RESEARCH PAPER

Pollination syndromes in a specialised plant-pollinator interaction: does floral morphology predict pollinators in *Calceolaria*?

M. Murúa[1,2] & A. Espíndola[3]

1 Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
2 Jardín Botánico Nacional, Viña del Mar, Chile
3 Department of Biological Sciences, University of Idaho, Moscow, ID, USA

ABSTRACT

Pollination syndromes are defined as suites of floral traits evolved in response to selection imposed by a particular group of pollinators (*e.g.*, butterflies, hummingbirds, bats). Although numerous studies demonstrated their occurrence in plants pollinated by radically different pollinators, it is less known whether it is possible to identify them within species pollinated by one functional pollinator group. In such a framework, we expect floral traits to evolve also in response to pollinator subgroups (*e.g.*, species, genera) within that unique functional group. On this, specialised pollination systems represent appropriate case studies to test such expectations. *Calceolaria* is a highly diversified plant genus pollinated by oil-collecting bees in genera *Centris* and *Chalepogenus*. Variation in floral traits in *Calceolaria* has recently been suggested to reflect adaptations to pollinator types. However, to date no study has explicitly tested that observation. In this paper, we quantitatively test that hypothesis by evaluating the presence of pollination syndromes within the specialised pollination system formed by several *Calceolaria* and their insect pollinators. To do so, we use multivariate approaches and explore the structural matching between the morphology of 10 *Calceolaria* taxa and that of their principal pollinators. Our results identify morphological matching between floral traits related to access to the reward and insect traits involved in oil collection, confirming the presence of pollinator syndromes in *Calceolaria*. From a general perspective, our findings indicate that the pollination syndrome concept can be also extended to the intra-pollinator group level.

INTRODUCTION

Floral specialisation has been widely discussed under the concept of pollinator syndromes, defined as suites of correlated floral traits (*e.g.,* corolla colour, shape or size) evolved in response to selection imposed by a particular group of pollinators (reviewed in Fenster *et al.* 2004). Some well-known examples of pollinator syndromes are those associating hummingbird pollination with diurnal tubular red or orange flowers (McDade 1992; Hargreaves *et al.* 2004; Martén-Rodríguez *et al.* 2010) or bat pollination in odorous and nocturnal flowers displaying light-coloured corollas (Flemming *et al.* 2009).

During the last decades, the conceptual framework of pollination syndromes has gained importance in evolutionary biology. Indeed, the concept was shown to provide a mechanistic explanation for floral diversity (*i.e.,* convergent adaptation) and allow inferring pollinator types based on floral traits (Fenster *et al.* 2004). In spite of this, the general validity of the concept has been sometimes questioned (*e.g.,* Waser *et al.* 1996; Johnson & Steiner 2000) since it appeared to be unreliable in predicting major pollinators in particular cases (Ollerton *et al.*

2009). For example, it has been observed that the accuracy of predictions differs across plant families, being more appropriate in Fabaceae, Apocynaceae and Asteraceae than in other groups (Ollerton *et al.* 2009). Furthermore, pollinator observations showed that plants are often visited by diverse pollinator species and that, in some cases, the most abundant pollinator is not necessarily the most efficient (Waser *et al.* 1996; Ollerton 1998; Rivera-Marchard & Ackerman 2006; Li & Huang 2009). Despite the critics, the applicability of this concept has been successfully demonstrated in numerous plant–pollinator systems (*e.g., Loasa*, Ackermann & Weigend 2006; *Silene*, Reynolds *et al.* 2009; *Raphanus*, Sahli & Conner 2006), and a recent meta-analysis across angiosperms provided an overwhelming demonstration on its reality (Rosas-Guerrero *et al.* 2014).

Although the concept is not new and many studies have been done on the subject, most have been performed on plants visited by a wide array of pollinators. In contrast, only a handful has been done in plant species pollinated exclusively by one functional group (*e.g.,* Hargreaves *et al.* 2004; Pauw 2006). This omission is unfortunate since, in highly specialised pollination systems, the floral traits responsible for the maintenance of the

specific interaction (*e.g.,* flower aperture size, amount of floral reward) are expected to be strongly correlated with the pollinator morphology (*e.g.,* body size, structures for floral reward collection), enhancing the syndromes.

A widespread case of highly specialised pollination involves oil-collecting bees from genera *Centris* Fabricius (Apidae: Centridini) and *Chalepogenus* Holmberg (Apidae: Tapinotaspidini), and the Andean–Patagonian oil-rewarding and nectar-less *Calceolaria* L. (Calceolariaceae; Vogel 1974; Molau 1988; Cosacov *et al.* 2009). *Calceolaria* species differ in several floral traits, especially those related to the corolla and the elaiophore (*i.e.,* the oil gland). These differences have been recently qualitatively evaluated by Sérsic (2004) to suggest two pollinator syndromes in the genus. Based on Sérsic's observations, the Tapinotaspidini syndrome is present in flowers displaying a flattened and not so much inflated lower lobe, which is larger than the upper one, and is used as a pollinator-landing surface. These flowers have been associated with visits by *Chalepogenus*, who collect oils through capillarity using hairs located in their fore-basitarsi (Cocucci *et al.* 2000). In this plant group, the elaiophore is immediately accessible and is located close to a reduced corolla aperture. In contrast, the Centridini syndrome is present in more morphologically diverse flowers. In these species, there is strong floral size variation and less clear correlations with pollinator shape. Moreover, the floral upper lobes can be hooded and the lower lobes are generally inflated, but never flattened. The latter are supposed to be used for floral handling during oil collection, as *Centris* actively scratches the elaiophore to collect oils using combs present on the fore- and mid-legs.

In a scenario of rarely tested pollination syndromes in specialised plant–pollination systems, the *Calceolaria*–pollinators interaction emerges as an appropriate biological model to further investigate the topic. Indeed, further understanding pollination syndromes in specialised interactions can deepen our knowledge on the evolutionary and ecological scales at which plant–pollinator relationships and adaptations emerge, evolve and establish. Here, we attempt to quantitatively test the presence of pollination syndromes in several *Calceolaria* species. Specifically, we ask the following questions: (i) do morphologically different *Calceolaria* species interact with different pollinator groups; and (ii) is there structural covariation between floral and pollinator traits in this plant group? To answer these questions we first identify and measure the main pollinators of ten *Calceolaria* taxa. Later, we quantify the floral morphology of these plants and use these data to explore morphological plant–pollinator correspondence using a multivariate approach.

MATERIAL AND METHODS

Sampling

This study was conducted during the spring–summer seasons of 2011–2013 on nine *Calceolaria* species (and two subspecies) from populations in central and southern Chile (Table 1). In each population, between ten and 20 plants of each species or subspecies were randomly chosen and floral traits were measured. An exception to this is the case of *C. arachnoidea*, for which six locations were sampled (110 plants).

During each visit, we took pictures of the flowers (see below) and recorded and collected pollinators. To do so, in each population and during four sunny days, we recorded and collected all insect species visiting the plant and entering into contact with the plant reproductive structures. The captured insects were afterwards identified and further measured in the laboratory (see below).

Floral and pollinator traits

We digitally measured seven floral traits (Fig. 1A–C) from pictures, using the software ImageJ 1.46r (http://rsb.info.nih.gov/ij/). For each species, the following floral traits were considered: (1) upper lobe length (ULL), (2) upper lobe width (ULW), (3) lower lobe length (LLL), (4) lower lobe width (LLW), (5) corolla area (CA), (6) aperture area (AA), and (7) elaiophore area (EA).

We identified pollinators using entomological keys, and classified them as either oil or pollen collectors. Based on pictures taken under a binocular microscope, we measured four insect morphological traits (Fig. 1D–F): (1) total length (TL), (2) thorax width (TW), (3) maximum extension for oil collection (MOC) and (4) length of the oil collecting zone (LOZ). Specifically, the maximum extension for oil collection (MOC) was estimated as the sum of the leg total length and the thorax height. As mentioned above, the two main pollinator groups

Table 1. Geographic coordinates and mean floral trait values of sampled *Calceolaria* species.

species	coordinates	ULL	ULW	LLL	LLW	CA	AA	EA
C. arachnoidea	36°36′ S/72°00′ W	0.21 ± 0.004	0.47 ± 0.01	0.78 ± 0.01	1.01 ± 0.01	0.77 ± 0.02	0.21 ± 0.01	0.08 ± 0.002
C. biflora	50°52′ S/72°44′ W	0.28 ± 0.03	0.77 ± 0.03	1.56 ± 0.05	2.27 ± 0.09	4.32 ± 0.24	0.53 ± 0.02	0.51 ± 0.03
C. cana	35°35′ S/70°00′ W	0.23 ± 0.03	0.40 ± 0.03	0.88 ± 0.03	1.01 ± 0.04	1.20 ± 0.02	0.16 ± 0.01	0.06 ± 0.004
C. corymbosa	37°46′ S/72°47′ W	0.45 ± 0.02	0.51 ± 0.03	0.92 ± 0.05	1.26 ± 0.06	1.50 ± 0.14	0.25 ± 0.03	0.08 ± 0.01
C. crenatiflora	38°28′ S/71°38′ W	0.43 ± 0.03	0.85 ± 0.02	2.43 ± 0.07	2.18 ± 0.07	5.61 ± 0.26	0.39 ± 0.02	0.13 ± 0.01
C. filicaulis	36°36′ S/72°00′ W	0.95 ± 0.03	1.21 ± 0.03	1.07 ± 0.04	2.30 ± 0.07	0.25 ± 0.004	ND	0.28 ± 0.01
C. fil. ssp. *luxurians*	33°19′ S/70°16′ W	0.23 ± 0.02	0.45 ± 0.01	1.26 ± 0.03	1.78 ± 0.04	2.01 ± 0.08	0.20 ± 0.02	0.60 ± 0.02
C. paralia	35°02′ S/70°36′ W	1.06 ± 0.03	1.19 ± 0.02	1.71 ± 0.05	0.38 ± 0.01	0.61 ± 0.02	0.42 ± 0.02	0.19 ± 0.02
C. purpurea	33°23′ S/70°27′ W	0.47 ± 0.02	0.60 ± 0.03	0.40 ± 0.03	0.87 ± 0.04	0.60 ± 0.05	0.11 ± 0.01	0.04 ± 0.003
C. valdiviana	37°49′ S/72°57′ W	0.49 ± 0.01	0.70 ± 0.01	0.83 ± 0.02	1.43 ± 0.04	1.37 ± 0.05	0.09 ± 0.01	0.09 ± 0.01

ULL, upper lobe length; ULW, upper lobe width; LLL, lower lobe length; LLW, lower lobe width; CA, corolla area; AA, aperture area; EA, elaiophore area; ND, no data.
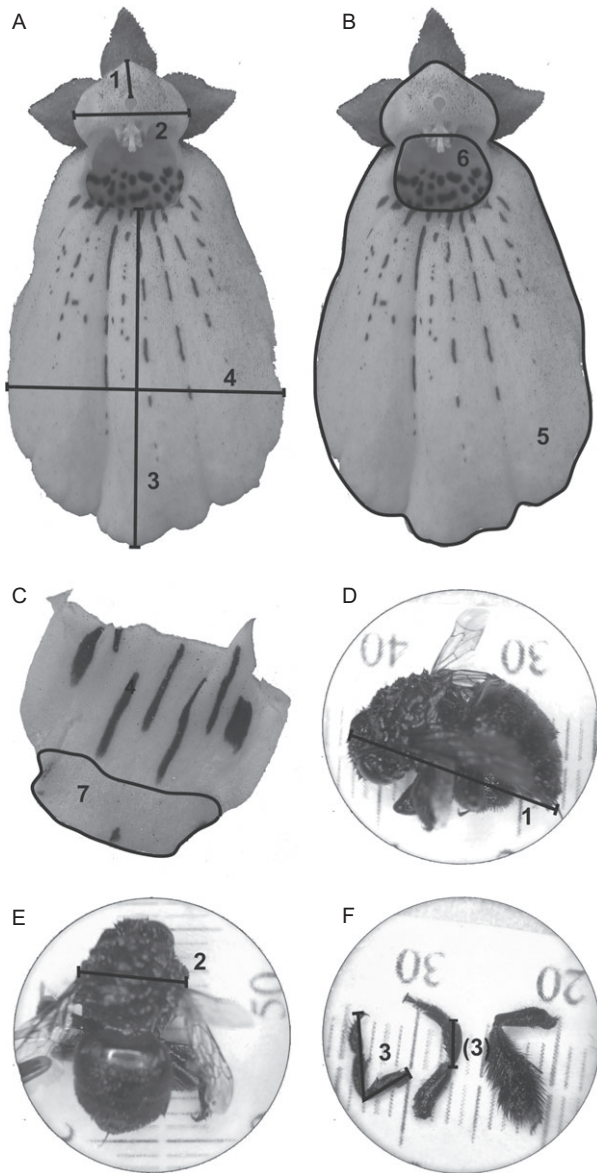Values are mean ± SD.

Fig. 1. Floral and pollinator traits measured from digital pictures. (A–C) *Calceolaria* morphology: (1) upper lobe length (ULL), (2) upper lobe width (ULW), (3) lower lobe length (LLL), (4) lower lobe width (LLW), (5) corolla area (CA), (6) aperture area (AA) and (7) elaiophore area (EA). (D–F). Pollinator morphology: (1) total length (TL), (2) thorax width (TW) and (3) length of the oil-collecting zone (LOZ).

display oil collection structures in either the first (*Chalepogenus*) or the first and second (*Centris*) pair of legs. Then, LOZ was calculated as the sum of the lengths of the first two leg pairs in *Centris* and as the length of the first pair in *Chalepogenus*. Because these structures are absent from pollen collectors (*i.e.,* non-oil-collecting bees), we did not measure LOZ in those cases.

Statistical analyses

First, to determine the morphological association between flowers and insects, we calculated Pearson's correlation coefficients between all measured traits. In this test, a significant

correlation between plant and insect group traits would support the idea of presence of pollination syndromes in the system. Furthermore, such an analysis helps identify traits that could play key ecological and evolutionary roles in this specialised pollination system.

Second, we used a multivariate statistical approximation to evaluate the link between plant morphology and pollinator identity. For this, we used a principal components approach, and evaluated the clustering of all plant samples in their morphological multidimensional space. In this same analysis, we also evaluated the correlation between the trait vectors and the first three principal components. Afterwards, we examined our data to identify an eventual clustering of plant traits based on pollinator composition. The observation of such a clustering would further support the presence of pollination syndromes in the group, since it would demonstrate that particular floral morphologies are associated with particular pollinator groups. All statistical analyses were performed on standardised log-transformed data in JMP 8.0 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

General floral structure and pollinators

From a morphological point of view, all *Calceolaria* species had an upper lobe almost twofold larger than the lower lobe (Table 1). While *C. paralia* was the species with the largest upper lobe (ULL: 1.06 cm, ULW: 1.19 cm), *C. arachnoidea* (ULL: 0.21 cm; ULW: 0.47 cm) and *C. cana* (ULL: 0.23; ULW: 0.4 cm) had the smallest. Regarding the lower lobe, *C. crenatiflora* presented the longest (2.43 cm) and *C. purpurea* the shortest (0.4 cm). *Calceolaria crenatiflora* displayed the largest corolla area (CA), whereas *C. filicaulis* presented the smallest. The corolla aperture (AA) was one of the traits with the highest variability among species [coefficients of variation (CV): 15–29%], with *C. biflora* displaying the largest aperture and *C. valdiviana* the smallest. Finally, the elaiophore area (EA) was highly variable (CV: 14–56%), with *C. filicaulis* ssp. *luxurians* displaying the largest gland area (0.6 cm^2) and *C. purpurea* the smallest (0.04 cm^2).

Pollination was performed only by Hymenoptera, which for the purposes of this study, may be classified as oil or pollen collectors. The two known genera of oil-collecting bees in *Calceolaria* (*i.e.,* *Centris* and *Chalepogenus*) were responsible for pollination in six of the ten *Calceolaria* taxa (Table 2) studied here. On this, *C. arachnoidea* and *C. paralia* were exclusively pollinated by *Centris nigerrima*, while *C. filicaulis* and *C. valdiviana* were exclusively pollinated by *Chalepogenus* spp. Both *Centris* and *Chalepogenus* were involved in the pollination of *C. corymbosa*, *C. filicaulis* ssp. *luxurians* and *C. purpurea*. *Calceolaria biflora*, *C. cana* and *C. crenatiflora* were exclusively visited by pollen-collecting bees: *Megachile semirufa*, *Anthidium* sp. and *Bombus terrestris*.

Regarding the morphology of pollinators, most traits were larger in *Centris* than in *Chalepogenus* (Table 2). As expected, this was particularly so for the structures responsible for flower handling and oil collection (MOC and LOZ). All pollen-collecting bees had a size similar to *Centris*, with *Bombus terrestris* and *Megachile semirufa* having the largest values, and *Anthidium* sp. the smallest.

Table 2. Mean ± SD for traits measured on the principal pollinators of the sampled *Calceolaria* species.

pollinator type	plant species	TL	TW	MOC	LOZ	pollinator species
only *Centris*	*C. arachnoidea*	1.57 ± 0.06	0.73 ± 0.02	1.75 ± 0.06	0.68 ± 0.05	*Centris nigerrima*
	C. paralia	1.54 ± 0.02	0.69 ± 0.03	1.80 ± 0.09	0.55 ± 0.04	*Centris nigerrima*
only *Chalepogenus*	*C. filicaulis*	0.96 ± 0.05	0.37 ± 0.02	0.94 ± 0.04	0.14 ± 0.01	*Chalepogenus caeruleus*
	C. valdiviana	1.04*	0.35*	0.89*	0.16*	*Chalepogenus caeruleus*
both	*C. corymbosa*	1.39 ± 0.02	0.60 ± 0.01	1.72 ± 0.18	0.53 ± 0.06	*Centris nigerrima*
		0.86*	0.32*	0.83*	0.13*	*Chalepogenus caeruleus*
	C. fil. ssp. *luxurians*	1.60 ± 0.04	0.72 ± 0.02	1.67 ± 0.04	0.60 ± 0.01	*Centris nigerrima*
		1.19*	0.45*	0.59*	0.19*	*Chalepogenus caeruleus*
	C. purpurea	1.22 ± 0.28	0.95 ± 0.27	1.85 ± 0.14	0.61 ± 0.05	*Centris nigerrima*
		0.57 ± 0.12	0.39 ± 0.10	0.84 ± 0.28	0.13 ± 0.61	*Chalepogenus* sp.
pollen Hymenoptera	*C. biflora*	1.46 ± 0.17	0.71 ± 0.03	1.65 ± 0.08		*Megachiles semirufa*
	C. cana	0.94 ± 0.07	0.45 ± 0.01	0.97 ± 0.01		*Anthidium* sp.
	C. crenatiflora	1.69 ± 0.22	0.89 ± 0.23	5.52 ± 1.16		*Bombus terrestris*

TL, total length; TW, thorax width; MOC, maximum extension for oil collection; LOZ, length of the oil collection zone. Asterisks indicate N equal to one.

Plant–insect trait correlation

Overall, the Pearson's correlation matrix showed that all floral traits were highly correlated, while the same was true for all insect traits (Table 3). Most floral traits were positively correlated, with the exception of corolla area (CA), which was negatively correlated with the length of the upper lobe (ULL). Only two traits combinations were not significantly correlated (LLW *versus* ULL and CA *versus* ULW). Most pollinator traits were positively correlated, with the exception of the length of the oil-collecting structures (LOZ), which were not significantly correlated with the total body length (TL; Table 3).

The correlations between floral and insect traits indicated that insect total length was the only trait not significantly correlated with any floral character. Thorax width (TW) was negatively correlated with the length of the upper lobe (ULL, −0.16). The maximum leg extension (MOC) showed a significant positive correlation with LLL (0.15), CA (0.15) and AA (0.14). The length of the

oil collection zone (LOZ) was negatively correlated with the length and width of the upper lobe (−0.21 and −0.18, respectively).

Plant trait clustering

The principal components analysis (PCA) of floral traits indicated that the first three PC axes explained 48%, 26% and 12% of the total variance, respectively (Fig. 2). The first axis was positively correlated with high values of SLL, SLW, ILW, CA and EA (Fig. 2A). The second was positively correlated with high values of SLL and SLW, and negatively correlated with high values of ILW, CA and EA (Fig. 2A). ILL and AA were the only traits not directly correlated with either of the two axes (Fig. 2A). The third axis was positively correlated with high values of SLL, SLW, ILW and EA, and negatively correlated with high values of ILL, CA and AA (Fig. 2B). The evaluation of PCA loading factors revealed that multiple floral traits were responsible for the explained variances. Specifically, the length of the lower

Table 3. Pearson's correlation matrix per species between mean floral traits and mean pollinator traits.

	floral traits						pollinator traits			
	ULW	LLL	LLW	CA	AA	EA	TL	TW	MOC	LOZ
floral										
ULL	**0.88**	**0.38**	−0.03	**−0.19**	**0.31**	**0.15**	−0.1	**−0.16**	−0.09	**−0.21**
ULW		**0.2**	**0.58**	0.04	**0.54**	**0.26**	−0.07	−0.11	−0.05	**−0.18**
LLL			**0.38**	**0.68**	**0.68**	**0.44**	0.08	0.05	**0.15**	0.01
LLW				**0.57**	**0.21**	**0.51**	−0.03	−0.08	−0.03	−0.14
CA					**0.48**	**0.39**	0.06	0.09	**0.15**	0.03
AA						**0.39**	0.11	0.1	**0.14**	0.04
pollinator										
TL							0.02	−0.03	−0.04	−0.09
TW								**0.44**	**0.47**	0.1
MOC									**0.6**	**0.42**
LOZ										**0.52**

Floral traits: ULL, upper lobe length; ULW, upper lobe width; LLL, lower lobe length; LLW, lower lobe width; CA, corolla area; AA, aperture area; EA, elaiophore area. Pollinator traits: TL, total length; TW, thorax width; MOC, maximum extension for oil collection; LOZ, length of the oil collection zone.
Bold numbers indicate significant correlations at $P < 0.05$.
Empty cells correspond to traits absent in pollen-collecting bees; asterisks indicate N equal to one.
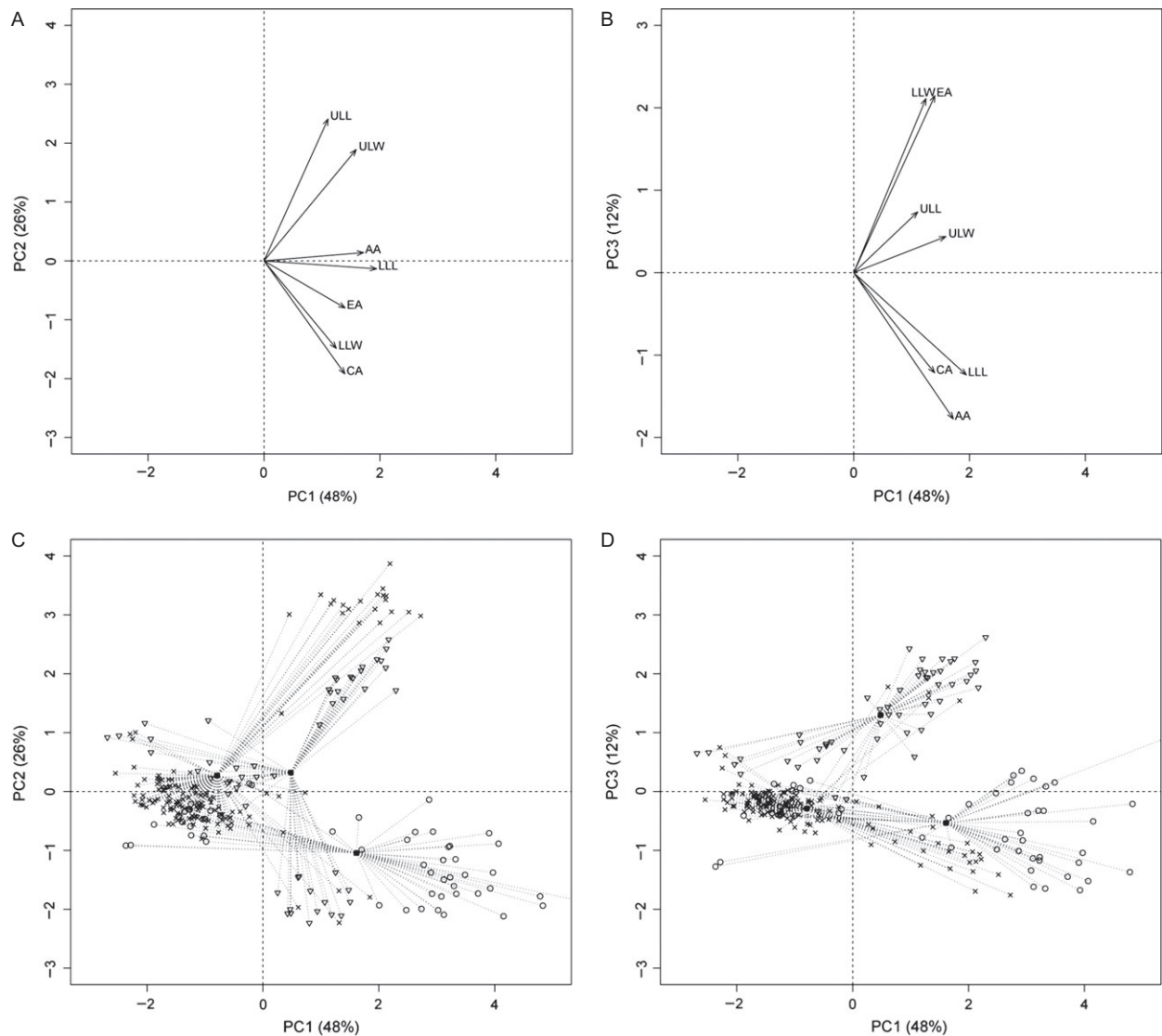
Fig. 2. Principal components analysis (PCA) for all floral traits highlighted in the observed pollinators for each measured *Calceolaria* species. (A) PCA vectors for the first and second PCA axes. (B) PCA vectors for the first and third PCA axes. Codes for floral traits are: upper lobe length (ULL), upper lobe width (ULW), lower lobe length (LLL), lower lobe width (LLW), corolla area (CA), aperture area (AA) and elaiophore area (EA). (C) Pollinator clustering of the first and second PCA axes. (D) Pollinator clustering of the first and third PCA axes. Circles: pollen collectors; triangles: *Chalepogenus*; crosses: *Centris*. Percentage of explained variance is shown on the axes.

lobe (LLL: 0.49) and the corolla aperture (AA: 0.43) similarly explained the first component. While the second component was mostly explained from the length of the upper lobe (ULL: 0.6), the third component was mostly explained from both the width of the lower lobe (LLW: 0.54) and the elaiophore area (EA: 0.53).

The clustering observed in the plant samples when using the first two principal components could be partially correlated with the pollinator groups (Fig. 2A,C). The first group was formed mainly of plants with large upper lobes (ULL and ULW), which appeared to be visited by oil-collecting bees (blue, Fig. 2C,D). The second group contained all plants with larger elaiophore areas (EA) and bigger lower lobes (LLL and LLW), which were visited by all pollinator types. The clustering defined by the first and third principal components roughly formed two groups (Fig. 2B,D). The first one included plants

displaying large upper (ULL and ULW) and lower lobes (LLW), as well as large elaiophore areas (EA), which were solely visited by *Chalepogenus* (triangles). The second contained plants with small lower lobes and elaiophore areas that were visited both by *Centris* and pollen-collecting Hymenoptera.

DISCUSSION

In this study, we aimed to evaluate the presence of pollinator syndromes in a specialised pollination. To do so, we tested the correlation and clustering between floral and pollinator morphology in ten *Calceolaria* taxa. Globally, our results support the existence of the pollinator syndromes previously proposed in Sérsic (2004), indicating that floral traits in *Calceolaria* are good predictors of the pollinator group. In the following

section, we review the structural correlation between flowers and pollinators identified in this work, and we discuss the general applicability of the pollination syndrome concept to specialised pollination systems.

Floral and pollinator morphological correlations

The relationship between floral parts and pollinator structures are well known for different plant systems (Fenster *et al.* 2004; Rosas-Guerrero *et al.* 2014). Some known cases are the strong correlation between floral tube and proboscis length in *Aquilegia* (Whittall & Hodges 2007) and *Platanthera* (Boberg & Agren 2009). The same type of relationship has been also documented for different oil-secreting flowers and their oil-collecting bees, including *Diascia* (Steiner & Whitehead 1990), *Bowkeria* (Steiner & Whitehead 1991) and *Colpias* (Steiner & Whitehead 2002). All these studies concluded that both leg size and the structures responsible for oil collection condition the access to the floral reward, promoting the evolution of longer spurs.

Our results showed that all the floral traits studied here were strongly and significantly correlated, while the same was true for the insect traits (Table 3). Specifically, most floral traits were positively correlated, which is not surprising since they originate in the same floral whorl (Conner & Sterling 1995; Diggle 2002). Pollinator traits were also positively and significantly correlated, with the exception of total body length (TL), which did not significantly correlate with any other pollinator traits (Table 3). The absence of correlation between TL and MOC agrees with developmental studies (Steiner & Whitehead 1990) and can be explained in our study as a morphological decoupling between traits strongly related to the pollination interaction and those not directly involved in it.

The correlation analysis between floral and pollinator traits showed that all pollinator traits (besides pollinator total length, TL) were significantly correlated with some plant trait (Table 3), supporting the idea of plant–pollinator morphological fitting. The lack of correlation displayed by TL could indicate that traits directly related to oil collection (*e.g.,* LOZ, MOC) play a more important role in the evolution of floral morphology and the establishment of pollination syndromes in *Calceolaria* than traits that are not directly involved in pollination.

The insect thorax width (TW) and length of the oil collection zone (LOZ) were negatively correlated with the length and width of the upper floral lobe (ULL and ULW). The correlation between TW and the two plant traits agrees with previous knowledge on the *Calceolaria* interaction. Indeed, it has been proposed that in this plant group, the upper lobe plays a role in protection of the reproductive structures, but also in increasing the accuracy of pollen deposition (Sérsic 1991). In this framework, the correlation observed in our dataset can be a direct signature of the pollination interaction. Indeed, a larger upper lobe could increase the precision in pollen deposition on the insect body, which might be more necessary in narrow insects. Instead, the negative correlation between LOZ and the length and width of the upper lobe is likely related to the strong correlation present between TW and LOZ (Table 3), more than to a direct functional relationship between the upper lobe size and pollinator oil-collecting structures.

The maximum leg extension (MOC) exhibited a positive and significant correlation with the length of the lower lobe (LLL), the corolla (CA) and the aperture area (AA). As discussed above, this association is probably related to the pollinator ability to handle and forage on these flowers. Thus, insects with long legs may be able to pollinate larger flowers (*i.e.,* flowers with large lower lobes and corolla areas) as they can easily hold and manipulate them (Sérsic 2004). Last, MOC was positively correlated with the corolla aperture area (CA), which may suggest the filtering role that the CA plays during pollination. Indeed, corolla aperture has been suggested to play a dual role as a facilitator of pollination and as a restrictor of other floral visitors (Schemske & Bradshaw 1999; Castellanos *et al.* 2004; Muchhala 2009). In the case of *Calceolaria*, it is likely that corolla aperture acts as a filter against less effective pollinators. The same process may favour the maintenance of the Tapinotaspidini syndrome, as it hinders oil collection by *Centris* on *Chalepogenus* flowers.

Pollination syndromes

The PCA on floral traits showed that floral lobes (*i.e.,* length and width of upper and lower lobes) are useful traits to identify the main specialised pollinators in this system (Fig. 2), which further supports Sérsic (2004) hypothesis of pollination syndromes in *Calceolaria*. Specifically, we found that large upper floral lobes were only associated with oil-collecting bees (triangles and crosses, Fig. 2C). We observed that when comparing the first and third PC axes, wide lower lobes (LLW) and long and wide upper lobes (ULL and ULW) were only associated with *Chalepogenus* (triangles, Fig. 2D). Moreover, even though *Centris* was associated with different floral shapes, it was more often observed pollinating flowers with short upper lobes (ULL; crosses in Fig. 2D). Finally, flowers displaying long lower lobes (LLL) and large corolla apertures (AA) were preferentially visited by pollen collectors (circles, Fig. 2C,D).

These relationships may be explained by the different functions played by each corolla part (Faegri & van der Pijl 1966; Stebbins 1970; Fenster *et al.* 2004) and by different pollinator foraging behaviour during floral visits (Sérsic 2004). In terms of the relationship between the upper lobe size and oil collectors, our results agree with Sérsic's (1991) idea on the role played by these structures in the pollen deposition process. Specifically, oil-collecting bees behave as buzz pollinators, shaking anthers and increasing the liberation and deposition of pollen on the body of insects (Vogel 1974). The shape of the upper lobe can then largely define and contribute to the specificity in pollen deposition, both on the insect and on the floral stigma. A morphological fitting between insect types and floral shape for this trait can thus very likely increase plant fitness, benefiting the establishment of such an oil-collecting syndrome. The association between wide lower lobes and *Chalepogenus* has been previously proposed (Sérsic 2004), and we were able to identify it here using quantitative methods. As suggested in previous studies, wider lower lobes might facilitate insect landing and floral manipulation, which can be particularly important for small bees like *Chalepogenus*. We also identified a more generalised floral preference in *Centris*, but we observed that this insect group was generally associated with short upper lobes. This suggests that it might be more advantageous for the plant to display smaller upper lobes if regularly visited by this massive insect. Such a floral character can improve pollen exposition by better exposing the pollen while

at the same time reducing floral damage. Finally, flowers visited by pollen collectors had long lower lobes and large corolla apertures, which could be facilitating pollinator landing and pollen collection by these non-oil-seeking insects.

Even though the pollinator syndrome hypothesis in *Calceolaria* was first suggested by Sérsic (2004), our study represents the first intent to quantitatively test it. On the one hand, this study confirms the existence of the previously suggested pollinator syndromes in *Calceolaria*. On the other hand, our work provides further support to the idea that pollination syndromes can be identified not only for different pollinator functional groups, but also for subgroups within them.

ACKNOWLEDGEMENTS

REFERENCES

Ackermann M., Weigend M. (2006) Nectar, floral morphology and pollination syndrome in *Loasaceae* subfam. *Loasoideae* (Cornales). *Annals of Botany*, **98**, 503–514.

Boberg E., Agren J. (2009) Despite their apparent integration, spur length but not perianth size affects reproductive success in the moth-pollinated orchid *Platanthera bifolia*. *Functional Ecology*, **23**, 1022–1028.

Castellanos M.C., Wilson P., Thomson J.D. (2004) 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, **17**, 876–885.

Cocucci A.A., Sérsic A.N., Roig-Alsina A. (2000) Oil-collecting structures in Tapinotaspidini (Hymenoptera: Apidae): their diversity, function, and probable origin. *Mitteilungen der Münchner Entomologischen Gesellschaft*, **90**, 51–74.

Conner J.K., Sterling A. (1995) Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in five insect-pollinated plants. *American Journal of Botany*, **82**, 1399–1406.

Cosacov A., Sérsic A.N., Sosa V., De-Nova J.A., Nylinder S., Cocucci A.A. (2009) New insights into the phylogenetic relationships, character evolution, and phytogeographic patterns of *Calceolaria* (Calceolariaceae). *American Journal of Botany*, **96**, 2240–2255.

Diggle P.K. (2002) A developmental morphologist's perspective on plasticity. *Evolutionary Ecology*, **16**, 267–283.

Faegri J., van der Pijl L. (1966) *The principles of pollination ecology*. Pergamon, Oxford, UK, 248 pp.

Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, **35**, 375–403.

Flemming T.H., Geiselman C., Kress W.J. (2009) The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany*, **104**, 1017–1043.

Hargreaves A.L., Johnson S.D., Nol E. (2004) Do floral syndromes predict specialization in plant pollination systems? An experimental test in an "ornithophilous" African *Protea*. *Oecologia*, **140**, 295–301.

Johnson S.E., Steiner K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, **15**, 190–193.

Li J.-K., Huang S.-Q. (2009) Effective pollinators of Asian sacred lotus (*Nelumbo nucifera*): contemporary pollinators may not reflect the historical pollination syndrome. *Annals of Botany*, **104**, 845–851.

Marten-Rodriguez S., Fenster C.B., Agnarsson I., Skog L.E., Zimmer E.A. (2010) Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist*, **188**, 403–417.

McDade L.A. (1992) Pollinator relationships, biogeography and phylogenetics. *BioScience*, **42**, 21–26.

Molau U. (1988) Scrophulariaceae – Part I. Calceolarieae. *Flora Neotropica*, **47**, 1–326.

Muchhala N. (2009) The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. *American Journal of Botany*, **93**, 1081–1089.

Ollerton J. (1998) Sunbird surprise for syndromes. *Nature*, **394**, 726–727.

Ollerton J., Alarcón R., Waser N.M., Price M.V., Watts S., Cranmer L., Hington A., Peter C.I., Rotenberry J. (2009) A global test of the pollination syndrome hypothesis. *Annals of Botany*, **103**, 1471–1480.

Pauw A. (2006) Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). *American Journal of Botany*, **93**, 917–926.

Reynolds R.J., Westrook M.J., Rohde A.S., Cridland J.M., Fenster C.B., Dudash M.R. (2009) Pollination specialization and pollination syndromes of three related North American *Silene*. *Ecology*, **90**, 2077–2087.

Rivera-Marchard B., Ackerman J.D. (2006) Bat pollination breakdown in the Caribbean columnar cactus *Pilosocereus royenii*. *Biotropica*, **38**, 635–642.

Rosas-Guerrero V., Aguilar R., Martén-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Bastida J.M., Quesada M. (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters*, **17**, 388–400.

Sahli H.F., Conner J.K. (2006) Testing for conflicting and nonadditive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution*, **65**, 1457–1473.

Schemske D.W., Bradshaw H.D. (1999) Pollinator preferences and the evolution of floral traits in monkey flowers (*Mimulus*). *Proceeding of the National Academy of Sciences of the USA*, **96**, 11910–11915.

Sérsic A.N. (1991) Observaciones sobree el mecanismo floral de *Calceolaria* (Scrophulariaceae). *Kurtziana*, **21**, 153–164.

Sérsic A.N. (2004) Pollination biology in the genus *Calceolaria* L. (Calceolariaceae). *Stapfia*, **82**, 121.

Stebbins G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms. I: pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.

Steiner K.E., Whitehead V.B. (1990) Pollinator adaptation to oil-secreting flowers – *Rediviva* and *Diascia*. *Evolution*, **44**, 1701–1707.

Steiner K.E., Whitehead V.B. (1991) Oil flowers and oil bees: further evidence for pollinator adaptation. *Evolution*, **45**, 1493–1501.

Steiner K.E., Whitehead V.B. (2002) Oil secretion and the pollination of *Colpias mollis* (Scrophulariaceae). *Plant Systematics and Evolution*, **235**, 53–66.

Vogel S.P. (1974) Ölblumen und ölsammelnde Bienen. *Tropische und Subtropische Pflanzenwelt*, **7**, 1–267.

Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.

Whittall J.B., Hodges S.A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, **447**, 706–712.