

The reproduction of *Colletia hystrix* and late-flowering in *Colletia* (Rhamnaceae: Colletieae)

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Abstract Plant fitness strongly depends on the timing of flower production. In temperate climates most plants bloom during a relatively well-defined peak, while comparatively few species flower before or after the community peak. Since a phylogenetical signal has been shown to exist in the determination of reproductive phenology, it is of interest to identify characters associated with the emergence of either type of behaviour. Here we report on the reproduction of the late-flowering shrub *Colletia hystrix* and discuss the results in the context of the whole genus *Colletia*. *Colletia hystrix* shares with its congeners deep flowers, associated with assemblages of long-mouthpart pollinators, and characters that maximise the chances of successful pollen receipt and export in a single pollinator visit (homogamy, a large stigma, and an extragynoecial compitum). Leaflessness and extreme spinescence of *Colletia* are suggested to be related (via compromised resource acquisition) to phenological displacement and its flower-level correlates.

Keywords *Colletia hystrix* · Breeding system · Pollination experiments · Reproduction · Phenology

Introduction

Plant fitness strongly depends on the seasonal timing of flower production, and several abiotic and biotic interactions are thought to shape the distribution of flowering times (Pilson 2000; Sola and Ehrlén 2007). In plant

communities of temperate climates, most species tend to bloom during a relatively well-defined spring peak, while few species flower before or after the community flowering peak. Concentration of flowering phenologies around a central peak may have no particular biological meaning in itself (randomly placed flowering periods will tend to overlap most frequently at the middle of the growing season, i.e. the ‘mid-domain’ effect, Morales et al. 2005). However, extremely early- or late-flowering implies exposure to both abiotic and biotic risks. These include harsh weather, insufficient pollination, increased herbivory, and others, which presumably have selected morphological and behavioural compensating traits (Kudo and Suzuki 2002; Mahoro 2003; Aizen 2003, 2005; Valtueña et al. 2008; Ronel et al. 2010; Munguía-Rosas et al. 2011; Agrawal et al. 2013). A recent meta-analysis (Munguía-Rosas et al. 2011) concluded that, rather than pollinators and seed predators, environmental conditions seem to be more important moderators of phenotypic selection on flowering time, although additional research is called for. This need seems particularly relevant for extremely early- and late-flowering species, because temperature is unlikely to directly select those uncommon reproductive behaviours (Aizen 2003).

Taxonomically related species tend to concur in particular flowering periods, indicating the existence of a phylogenetical signal in the determination of the reproductive phenology (Kochmer and Handel 1986; Johnson 1993). A given phenological behaviour can be a family-level trait, but it can also characterise taxa of lower ranks, like genera (Johnson 1993). Previous studies detected autumn- and winter-flowering in the small genus *Colletia* (Rhamnaceae) and provided relevant data on the reproductive biology of four of its five species (Skottsberg 1928; Tortosa 1989; D’Ambrogio and Medan 1993; Medan and

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Basilio 2001; Basilio and Medan 2001; Medan and Montaldo 2005; Basilio et al. 2006; Medan et al. 2006; Torretta et al. 2006; Aizen et al. 2012), but lack of data on *C. hystrix* Clos made it difficult to draw solid conclusions about which morphological and behavioural traits, if any, were consistently associated with late-flowering in *Colletia*.

In this study we first address the reproductive biology of *C. hystrix*, then analyse the results in the context of available information on other *Colletia* spp. We specifically asked (a) Which traits display *C. hystrix* as regards reproductive phenology, flower biology, and breeding system? (b) Are those traits consistent with those already known for other *Colletia* spp.? (c) Is there in *Colletia* a set of reproductive traits functionally related to late-flowering?

Materials and methods

Colletia hystrix The focal species grows in Chile and Argentina between sea level and 2,000 m a.s.l. as a spiny, nitrogen fixing, virtually leafless shrub that, depending on altitude and exposition, reaches a height of 0.4–4.5 m at reproductive age (Tortosa 1989; Tortosa and Medan 1989, D. Medan pers. obs.) (Fig. 1). Flowers are presented in condensed, 1–7 flowered brachyblasts which may be active for 2 years. Two such synflorescences are opposed at each node of flowering branches (Tortosa et al. 1996). Flowers are pedicellate, perfect, actinomorphic, 5-merous, 4 mm in diameter, with a 3 mm long \times 1.7 mm wide, campanulate, white floral tube, the lowermost part of which persists as a fruit pedestal (Medan and Aagesen 1995). The deltoid sepals and the stamens alternate at the rim of the floral tube. The anthers are raised 1.5 mm above the level of the floral tube opening. A revolute nectary is located at the lower half of the floral tube, delimiting a nectar chamber which is connected with the upper part of the tube by a circular opening 1.7 mm in diameter, which is traversed by the style (Medan and Aagesen 1995). The 3(4)-carpellate gynoecium is composed of a semi-inferior, 3(4)-ovulate ovary, a style, and a terminal stigma located at anther height or up to 1 mm above. Fruits are 1-3(4)-seeded explosive capsules. Voucher specimens (BAA 25677) are kept at the Gaspar Xuarez Herbarium (BAA, see Thiers 2014).

Study sites During the period 1988–2013 we worked on five populations, two of which were located in Chile and three in Argentina (Table 1). Short visits to populations 1–5 were devoted to recording plant phenological status, collecting flower visitors and assessing reproductive output and seed dispersal. Longer stays in 2006 and 2007 were devoted to further studying flower biology and undertaking controlled pollination experiments at population 4. At the

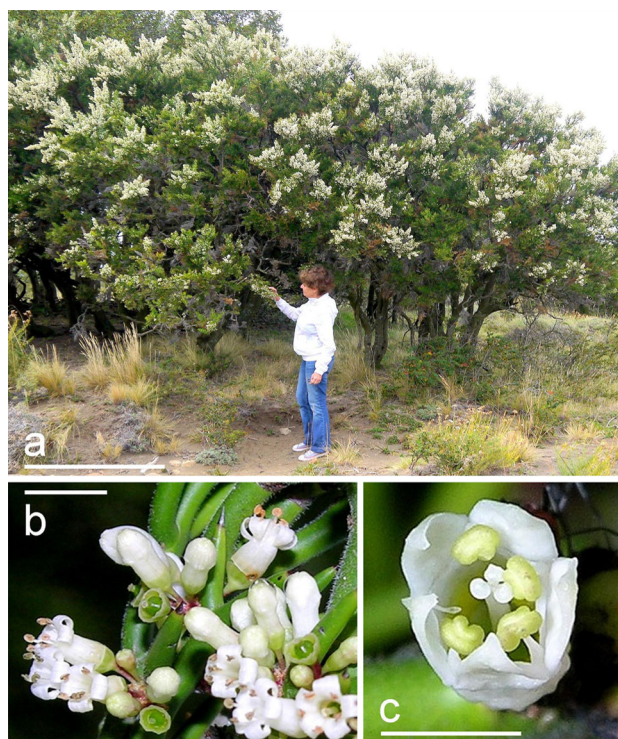


Fig. 1 *Colletia hystrix*. **a** habit, **b** three flowering nodes displaying from flower buds to recently initiated fruits, **c** a flower at stage 1 of anthesis, where one stamen is lacking, and another stamen adheres to the stigma. Bar 1 m (a), 5 mm (b) and 2 mm (c). Photos by D. Medan

latter site *C. hystrix* grows on rocky slopes dominated by *Nothofagus dombeyi* (Nothofagaceae), *Austrocedrus chilensis* (Cupressaceae), and *Lomatia hirsuta* (Proteaceae). Flowering zoophilous species in the plant community included *Carduus thoermeri*, *Haplopappus glutinosus*, *Hypochaeris radicata*, *Madia sativa*, *Mutisia oligodon*, *Senecio patagonicus*, *Solidago patagonica* and *Taraxacum officinale* (Asteraceae), *Echium plantagineum* (Boraginaceae), *Trifolium repens* (Fabaceae), and *Rosa eglanteria* (Rosaceae). Climate is cold temperate, with an annual rainfall of 1,500 mm. For further information on climate, soil, and vegetation of the study area see Movia et al. (1982), Roig (1998), Paruelo et al. (1998) and Eskuche (1999).

Reproductive phenology and pollination biology

Since *C. hystrix* populations are located farther than 1,200 km to the authors' laboratory, logistic reasons prevented the continuous monitoring of reproductive phenophases at any single population. However, the *Nothofagus* forests to which the focal species is associated have been intensively sampled by botanists over the last century, and herbarium specimens of *C. hystrix* are available for every month of the year and for the entire geographical area of

Table 1 Location of study sites, estimated size of populations, and dates of field work on *C. hystrix*

No.	Location	Population			Date(s) of work at population
		Estimated no. individuals	Geographic coordinates	Altitude (m a.s.l.)	
1	Near San Carlos de Apoquindo, Chile	50	33°21'55"S 70°28'02"W	960	11.01.1988
2	Cuesta La Dormida, near Til Til, Chile	50	33°04'00"S 70°59'00"W	1,000	24.01.1988
3	3.7 km SW of San Martín de los Andes, Argentina	40	40°10'34"S 71°23'26"W	720	31.03–03.04.1996
4	2.6 km S of San Martín de los Andes, Argentina	170	40°11'03"S 71°20'54"W	945	18–24.03.2006 19–23.03.2007
5	1.4 km SW of San Martín de los Andes, Argentina	100	40°10'03"S 71°22'10"W	750	30.03.2013

Table 2 Flower phenology of *Colletia hystrix*

	Day 0	Day 1	Day 2	Day 2	Day 3	Day 3
Floral trait/floral stage	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Sepals	Closed	Erect	Horizontal	Starting reflexion	Curved downwards	Pointing to flower tube
Pollen	Unavailable	Most anthers open	Most to all anthers open, pollen partly removed	Pollen mostly removed	Pollen removed	Pollen removed
Stigma	Lobes divergent, secretion incipient, peroxidase test+	Lobes divergent, secretion abundant, peroxidase test+	Lobes divergent, secretion abundant, peroxidase test+	Lobes divergent, secretion diminishes, peroxidase test+	Lobes connivent, secretion very scarce, peroxidase test+	Lobes wilting, no secretion, peroxidase test negative
Nectar	None	Present	Present	Present	Present	None

the species. Therefore, dates of blooming start and end are based on label data of 160 herbarium specimens cited in the *Colletia* taxonomic revision by Tortosa (1989), and on data from 15 additional specimens deposited at the BAA herbarium. As regards the use of herbarium specimens for phenological reconstructions see, e.g. Lavoie and Lachance (2006), Gallagher et al. (2009), and Robbirt et al. (2011). We confirmed and supplemented herbarium records with data gathered at the field, both at our main work sites (Table 1) and at other *C. hystrix* populations visited at mid December in the period 1997–2001 (Devoto et al. 2005).

The total number of flowers per flowering node was determined for a sample of 435 nodes taken from 4 individuals, and the number of open flowers per flowering node was determined for a sample of 270 nodes from a subset of 2 individuals. Twenty-five flower buds and open flowers were dissected and inspected under a 50 × stereomicroscope in the field, and 20 flowers from 2 individuals were individually labelled and monitored from the unopened bud stage until completed anthesis, to define floral morphological stages according to the position of sepals and the

status of pollen, stigma, and nectar presentation (Table 2). Stigmatic receptivity was tested with the Peroxtestmo Ko peroxidase test paper (Dafni and Motte Maués 1998). Nectar was extracted with handmade microcapillaries and sugar concentration was measured with a hand-held refractometer modified for small volumes.

Flower visitors were observed and sampled across populations 3–4 on different days and at different times of the day (cumulative time = 25 h) to record visitor profiles in as much detail as possible. Thirty-five insect individuals were caught for identification, and individuals from five additional visitor species were identified on the wing. Sixty-five per cent of the visitors' taxa were identified to the species level. When specific identification was not possible, insects were morphotyped. All collected material is deposited at the Entomological Collection of the General Botany Unit (FAUBA), at the School of Agronomy, University of Buenos Aires, or in collections of the assisting entomologists. The number of collected individuals was used as a primary measure of species abundance. Eight 10-minute visitor censuses were conducted at population 4

on two non-consecutive days in March 2006. Censuses involved 4 different plant individuals and included between 60 and 180 open flowers per census. For the study of pollen loads carried by the insects, individuals of the most abundant species were killed and stored in 70 % ethanol in separate vials. Two months later the insects were removed, the liquid was evaporated, and permanent microscopical slides were prepared with the solid residual. For comparison purposes, pollen samples taken from unopened flower buds, which were removed from field-collected plants or from herbarium specimens, were also made into microscopic slides. Pollen loads carried by one visitor species (*Bombus terrestris*) were reported elsewhere (Torretta et al. 2006).

Breeding system

To assess the effect of wind, insect visitors, and pollen origin (self vs. outcross) on fruit set, an experiment was conducted at population 4 in March 2006. Five treatments were applied to a total of 11 individuals: (1) *Open pollination* Flower buds were left uncovered and experienced natural pollination ($n = 1,905$ flowers). (2) *Mesh bagging* Flower buds were covered with 1 mm-mesh bridal veil bags, to prevent insect visits while enabling wind pollination ($n = 595$ flowers). (3) *Cloth bagging* As in (2), but using cloth bags, excluding both insects and airborne pollen ($n = 568$ flowers). (4) *Hand selfing* As in (3), but all flowers (except flower buds expected to open on the next few days) were removed and bags were closed, to be reopened 2 days later. Then, flowers with abundant stigmatic secretion (considered as an indication of stigmatic receptivity) were selected for manipulation. With the aid of a $10 \times$ hand lens, stigmas were visually controlled for lack of self-pollen and then pollinated by gently touching them with an anther of a freshly opened flower of the same individual. Pedicels of pollinated flowers were marked with fluorescent dye ($n = 51$ flowers). (5) *Hand outcrossing* As in (4), but pollen from a designated co-specific individual was used (mean distance between pollen donor and pollen receptor = 8.9 m) ($n = 66$ flowers). Experimental branches were collected 30 days after the start of the experiment, when all flowers had abscised or were developing a fruit. Fruit-to-flower ratios were computed and analysed with Kruskal–Wallis and Median tests.

To assess the size of stigmatic loads and the performance of pollen tubes under open pollination, late anthetic flowers of experimental plants were collected, fixed in FAA, transported to the laboratory, soaked for 60 h in 5 % NaOH (w:v) at 30 °C, cleared in diluted NaClO, mounted on 0.1 % decolorized aniline blue, and viewed with a fluorescence microscope. For each flower, the number of pollen grains per stigma was recorded, as well as the pistil

section reached by the farthest-growing pollen tubes (coded as 1 = stigma + upper style, 2 = mid style, 3 = lower style, 4 = upper ovary, or 5 = ovary bottom). The number of pollen tubes reaching the bottom of the ovary and the number of ovules penetrated by pollen tubes were also counted ($n = 105$ flowers). A limited number of hand self- and cross-pollinated flowers were also inspected in this way ($n = 12$ flowers).

Comparisons within *Colletia* and among Colletieae

Data on flowering times and on the proportion of long-mouthpart species (Hymenoptera, Lepidoptera and hummingbirds) in the visitor assemblages of *Colletia* spp. other than *C. hystrix* were taken from published sources (cited in the “Introduction”). Quantitative data concerning width of the stigma, used as a proxy of stigma size, and depth of the flower tube, were taken from Skottsberg (1928) (*Colletia spartioides*) and Medan and Aagesen (1995) (all other species of the tribe Colletieae) and compared using Mann–Whitney tests.

Results

Phenology and flower biology

Blooming extends from early January to end April. Within populations, individuals at different phenophases coexisted (e.g. plants opening their first flowers and plants already ripening fruit) indicating inter-individual variation in blooming start date.

Flowering nodes displayed 3.9 ± 2.5 flowers (arithmetic mean ± 1 SD) (range 1–12), of which 1.6 ± 1.0 were open at the same time. Flowers started anthesis at different times of the day and were active in pollen export and receipt for 2 days (Fig. 1b, c, stages 1–4 in Table 2). In flower buds dissected just before anthesis (stage 0) stigmatic lobes were already divergent and were covered by a clear and somewhat sticky secretion, also giving positive reaction to peroxidase test. No nectar was present yet. At stage 1 sepals opened, some anthers dehisced, stigmatic secretion increased, and nectar secretion started (Fig. 1c). At stages 2–3 sepals became horizontal (i.e. transversal to floral axis), then started curving downwards, all anthers completed dehiscence, pollen was mostly removed by flower visitors, and stigmatic secretion, receptivity and nectar secretion peaked. Stigmatic lobes were often covered by a single drop of secretion. In most individuals the stigma remained at anthers level, but in ca. 20 % of the plants the style elongated, raising the stigma 1 mm above the anthers. At stages 4–5 sepals completed their reflexion, pollen was completely removed, and stigmatic secretion,

receptivity, and nectar production declined and stopped. During stages 1–3 automatic (through direct contact of anthers and stigma, Fig. 1c) or visitor-facilitated deposition of self-pollen on stigma was possible. Nectar was generally present, but extractable amounts were found in only 30 % of the inspected flowers ($n = 40$; sugar concentration 20.8 ± 14.2 %, nectar volume 0.41 ± 0.4 μ l). A pleasant vanillin-like scent perceptible in full-bloom individuals probably originated from flowers at stages 1–4. Visits of insects occurred to flowers at those same stages between 9:00 am–04:00 pm, with temperatures around 9 °C or higher. After anthesis (stage 5 and later) the pedicel of fruiting flowers thickened, the ovary enlarged, and most of the flower tube with the sepals, stamens, and style gradually wilted and dropped.

Pollinators and pollination service

Seventeen species of insects belonging to nine families of Diptera, Hymenoptera and Lepidoptera were recorded as flower visitors (Table 3). One half of the visitors were butterflies or moths, but all species in this group were infrequent and played a secondary role in pollen transport.

Table 3 Identity and abundance of insect visitors to *Colletia hystrix* flowers

Order	Family	Species	Population	
			3	4
D	Syrphidae	<i>Allograpta hortensis</i> Philippi	1	1
D	Syrphidae	<i>Palpada meigenii</i> Wiedemann		1
D	Tachinidae	T. sp. 1		x
H	Apidae	<i>Apis mellifera</i> L.		x
H	Apidae	<i>Bombus dahlbomii</i> Guérin-Ménéville	4	10
H	Apidae	<i>Bombus ruderatus</i> Fabricius		2
H	Apidae	<i>Bombus terrestris</i> L.		7
H	Halictidae	<i>Ruizantheda proxima</i> (Spinola)	2	
L	Hesperiidae	<i>Hylephila</i> cf. <i>fasciolata</i> (Blanchard)		x
L	Noctuidae	N. sp. 1		1
L	Noctuidae	N. sp. 2		1
L	Noctuidae	N. sp. 3		2
L	Noctuidae	<i>Helicoverpa zea</i> (Boddie)		1
L	Nymphalidae	<i>Vanessa braziliensis</i> Moore	1	
L	Nymphalidae	<i>Yramea</i> cf. <i>lathonioides</i> (Blanchard)		x
L	Pyalidae	P. sp. 1		1
L	Satyridae	S. sp. 1		x

Putative pollinators appear in bold type. Figures indicate number of collected individuals. One ‘x’ indicates that the visitor was observed but not collected

D Diptera, H Hymenoptera, L Lepidoptera

Dipterans were both scarce and infrequent and their importance as pollinators was even lower. Hymenopterans were represented by five species and were much more frequent as visitors. The native bumblebee *Bombus dahlbomii* and the honey bee *Apis mellifera* were the only species recorded in the censuses. *B. dahlbomii* accounted for 25 times more visits to flowers of *C. hystrix* than *A. mellifera* did (cumulative visits during censuses = 500 vs. 20 flowers, respectively). *B. dahlbomii* visited flowers of *C. hystrix* in sequences of 2–10 flowers per flowering branch and up to ca. 50 flowers per individual, spending on average ca. 2.5 s/flower ($n = 442$ flowers). Individuals of *B. dahlbomii* carried 129.6 ± 209.6 pollen grains of *C. hystrix*, which amounted to 53.5 % of their complete body pollen load. Other plant species in loads of *B. dahlbomii* included *Carduus thoermeri*, *Solidago patagonica*, *Senecio patagonicus*, *Echium plantagineum*, and at least 4 additional species. Assuming constant visitation rates, 5 h a day of insect activity, samples of 100 flowers per census, and a duration of anthesis of 2 days, a typical *C. hystrix* flower of population 4 would receive 4.7 visits over its lifetime, i.e. ca. 2.3 visits per day, only from *B. dahlbomii*. Two exotic bumblebee species (*B. ruderatus* and *B. terrestris*) were far less frequent than *B. dahlbomii*, but one of these (*B. terrestris*) carried abundant *C. hystrix* pollen (Torretta et al. 2006). All three *Bombus* spp. and *Apis mellifera* are tentatively considered effective pollinators of *C. hystrix*.

Breeding system

The bagging experiment showed that pollination conditions affected fruit set (Table 4; Kruskal–Wallis $H = 14.63$, $p = 0.01$). Mean fruit set, which was 55.0 % in control branches, significantly dropped to 15.1 % under mesh bagging, 12.3 % under cloth bagging, and 5.9 % under hand selfing. As compared to selfing, hand outcrossing increased fruit set by a factor of 5, reaching a value (28.4 %) statistically close to that of open pollination ($p = 0.04$), suggesting that between-plant pollen transport by insects explained the fruit set of control plants. Between-plant flights were favoured by low inter-individual distance (mean = 3.4 m).

Pollen tube growth reflected, to some degree, pollination conditions and pollen identity. All sampled open-pollinated flowers showed large stigmatic loads ($n = 221.3 \pm 62.7$ pollen grains/stigma). Many pollen grains did not germinate and many pollen tubes were arrested in the stigma or style; however, pollen tubes reached the base of the ovary (at which level the micropyles of ovules are located) in 64.9 % of open-pollinated flowers ($n = 105$ flowers), a figure comparable to that of fruit set in the same pollination conditions (55.0 %, Table 4). Pollen tubes reached ovary bottom in all hand-crossed flowers but were arrested at

Table 4 Per cent fruit set of *Colletia hystrix* under five pollination treatments

	Treatment				
	Open pollination	Mesh bagging	Cloth bagging	Hand selfing	Hand outcrossing
Per cent fruit set	55.0 ± 33.9 ^a	12.3 ± 10.0 ^b	15.1 ± 17.5 ^b	5.9 ± 10.6 ^b	28.4 ± 33.7 ^b
<i>n</i> flowers/ <i>n</i> individuals	1,905/11	595/9	568/9	51/9	66/9

Values are mean ± SD. Kruskal–Wallis test for the whole experiment: $H = 14.63$, $p = 0.01$. Different exponents indicate significant differences among treatments (Median tests, $p < 0.05$)

mid-style in all hand-selfed flowers ($n = 6$ and 6 flowers, respectively).

Comparisons within *Colletia* and among Colletieae

The proportion of species with long mouthparts in the assemblage of visitors to *C. hystrix* (82.3 %) was within the range known for the genus *Colletia* (57.1–100 %). The stigma is wider in *Colletia* (genus average = 0.73 mm) than in the remainder of the tribe Colletieae (average = 0.44 mm) (Mann–Whitney $U = 51$, $p = 0.0088$, $n = 4$, 11). Likewise, the flower tube is deeper in *Colletia* (average = 5.62 mm) than in other genera of Colletieae (average = 3.17 mm) (Mann–Whitney $U = 54$, $p = 0.0015$, $n = 4$, 11).

Discussion

Colletia hystrix and the reproductive profile of *Colletia*. *Colletia hystrix* does not deviate from a set of characteristics collectively considered as a reproductive fingerprint of Colletieae (Medan et al. 2013): many small, coloured, relatively short-lived zoophilous flowers are displayed during a single annual reproductive episode, which extends over several weeks, allowing for both xenogamous and geitonogamous pollination, with selfing being at least partially hindered by self-incompatibility.

Colletia hystrix shows the genus-dominant white floral colour (flowers are red in *C. ulicina* only, Medan and Montaldo 2005). The pleasant vanillin-like scent of *C. hystrix* was also reported for *C. paradoxa* (D'Ambrogio and Medan 1993), *C. spinosissima* (Medan and Basilio 2001), and to a lesser degree for *C. ulicina* (Medan and Montaldo 2005). All four species offer pollen and nectar as rewards. The mean nectar concentration in *C. hystrix* (20.8 %) slightly expands the range known for the genus *Colletia* (14.2–18.8 %).

Homogamy, reported here for *C. hystrix* and present in all other *Colletia* spp. (including *C. spartioides*, Skottsberg 1928), is otherwise known only in the monotypic genus *Kentrothamnus* (Medan et al. 2013), thus being a nearly exclusive generic trait. The fixation of homogamy

increases pollen-stigma interference (Lloyd and Webb 1986; Bertin and Newman 1993) but it also allows pollen export and receipt in a single pollinator visit, which is adaptive in unpredictable pollination environments. In *C. hystrix* interference is apparently counterbalanced by two traits, one of which is common to all Colletieae (self-incompatibility) and other genus-exclusive: a significantly large stigma with abundant stigmatic secretion, which links the stigmatic lobes forming a single field where pollen grains stick and germinate (an extragynoecial compitum, Endress 1982; see Medan and Basilio 2001 for discussion). As reported here, the stigma is significantly wider in *Colletia* than in the remainder of the tribe Colletieae.

Likewise, the flower tube is significantly deeper in *Colletia* than in other genera of Colletieae, which probably explains the prevalence of species with long mouthparts in the assemblages of visitors to *Colletia* flowers. The native bumblebee *Bombus dahlbomii* was the main pollinator of *C. hystrix* and also a first-rank pollinator of the otherwise ornithophilous and partly sympatric *C. ulicina* (Medan and Montaldo 2005). *B. dahlbomii* is a generalist bumblebee widely distributed throughout southern South America, known to visit many species from at least seven plant families (Aizen et al. 2002). The exotic, invasive *B. terrestris* was the second most important visitor to *C. hystrix* when it was first discovered in Argentina (Torretta et al. 2006) during the field work for the present study, but in the meantime *B. terrestris* may have replaced *B. dahlbomii* as prime pollinator of *C. hystrix*, as it occurred with other plant species across most of *B. dahlbomii* range (Morales et al. 2013).

Species of social bees dominated the pollinator assemblages of *C. hystrix* and *C. ulicina*. In the *Nothofagus* forests such bees tend to remain active for longer periods of the year than solitary bees do. In fact, *Bombus* spp. and *Apis mellifera* were among the very few bees active at our field sites in autumn. Similarly, *C. ulicina* is co-pollinated by the native hummingbird *Sephanoides sephanioides*, of which only a few populations remain in the *C. ulicina* area, while most populations leave for central Chile during the fall (Medan and Montaldo 2005).

The bagging experiment and pollen-tube growth observations indicated that in *C. hystrix* wind pollination is unimportant, exclusion of flower visitors significantly

lowers fruit set, and access of pollen tubes to ovules is what triggers fruit initiation. Moreover, data from hand pollinations suggested a key role of outcross pollen in fruit set. Mean per-flower visitation rates and size of stigmatic loads of *C. hystrix* were large enough to suggest that its reproductive output, at least at the experimental sites and years studied, was not limited by pollen quantity. Limitation by pollen quality may have played a role, since pollen tubes failed to reach the micropyle level in ca. 35 % of open-pollinated flowers.

Late flowering in *Colletia*. The blooming period of *C. hystrix* (January to April) falls within the flowering period known for the remainder of the genus *Colletia* (January–September). All *Colletia* species start blooming in (austral) summer or later in the year, often peaking in autumn or winter, i.e. after their respective community's phenological peaks. In the Chilean temperate forests where *C. ulicina* belongs, peak occurs in December (Riveros and Smith-Ramírez 1996) before *C. ulicina* starts blooming (January–April). In April, the plant-pollinator network at the *C. ulicina* experimental sites included only 5 plant species (Medan and Montaldo 2005). Flowering of *C. spartioides* in the Juan Fernández islands occurs very late in the season (March–August), i.e. clearly after the community peak (Skottsberg 1928). The scenario on the eastern side of the Andes is similar, with community peak occurring in December (Devoto et al. 2005) and flowering of *C. hystrix* taking place from January to April in a gradually contracting interaction network (a dozen plant species co-flower with *C. hystrix* in March, out of ca. 30 species at peak bloom). Flowering of *C. spinosissima* at the 'Talar' xeric woods (NW of the Buenos Aires province in Argentina) peaks in winter, when the blooming plant community includes 4–6 plants, after the second (autumn) community peak in which 12 plant species participate (Basilio et al. 2006). Likewise, the autumn flowering peak of *C. paradoxa* in the hills of the Balcarce area (SE of the Buenos Aires province) follows the second (late summer) peak of its community (M. Sabatino, pers. comm.). *C. paradoxa* also shows late flowering when cultivated in the Northern Hemisphere (Lindley 1850; Fitzherbert 1911).

Correlates of late flowering in *Colletia*. As a consequence of their delayed flowering time, *Colletia* spp. integrate comparatively small plant-pollination networks, where competing plant species are fewer but available pollen vectors are also scarcer. The *Colletia* 'solution' to achieve adequate pollination service in such circumstances combines four traits, most of which are genus-exclusive: a restrictive flower morphology, which selects for long-mouthpart and probably more efficient pollinators, and three characters that maximise the chances of successful pollen receipt and export in a single pollinator visit: homogamy, a larger stigma, and a extragynoecial

compitum. At first view, as regards flowering time *Colletia* contrasts with all other genus of Colletieae, which tend to bloom during- or near the spring/early summer communal peaks (Primack 1979; Medan 1991, 2003; Medan and Arce 1999; Medan and D'Ambrogio 1998; Medan and Devoto 2005; Medan et al. 2013). A critical evaluation of the apparent singularity *Colletia* is called for.

Ronel et al. (2010) found that spinescence of plants in the semi-arid east Mediterranean region is associated with a delayed flowering season, which is of interest in view that the habit of all *Colletia* spp. is characterised by leaflessness and extreme spinescence (Tortosa 1989). The spiny habit of *Colletia* could have been acquired early in the evolution of the genus as an adaptation to drought, as increasing dryness developed in southern-mid latitudes in South America in post-Miocene times following the uprise of the Andes (Markgraf et al. 1995; Arroyo et al. 1996; Hinojosa and Villagrán 1997; Villagrán and Hinojosa 1997; Aizen and Ezcurra 1998; see also Houston and Hartley 2003). The leafless condition might compromise resource acquisition, causing a delay in the date of flowering. Recent micro-evolutionary work in *Oenothera* showed consistent selection of late-flowering genotypes, presumably because this allowed for greater resource acquisition before the onset of reproduction (Agrawal et al. 2013). If spinyess was an early acquisition in *Colletia*, phenological displacement and the flower-level correlates identified in this paper would be later evolutionary responses.

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