Do floral syndromes predict specialisation in plant pollination systems? Assessment of diurnal and nocturnal pollination of *Escallonia myrtoidea*

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Abstract   Pollination syndromes lead to questions concerning the role of different suites of pollinators on the pollination success of plants. *Escallonia myrtoidea* exhibits floral traits associated with pollination by diurnal butterflies; however, flowers remain open during the night and, thus, may also be pollinated by nocturnal moths. We assessed frequency of pollinator visits and pollination success in flowers exposed to diurnal and nocturnal pollinators in a factorial design. We also assessed pollinator dependency and limitations for seed set through hand self- and hand cross-pollination tests. Flowers were visited mainly by bees rather than by butterflies or moths. Neither diurnal nor nocturnal pollinators had any effect on seed set over the seed set of always-bagged inflorescences, while hand cross-pollinated flowers produced significantly more seeds than hand self-pollinated flowers or flowers exposed to diurnal and/or nocturnal pollinators. Thus, *E. myrtoidea*, a tree strongly pollinator-limited for seed setting, exhibited a lack of coupling among floral traits, pollinators, and reproductive success.

Keywords   floral traits; butterflies; moths; pollination success

INTRODUCTION

The correspondence between a suite of certain features in a flower with its proclivity to be pollinated by a specific suite of pollinators is still a pivotal subject in plant reproductive ecology (Ollerton & Watts 2000; Pellmyr 2002; Fenster et al. 2004). In fact, the concept of pollination syndromes, coined by Federico Delpino between 1868 and 1874, emerged in recognition that certain traits over-represented in flowers attract specific types of pollinators. The origin and/or maintenance of such particular traits would reflect the past or present selective pressures elicited by these pollinators. One of the major advantages of this concept is its apparent capacity to specify the type of pollinators of a given plant based only on observations of a certain number of floral traits (Pellmyr 2002; Fenster et al. 2004), namely flowering time, flower colour, flower scents, floral shape, floral position, type of reward, and nectar guides (Faegri & van der Pijl 1979). Nevertheless, this concept misled numerous biologists into thinking that specialisation in pollination systems, whereby few and specialised pollinators account for the reproductive success of a plant, is of more general occurrence than generalised systems, where numerous and unspecialised pollinators account for the reproductive success of plants (e.g., Stebbins 1970; Crepet 1984). Currently, however, it is widely accepted that generalism is more broadly represented than specialism in plant-pollinator relationships. This has led to a great controversy about the importance of the concept of pollination syndromes (Waser et al. 1996; Gómez 2002), and pollination syndromes are now considered as a criterion for inclusion rather than for exclusion, i.e., they do not imply the exclusion of other visitors to flowers (Pellmyr 2002; Fenster et al. 2004; but see Newstrom & Robertson (2005) for a current revision).

The recognition of generalism in pollination systems and the current concept of pollination syndromes have inspired work on the relative importance of different suites of pollinators in the reproductive success of a given plant species, and

whether the pollination syndrome allows one to predict the types and efficiency of such pollinators (e.g., Hingston & McQuillan 2000; Hargreaves et al. 2004).

   Escallonia myrtoidea Bert. ex DC. (Escalloniaceae) is a small tree of the Andes of central Chile. It has whitish, bell-shaped flowers producing a weak to moderate sweet scent, without nectar guides; hence, according to the pollination syndromes, *E. myrtoidea* should be a butterfly-pollinated tree (Faegri & van der Pijl 1979). Nevertheless, flowers stay open and sexually mature throughout two days and nights, and emit their scent throughout this time (pers. obs.). Because nocturnal lepidopterans are roughly similar to diurnal lepidopterans in terms of mouth morphology (Proctor et al. 1996), moths might successfully visit flowers of *E. myrtoidea* and contribute to its reproductive success (see Wolff et al. 2003). The aim of this work was to evaluate the relative importance of diurnal and nocturnal pollinators on the female reproductive success of *E. myrtoidea*. If this tree is specialised to being pollinated only by butterflies, seed set attained under a diurnal pollination regime will be higher than that produced under a nocturnal pollination regime (Young 2002; Fenster et al. 2004; but see Wolff et al. 2003). In addition, if *E. myrtoidea* is a butterfly-pollinated tree, diurnal lepidopterans will be the main floral visitors with respect to other diurnal visitors in terms of the frequency of visits, and probably also in terms of number of species (Proctor et al. 1996; Fenster et al. 2004; but see Vaughton 1992; Gómez & Zamora 1999; Potts et al. 2001).

MATERIALS AND METHODS

Species and study site

Escallonia myrtoidea is a small tree (6 m average height), inhabiting the Andes of central Chile from 30°S to 38°S and up to 2500 m a.s.l., mainly associated with humid habitats such as ravine bottoms, south-facing slopes, and areas near river banks (Rodríguez et al. 1983). It bears hermaphroditic flowers that produce an average of 90 300 pollen grains and 240 ovules per flower (Uslar 1982). Flowering and fruiting occur from December to March, during the austral summer, and seed dispersal begins in April (Rodríguez et al. 1983).

   The present study was conducted from January to April 2004 at the Yerba Loca Natural Sanctuary (33°10′–33°22′S, 70°13′–70°24′W), where vegetation consists of sub-Andean mountain scrublands and high-Andean plant communities, characteristic of the Mediterranean region (Uslar 1982).

Frequency of visits by floral visitors

A preliminary survey was carried out of visitors to flowers of *E. myrtoidea* in which all visitors to flowers over 5 days were caught and identified. Thereafter, a quantitative evaluation of visit frequency by each animal species was made through direct observations of inflorescences throughout a day in the peak of flowering time. Observations were made by simultaneously observing c. 5 inflorescences for a period of 15 min every hour throughout one day. A total of 8 days of observations was completed in order to describe the time course of visitation to *E. myrtoidea* throughout an average day.

Diurnal and nocturnal pollination

The net effect of floral visitors on the reproductive success of plants may be very different from that predicted by summing the effect of each visitor species in isolation (Fenster et al. 2004). Hence, in order to evaluate the relative importance of diurnal and nocturnal pollinators on seed production of *E. myrtoidea*, a fully balanced 2 × 2 factorial design was applied, thus allowing for a statistical dissection of the isolated and combined action of both types of pollinators. The two level treatments, i.e., diurnal (D) and nocturnal pollinators (N), were factorially combined into the following four combinations: (1) control flowers (+D+N), i.e., flowers exposed to diurnal and nocturnal pollinators (completely natural pollination), (2) diurnal treatment (-D+N), i.e., flowers only exposed to nocturnal pollinators (partially natural pollination), (3) nocturnal treatment (+D-N), i.e., flowers only exposed to diurnal pollinators (partially natural pollination), and (4) diurnal plus nocturnal treatment (-D-N), i.e., flowers excluded from both diurnal and nocturnal pollinators (autogamic pollination).

   Fifty flowers uniformly distributed among 7 inflorescences (c. 7 flowers per inflorescence), each from a different tree, were bagged at the bud stage with a tulle-mesh bag in order to prevent plant-pollinator interactions. When flowers were sexually mature, inflorescences were uncovered depending on the treatment described above. Thus, for the assessment of the whole natural pollination (+D+N), flowers were uncovered during two days and their following nights. For an autogamic pollination assessment (-D-N), flowers stayed bagged throughout the time of sexual maturity. Special care was taken

in avoiding any flower-bag contact that could allow self-pollination. For the assessments of the two trials of partial natural pollination, flowers were uncovered for 12 h depending on whether they correspond to diurnal (8:00 to 20:00 h) or nocturnal pollination treatment (20:00 to 8:00 h). The time at which flowers were covered or uncovered was determined in a preliminary survey of the time of segregation of diurnal and nocturnal floral visitors (data not shown).

Just prior to seed dispersal, fruits from each treatment were severed and the number of seeds counted. The effect of diurnal and nocturnal pollinators on seed production was assessed through a two-way ANOVA.

Pollinator dependency and pollinator limitations for seed set

To evaluate the compatibility system and whether *E. myrtoidea* is dependent on animal vectors for seed setting, an experiment involving hand pollination was done. Thus, in order to verify geitonogamy, 50 flowers uniformly distributed on 7 inflorescences (c. 7 flowers per inflorescence), each from a different tree, were emasculated (i.e., anther excision) at the bud stage and bagged with a tulle-mesh bag per inflorescence until sexual ripeness. Flowers were uncovered and hand pollinated with pollen produced by other flowers of the same tree. Similarly, in order to verify xenogamy, 50 flowers uniformly distributed on 7 inflorescences (c. 7 flowers per inflorescence), each from a different tree, were emasculated and bagged until sexual ripeness, and were hand pollinated with pollen from other trees. Pollen for the xenogamy test was obtained from trees growing at least 15 m away from the recipient tree (Sobrevila 1988).

Just prior to seed dispersal, fruits of both treatments were severed and the number of developed seed counted. In order to determine the degree of self-incompatibility, the index of self-incompatibility was determined as the ratio between seeds produced per crossed flower in hand self-pollinated flowers and hand cross-pollinated flowers (Ruiz-Zapata & Arroyo 1978).

In addition, in order to determine if *E. myrtoidea* is limited by pollinators for seed set, the number of seeds produced in the pollination trials of diurnal

Table 1　Floral visitors and type of foraging (D, diurnal; N, nocturnal) to *Escallonia myrtoidea* in the Andes of central Chile.

| Birds	Insects			
Trochiliformes	Coleoptera	Diptera	Hymenoptera	Lepidoptera
Trochilidae	Cantharidae	Bombyliidae	Apidae	Geometridae
Sephanoides sephaniodes (D)	*Haplous* sp. (D)	*Cyrtomia* sp. (D)	*Apis mellifera* (D)	Sp. 1 (N)
	Merillidae	Drosophilidae	*Bombus dahlbomii* (D)	Sp. 2 (N)
Oreotrochilus leucopleurus (D)	*Arthrobrachus varians* (D)	*Drosophila* sp. (D)	*Bombus terrestris* (D)	Sp. 3 (N)
		Nemestrinidae	Chrysididae	Lycaenidae
		Trichophthalma sp. (D)	*Chrysis carinata* (D)	*Pseudolucia chilensis* (D)
		Syrphidae	Colletidae	
		Copestylum bradleyi (D)	*Colletes murinus* (D)	Nymphalidae
		Scaeva sp. (D)	Halictidae	*Vanessa carye* (D)
		Tabanidae	*Corynura chilensis* (D)	Pieridae
		Dasybasis pruinivitta (D)	*Corynura lepida* (D)	*Tatochila mercedis*
			Corynura scitulus (D)	
			Ruizantheda proxima (D)	
			Sphecodes chilensis (D)	
			Megachilidae	
			Megachile saulcy (D)	
			Pompilidae	
			Pepsis limbata (D)	
			Vespidae	
			Hypodynerus chilensis (D)	
			Hypodynerus colocolo (D)	
			Polistes buyssoni (D)	
			Stenodynerus scabriusculus (D)	
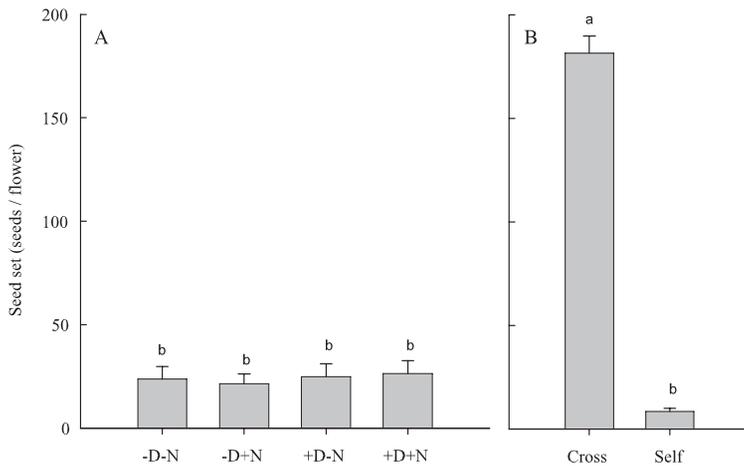
Fig. 1 A, Seed production of *Escallonia myrtoidea* exposed to (+) or excluded from (-) two contrasting pollination regimes; **B**, Seed production through hand self- and cross-pollination trials. Values depict the mean number of seeds ± 1SE. Fifty flowers were exposed to each treatment. Different letters depict significant differences. D, diurnal pollination; N, nocturnal pollination.

Table 2 Frequency of visits and types of floral insect visitors to *Escallonia myrtoidea.* Survey performed by observing 80 inflorescences during eight days and nights (mean ± 1SE). Ninety-six diurnal and 96 nocturnal censuses were performed. All visitors were diurnal, with the exception of the Geometridae.

Floral visitors	Family	Frequency of visits (No. of visits / inflorescence / 15 min)
Colletes murinus	Colletidae	0.150 ± 0.047
Hypodynerus chilensis	Vespidae	0.063 ± 0.027
Scaeva sp.	Syrphidae	0.050 ± 0.024
Pepsis limbata	Pompilidae	0.050 ± 0.024
Chrysis carinata	Chrysididae	0.038 ± 0.021
Corynura chilensis	Halictidae	0.038 ± 0.028
Drosophila sp.	Drosophilidae	0.038 ± 0.021
Trichophthalma sp.	Nemestrinidae	0.025 ± 0.017
Copestylum bradleyi	Syrphidae	0.025 ± 0.017
Megachile saulcy	Megachilidae	0.025 ± 0.017
Dasybasis pruinivitta	Tabanidae	0.013 ± 0.013
Hypodynerus colocolo	Vespidae	0.013 ± 0.013
Cyrtomia sp.	Bombyliidae	0.013 ± 0.013
Sp. 1	Geometridae	0.013 ± 0.013

Table 3 Summary of an ANOVA testing the effects of diurnal and nocturnal pollinators on seed set in *Escallonia myrtoidea.*

Source	d.f.	MS	*F*	*P*
Diurnal	1	441.05	0.257	0.613
Nocturnal	1	13.01	0.008	0.931
Diurnal × Nocturnal	1	174.85	0.102	0.750
Error	196	1715.94		

and nocturnal regimes (see diurnal and nocturnal pollination), together with seed set in hand-pollinated flowers, were assessed by applying a one-way ANOVA followed by Tukey HSD tests for multiple comparisons.

RESULTS

Frequency of visits by floral visitors

In the preliminary survey, *E. myrtoidea* was found to be visited by 31 different taxa of floral visitors, Hymenoptera being the most represented with 16 species (51.6%), followed by Lepidoptera with six species (19.4%), Diptera with five species (16.1%), and Coleoptera with two species (6.5%) (Table 1). Twenty-eight visitor taxa were diurnal and three moth taxa were nocturnal.

In the quantitative evaluation of the frequency of visits, Hymenoptera and Diptera were the main diurnal floral visitors, while only Lepidoptera visited flowers by night (Table 2). Diurnal floral visitors accounted for 97.7% of visits, while nocturnal floral visitors accounted for 2.3% of visits (Table 2).

Diurnal and nocturnal pollination

Seed set was not significantly improved by either nocturnal or diurnal pollinators over the seed set of always-bagged inflorescences (Fig. 1A; Table 3).

Pollinator dependency and pollinator limitations for seed set

The self-incompatibility index was 0.05, showing that *E. myrtoidea* is a xenogamous plant largely self-incompatible. In fact, hand cross-pollinated flowers set 21.5 times more seed than hand self-pollinated flowers (Tukey HSD test for a one-way ANOVA, $P < 0.001$; Fig. 1B). Concerning the effects of pollinators on seed set, hand cross-pollinated flowers set significantly more seeds than flowers exposed to diurnal pollinators ($P < 0.01$; Fig. 1), to nocturnal pollinators ($P < 0.01$; Fig. 1), and to diurnal plus nocturnal pollinators ($P < 0.01$; Fig. 1), and than flowers excluded from both types of pollinators ($P < 0.01$; Fig. 1). On the contrary, seed set attained in hand self-pollinated flowers did not differ significantly with respect to either seed set attained in flowers exposed to diurnal pollinators ($P = 0.351$; Fig. 1), nocturnal pollinators ($P = 0.621$; Fig. 1), diurnal plus nocturnal pollinators ($P = 0.262$; Fig. 1), or flowers excluded from both types of pollinators ($P = 0.431$; Fig. 1).

DISCUSSION

Escallonia myrtoidea, despite exhibiting floral traits that suggest pollination by butterflies, was more visited by diurnal Hymenoptera than by Lepidoptera. In fact, the relative importance of Lepidoptera, in term of frequency of visits, was lower than that of any other order of floral visitor, suggesting a minor role of butterflies for the reproductive succes of *E. myrtoidea* (Proctor et al. 1996; but see Vaughton 1992; Gómez & Zamora 1999; Potts et al. 2001). The very low number of diurnal lepidopteran species visiting this tree may be a result of the impoverishment of the lepidopteran fauna in central Chile due to Pleistocene glaciations (Peña & Ugarte 1996).

As far as pollination success is concerned, neither diurnal nor nocturnal floral visitors had a significant effect on seed set by *E. myrtoidea*. Seed set in hand cross-pollinated flowers was significantly greater than under natural pollination; hence, our population of *E. myrtoidea* may be considered a self-incompatible tree strongly limited by pollinators (sensu Bierzychudek 1981; but see Zimmerman & Pyke 1988). Moreover, given the large number of inflorescences in each tree, the large number of flowers per inflorescence, and the scarcity of pollinators, most pollen transfers will occur within the same tree (Snow et al. 1996), and only a small number of seed will set under natural pollination.

Nevertheless, the lack of coupling between floral syndrome, floral visitors, and pollination success leads to consideration of two mutually compatible explanations. The first deals with the ecological factors leading to the pollination success of *E. myrtoidea*. The strong limitation by pollinators for seed set may simply be due to the fluctuations in nature and quantity of pollinator populations within the season (Tables 1 and 2) and to the specific time and population assessed. A great variability in time and space has been observed in plant-pollinator relationships (Herrera 1996; Pellmyr 2002). For instance, the pollinator-dependent orchids *Anacamptis pyramidalis*, *Orchis palustris*, and *O. spitzelii* exhibit a great variability in pollinator-mediated selective pressures among years and among populations of insects acting upon traits such as flowering time and plant size (Fritz & Nilsson 1996). Because the present study was conducted in only one season and one population and no pollinator census is available for a longer period of time, there is no reason to suspect a constant pollinator assemblage across years and populations.

The second explanation deals with the evolutionary trends of plant-pollinator relationships. Plants are heavily constrained by their ecological context and evolutionary history (e.g., Herrera 1996; Armbruster 1997). Thus, a suite of floral traits currently observed in plants does not necessarily correspond to current selective pressures elicited by their pollinators since these traits may be a response to ancient selective pressures faced by the plant lineage (Herrera 1996; Pellmyr 2002). For instance, *Datura stramonium* may be considered as a moth-pollinated plant on account of bearing a suite of traits that attract moths. Nevertheless, its capacity for being self-pollinated and the observation of unpredicted visits by honeybees may strongly reduce the importance of moths as pollinators. This contrasts with congeneric counterparts that are successfully pollinated by moths as predicted by their pollination syndromes (Motten & Antonovics 1992; Motten & Stone 2000). A similar situation has been reported for species of *Nicotiana* which present a great number of species exhibiting both ecological and evolutionary constraints for variations in the amount and identity of volatile compounds emitted as floral scents (Raguso et al. 2003). Unfortunately, no information is available with respect to other *Escallonia* species which could bear on a phylogenetic explanation for the current pollination success observed in *E. myrtoidea*.

Consequently, at least for the population assessed and at the time assessment took place, the fact that the total floral visitors, and Lepidopterans in particular, did not represent a selective force conducting the phenotypic evolution of the particular suite of floral traits exhibited by *E. myrtoidea* suggests that the floral syndrome of *E. myrtoidea* is not a good predictor of its current relationship with pollinators, probably due to ecological and/or evolutionary constraints.

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