multiple linear regressions to evaluate the effect of the floral traits and nectar robbing intensity on the probability of successful pollination in each of the studied populations

Floral traits	Montmajor				Serrat dels Boixos				Coldecarrera			
	Coefficient	$\beta$	<i>t</i>	<i>P</i>	Coefficient	$\beta$	<i>t</i>	<i>P</i>	Coefficient	$\beta$	<i>t</i>	<i>P</i>
Angle	0.015	0.055	0.662	0.509	-0.039	-0.072	-0.665	0.508	0.026	0.045	0.465	0.643
PCA factor 1	0.189	0.099	1.136	0.258	<b>1.422</b>	<b>0.344</b>	<b>3.179</b>	<b>0.002</b>	-0.264	-0.054	-0.586	0.559
PCA factor 2	0.261	0.081	0.898	0.371	<b>-1.439</b>	<b>-0.222</b>	<b>-2.066</b>	<b>0.042</b>	-0.345	-0.050	-0.542	0.589
Nectar gland	<b>7.192</b>	<b>0.311</b>	<b>3.293</b>	<b>0.001</b>	10.429	0.169	1.559	0.123	<b>13.955</b>	<b>0.255</b>	<b>2.573</b>	<b>0.011</b>
Robbing intensity	<b>-1.533</b>	<b>-0.225</b>	<b>-2.667</b>	<b>0.009</b>	-0.209	-0.011	-0.103	0.919	<b>-3.944</b>	<b>-0.188</b>	<b>-1.988</b>	<b>0.049</b>

Coefficient = regression coefficient;  $\beta$  = regression coefficient with standardized variables with a mean of 0 and a standard deviation of 1. The coefficients with *P* < 0.05 are highlighted in **bold**

pollinator. In both populations, while the nectar gland size was positively correlated and presented a high contribution when explaining successful pollinations (regression coefficients of 7.2 and 14.0), the nectar robbing intensity was negatively correlated (regression coefficients of -1.5 and -3.9; Table 5). The remaining variables were not significantly correlated and presented low regression coefficients. In Serrat dels Boixos, a different scenario was observed, with factors 1 and 2 of the PCA being the only significant variables presenting opposite correlations (Table 5). Factor 1 of the PCA (where all corolla traits presented strong factor coordinates; Appendix) was positively correlated with the occurrence of an efficient visit, while factor 2 (where the crest presented a strong contribution; Appendix) was negatively correlated. Nevertheless, one should note that, despite being not significant, the nectar gland size presents a highly positive regression coefficient (10.4; Table 5).

Nectar robbing was a steady feature in all of the studied populations. The results of the multiple linear regression analysis of the effect of floral traits on the occurrence of nectar robbing are presented in Table 6. Positive and significant correlation coefficients were only obtained for the variables integrated in factor 2 of the PCA in the Coldecarrera population (where corolla tube and crest presented the strongest contributions; Appendix). No further morphometric traits exhibited significant regression coefficients for any of the studied populations (Table 6).

## Discussion

Several features are involved in floral traits selection, with legitimate pollinators and nectar robbers being two of the key factors in the dynamics of this process (Irwin et al. 2001; Irwin 2006). The behavior and preferences of visitors and their efficiency in pollen transfer has major impacts in the reproductive outputs of the plant and, subsequently, in the selection of several traits.

The elaborated corolla morphology of *P. vayredae* results in a long corolla tube where nectar is enclosed. Consequently, only a group of visitors with long proboscides are able to access the nectar through a legitimate visit, with the remaining visitors piercing the corolla tube or the upper sepal to access the nectar. This is similar to what was observed in other studies on tubular flowers, where illegitimate visits by nectar robbers were a frequent phenomenon (e.g., Navarro 1999; Urcelay et al. 2006). As nectar robbing was a steady element in all of the studied populations and as it can affect the pollen exportation in this species (Castro 2007), nectar robbers might play a major role in further plant-visitors interactions, being potentially involved in selective pressures over *P. vayredae* floral traits.

The existence of floral traits variability is considered to be the first step for selection by floral visitors (Herrera 1990, 1996). Flowers of *P. vayredae* exhibited considerable variability in several floral characteristics, with the corolla traits being generally positively correlated to

**Table 6** Results of the multiple linear regressions to evaluate the effect of the floral traits on the probability of receiving visit(s) by a nectar robber in each of the studied populations

Floral traits	Montmajor				Serrat dels Boixos				Coldecarrera			
	Coefficient	$\beta$	$t$	$P$	Coefficient	$\beta$	$t$	$P$	Coefficient	$\beta$	$t$	$P$
Angle	0.011	0.176	1.755	0.082	-0.004	-0.080	-0.686	0.495	-0.006	-0.073	-0.847	0.399
PCA factor 1	-0.045	-0.084	-0.883	0.379	0.094	-0.219	-1.885	0.064	-0.070	-0.127	-1.400	0.164
PCA factor 2	-0.107	-0.142	-1.500	0.137	-0.007	-0.011	-0.094	0.925	<b>0.251</b>	<b>0.270</b>	<b>2.921</b>	<b>0.004</b>
Nectar gland	-0.814	-0.137	-1.376	0.172	-0.332	-0.052	-0.446	0.657	-0.335	-0.050	-0.509	0.612

Coefficient = regression coefficient;  $\beta$  = regression coefficient with standardized variables with a mean of 0 and a standard deviation of 1. The coefficients with  $P < 0.05$  are highlighted in **bold**

each other. Thus, it is plausible that the increase in a corolla trait will result in a general increase of the corolla size and vice versa. This is in accordance with the assumption of better floral integration in species that depend on pollen vectors (Berg 1959; Armbruster et al. 1999). Furthermore, significant differences among the studied populations were observed for all of the analyzed characteristics, with exception of the nectar gland size. This characteristic presented considerable intrapopulation variability, resulting in a lack of significant differences among populations. The nectar gland dimension was positively correlated with nectar production, thus, it was a good measure to infer the main floral reward offered by the flowers of *P. vayredae*. This is in accordance with other studies among several species, where a good fit between flower length and the volume of secreted nectar was also found (e.g., Galetto and Bernardello 2004). As the nectar gland size was not correlated with the remaining characteristics in any of the studied populations, it seems that this floral trait varied independently within the flower, decoupled from the remaining corolla characteristics.

In general, successfully pollinated flowers of *P. vayredae* were smaller and produced larger nectar rewards. Moreover, they were subjected less to nectar robbing. Apparently, bigger flowers were more prone to be elected by nectar robbers, with legitimate pollinators being more likely to avoid these intensively robbed flowers (e.g., Irwin and Brody 1999). On the other hand, smaller flowers were less subjected to nectar robbing and presented higher female fitness. Therefore, in this species, it seems that the selection of some floral traits is indirectly driven by the nectar robbers, by potentially changing the pollinator's behavior. If so, the populations with greater nectar robbing will present smaller flowers. Actually, during the year of 2005, nectar robbing was more intense in Coldecarrera, the population that presents flowers with a smaller size. The robbers' preferences for larger flowers has already been observed in previous studies involving artificial flowers (Lara and Ornelas 2001) and in *Campsidium valdivianum* (Urcelay et al. 2006) and *Duranta repens* (Navarro and Medel 2007, unpublished data). Although variable, it has been previously shown that the indirect interactions of robbers on legitimate pollinators are important in driving the selection for some floral traits (Irwin 2006).

The correlation analyses within each population were not as straightforward, and different floral traits involved in successful pollination emerged. Nevertheless, the nectar gland, and, thus, the floral rewards, played an important role in female fitness, mainly in the Montmajor and Coldecarrera populations, with individuals with bigger nectar glands being generally selected. Also, in accordance with that exposed above and in spite of the low correlation coefficient, the behavior of nectar robbers seems to have influenced the subsequent behavior of legitimate pollinators. This was especially notorious in the Coldecarrera population, where robbers preferred flowers with bigger floral traits, while pollinators (which were negatively affected by nectar robbing) preferred flowers that were subjected less to robbing, i.e., indirect negative selection for smaller flowers. In Serrat dels Boixos, a complex correlation with floral traits was observed, with nectar robbing being an outside feature during 2005. On other hand, in Montmajor, robbers did not show any preference for the studied floral traits and, as a result, no significant effect of corolla traits in female fitness was detected. In this population, flowers with larger rewards, subjected less to nectar robbing were the ones presenting higher female fitness. Nevertheless, the selective pressures will vary with time according to the abundance and behavior of nectar robbers and legitimate pollinators, with further investigations involving the absolute frequencies of both types of visitors being needed to confirm the proposed hypothesis of the indirect negative selection of floral traits mediated by nectar robbers.

Despite several studies searching for the contribution of many factors on phenotypic selection, nectar robbing has been frequently disregarded (but see Irwin 2006). In the present study, although differences were obtained among populations, the results clearly revealed that nectar rewards and nectar robbing played an important role in the interactions between *P. vayredae* flowers and its legitimate pollinators. Thus, the contribution of nectar robbing, together with several other factors (e.g., herbivory, Herrera et al. 2002; Gómez 2003; Sánchez-Lafuente 2007; abiotic factors, Herrera 1993; diseases, Giles et al. 2006), cannot be overlooked and must be considered in future studies (Irwin 2006). Furthermore, whereas phenotypic variation has a genetic basis and, thus, if populations are able to respond to selection,

should also be assessed to further understand the mechanisms of floral evolution.

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## Appendix

Factor coordinates of the floral traits (corolla tube, keel, crest, and wing) obtained from the principal component analysis (PCA) performed for each of the studied populations of *Polygala vayredae*.

Population	Montmajor		Serrat dels Boixos		Coldecarrera	
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
Floral traits						
Corolla tube	-0.7300	0.5766	-0.7572	-0.4436	-0.5833	0.7265
Keel	-0.8525	-0.3337	-0.8375	0.2602	-0.8423	-0.2853
Crest	-0.7720	-0.5495	-0.6087	0.7203	-0.6810	-0.6250
Wing	-0.8096	0.3556	-0.8130	-0.3942	-0.8266	0.2931

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