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ORIGINAL ARTICLE

Plant reproduction in the high-Andean Puna: *Kentrothamnus weddellianus* (Rhamnaceae: Colletieae)

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Abstract The global picture of plant reproduction at high altitudes is still diffuse due to conflicting reports (e.g., about which are the prevalent breeding systems) and incomplete geographical and taxonomic coverage of high-altitude ecosystems. This paper reports on the reproductive biology of *Kentrothamnus wedellianus*, a shrub inhabiting the Puna semidesert in Argentina and Bolivia at ca. 3,600 m a.s.l. A set of four traits, including a high pollen/low nectar floral reward strategy, homogamy, a dry stigma, and partial self-compatibility, appear to be central for *K. weddellianus* to accomplish sexual reproduction in the high-altitude Puna. The existence of an entirely different set of characteristics in the related species *Ochetophila nana* suggests that adaptation to reproduction at high altitudes can be achieved through different pathways. Hence, the final results may strongly diverge from the patterns found in lowland species of each lineage, or not. More case studies still seem to be necessary before specific patterns can be discerned within the panorama of high-altitude plant reproduction.

Keywords Andes · Colletieae · Breeding system · *Kentrothamnus* · *Ochetophila* · Pollination experiments · Puna · Reproduction

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Introduction

Abiotic conditions at high-elevation environments are characterized by low temperatures, strong winds, unpredictable storms, overcast skies, and short snow-free seasons (Körner 1999; Torres-Díaz et al. 2011). Diversity, availability, and activity of insect pollinators decline with elevation above the timberline, as documented by many studies (Torres-Díaz et al. 2011 and references therein). The reduction in pollinator availability is paralleled by an altitudinal turnover in the major pollinator groups (Arroyo et al. 1982; Medan et al. 2002). Flower visitation rates may decline with increasing altitude, not only because of the scarcity of pollinators but also because of diminished floral rewards (Ladd and Arroyo 2009). At community level, the severe conditions found at high elevations result in decreased number of potential visitors per plant species and less organized pollination networks (Ramos-Jiliberto et al. 2010).

Under the reproductive assurance hypothesis, a low-quality pollination scenario should lead to increasing selfing at higher altitudes. While some studies have actually found increases in self-fertilization as a result of elevation, there is also evidence of increased out-crossing and decreased selfing rates in such situations (Kühn et al. 2006; Torres-Díaz et al. 2011). In order to capture scarce visitors and maintain out-crossing, plants may increase flower showiness (Fabbro and Körner 2004) and flower longevity (Arroyo et al. 1982; Rathcke 2003; but see Steinacher and Wagner 2010), or they can alter other phenological traits such as the intensity and the degree of synchrony of flowering (Fagua and Gonzalez 2007, but see Duan et al. 2007). Although out-crossers are particularly prone to suffering from pollination limitation (Larson and Barrett 2000), recent studies indicate that at community level increased elevation is neither associated with increased pollen limitation (García-Camacho and Totland 2009) nor decreased genetic diversity (Thiel-Egenter et al. 2009). The abundance and even prevalence of out-crossers at high altitudes may reflect the advantage of maintaining high genetic variability at population level, which is considered a prerequisite for long-term persistence in stochastic environments (Lande and Shannon 1996). The preferentially out-crossing perennials can accumulate resources over time, thus becoming able to endure unfavorable periods (Kühn et al. 2006).

Shifting to wind pollination is another strategy to escape from insufficient biotic pollination (Arroyo and Squeo 1987; Linder 1998; Anderson et al. 2000). A mixed insect-wind pollination strategy (ambophyly) has been shown as most effective in alpine environments with low and infrequent pollinator activity and high wind speeds (Totland and Sottocornola 2001).

Alpine vegetation, which occupies 3 % of Earth's land area (Körner 1999), has been the target of much research. However, the global picture of plant reproduction in high-mountain ecosystems is still diffuse, partly due to incomplete geographical and taxonomic coverage. Knowledge is particularly scarce regarding the Southern Hemisphere, where the Andes of South America offer large unexplored research scenarios (Muñoz and Arroyo 2006). Moreover, reports on several important topics are conflicting, perhaps because altitudinal comparisons are rarely placed in the appropriate phylogenetic context (Kühn et al. 2006). For instance, by applying phylogeny-independent contrasts, Medan et al. (2002) showed that the prevalence of selfers at higher elevations in the Andes of Argentina did not reflect a community-wide increase of self-compatibility with altitude, but it merely resulted from an increase in self-compatibility in two species-rich families.

This paper reports on the reproductive biology of *Kentrothamnus wedellianus*, a shrub inhabiting the Puna semidesert in Argentina and Bolivia. The Puna is a high plateau forming part of the Andes of South America between 14°S and 32° 40′S, at elevations ranging from 2,700 to 4,200 m a.s.l. The climate is cold and dry, with frosts occurring throughout the entire year (Cabrera and Willink 1973; Martínez Carretero 1995). Considered as the world's second largest high plateau, after the Tibetan plateau, the Puna is virtually unexplored regarding plant reproduction. The genus *Kentrothamnus* belongs to the monophyletic tribe Colletieae in the Rhamnaceae (Aagesen et al. 2005). Most Colletieae inhabit lowlands and mid-montane habitats, but three genera include species which can be found at 3,300 m a.s.l. or higher in the Andes of South-America (Table 1). The addition of data on *Kentrothamnus weddellianus* to the existing knowledge about reproduction of Colletieae (references summarized in Table 1) may help to identify evolutionary shifts associated with adaptation of this lineage to high-mountain environments. Specifically, we searched for deviations from tribe-prevalent reproductive traits such as dichogamy, use of pollen and nectar as floral rewards, and self-incompatibility.

Materials and methods

Plant species

Kentrothamnus weddelianus (Miers) M.C. Johnst grows in Bolivia and Argentina between 2,500 and 3,800 m a.s.l. as a spiny, nitrogen fixing, sparsely leaved shrub that, depending on altitude and exposition, reaches a height of 0.5–4 m at reproductive age (Johnston 1973; Medan and Tortosa 1981, D. Medan *pers. obs.*). Flowers are presented horizontally ±60° in 1–3- flowered cymes grouped in 1–10-flowered axillary, leafy synflorescences that proliferate after anthesis. Two such synflorescences are opposed at each node of flowering branches (Tortosa et al. 1996). Flowers are pedicellate, perfect, actinomorphic, 5-merous, 5 mm in diameter, with a 4.5 mm long × 4 mm wide, slightly campanulate floral tube, whose lower half persists as a fruit pedestal (Johnston 1973; Medan and Aagesen 1995). Among individuals, the floral tube varies from whitish to reddish. The deltoid sepals and the hooded petals alternate at the rim of the floral tube. The stamens are opposite the petals, with the anthers raised 1 mm above the level of the floral tube mouth. An adpressed nectariferous disc lines the lower half of the floral tube (Medan and Aagesen 1995). The tri-carpellate gynoecium is composed of a superior, 3-ovulate ovary, a style, and a terminal stigma located at anther height at dehiscence and 1.5 mm above anthers at the end of anthesis. Post-anthetic flowers temporarily retain the style and gradually become erect as a result of bending of the pedicel. Fruits are 1–3-seeded explosive capsules. Voucher specimens are kept at Gaspar Xuarez Herbarium (BAA 17203).

Study sites

During the period 1995–2011, we worked on seven populations, three of which were located in Argentina and four in Bolivia (Table 2). Short visits to populations 1 and 4 through 7 were devoted to recording plant phenological status, collecting flower visitors, assessing scent emission and measuring nectar volumes and pollen quantity. For longer stays in 2009 and 2010–2011, controlled pollination experiments were made on populations 1, 2, and 3, located at Cuesta de Toquero, near the Argentinean-Bolivian border. Annual rainfall in this area is 300–400 mm, and

Table 1 Diversity, altitudinal range and present knowledge on reproductive biology of the tribe Colletieae (Rhamnaceae)

Genus	Total no. species/species with known reproductive biology	Altitudinal range (m a.s.l.)	References
Retanilla	3/3	0–1,500	Medan and Arce (1999)
Discaria	6/4	0–2,000	Primack (1979), Webb (1985), Medan (1991, 1993), Medan and Vasellati (1996), Medan et al. (1999, 2012)
Trevoa	1/1	80–2,000	Medan and D'Ambrogio (1998)
Adolphia	1/0	200–2,600	–
Ochetophila	2/2	500–3,300	Medan (2003), Medan and Devoto (2005)
Kentrothamnus	1/1	2,500–3,800	this paper
Colletia	5/4	0–4,000	D'Ambrogio and Medan (1993), Medan and Basilio (2001), Basilio and Medan (2001), Medan and Torretta (2006)

Genera are listed according to increasing upper altitudinal limit. Before 2005, in the literature the species of *Ochetophila* were included in *Discaria* (see Kellermann et al. 2005)

Table 2 Location of study sites, estimated size of populations, and dates of field work on *Kentrothamnus weddellianus*

Population					Date(s) of work at population
No.	Location	Estimated no. individuals	Geographic coordinates	Altitude (m a.s.l.)	
1	14.6 km W of La Quiaca, Argentina	100	22°08′08.7″S, 65°44′10.7″W	3,627	11.12.1995 03–07.01.2009
2	16.8 km W of La Quiaca, Argentina	70	22°06′51.4″S, 65°45′39.1″W	3,591	03–07.01.2009
3	18 km W of La Quiaca, Argentina	200	22°06′33.1″S, 65°46′26.9″W	3,660	03–07.01.2009 28.12.2010–08.01.2011
4	12 km N of Tupiza, Bolivia	50	21°20′24″S, 65°40′27″W	3,480	01.01.2011
5	2 km W of Sucre, Bolivia	50	19°04′59.8″S, 65°13′45.3″W	2,655	02.01.2011
6	8 km W of Tarabuco, Bolivia	50	19°05′20.4″S, 64°57′16.5″W	3,308	02.01.2011
7	Between Potosí and Yocalla, Bolivia	50	19°23′34″S, 65°53′01″W	3,608	06.01.2011

grazing by domestic llama (*Lama glama glama*) is common. *K. weddellianus* occurs there associated with *Lophopappus foliosus* and *Porophyllum eremophilum* (Asteraceae), *Puya hofstenii* (Bromeliaceae), *Adesmia nordenskioldii* (Fabaceae), and *Menodora pulchella* (Oleaceae) (Ruthsatz (1977).

Reproductive phenology and pollination biology

Blooming start and end dates at population level are based on field observations. Twenty flower buds and open flowers were dissected and inspected under a 10× scope in the field, and 40 additional flowers, fixed in formalin–acetic acid–ethanol (FAA), were studied later in the laboratory with a 50× stereomicroscope. Five floral morphological stages were defined according to the position of sepals, petals, and stigma, status of pollen, stigma, nectar, and scent presentation, and color of floral parts (Table 3). Stigmatic receptivity was assayed with the Peroxtesmo 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. Scent emission was assessed by placing samples of anthetic flowers in small vials for 1 h, to allow for accumulation of volatiles, followed by smell tests by two untrained persons. The number of pollen grains per flower was determined based on an individual from population 1. Three unopened anthers were individually squashed on a slide, pollen grains were counted, and the average value was multiplied by five.

Flower visitors were observed and sampled across populations on different days and at different times of the day (cumulative time = 15 h) to record visitor profiles in as much detail as possible. Sixty-four insect individuals were caught for identification. Seventy-two percent of the

Table 3 Flower- and early fruit phenology of *Kentrothamnus weddellianus*

Floral trait	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Sepals	Closed	Slightly open	Erect	Wide open	Wide open	Wilting
Petals	Connivent	Concealing anthers	Erect, exposing anthers	Erect, exposing anthers	Somewhat reflexed, exposing anthers	Wilting
Pollen availability	Unavailable	Hardly available in spite of open anthers	Easily available	Mostly removed	Removed	Removed
Stigma	Dry	Dry	Dry, lightly swollen	Dry, shrivelled	Wilting	Wilting
Scent, nectar	None	Present	Present	Scarce	None	None
Ovary/flower tube	Unmodified	Unmodified	Unmodified	Enlarging/expanding proximally	Enlarging/expanding proximally	Enlarging/tearing apart in interstaminal areas

visitor taxa were identified to species or genus. When identifications to species were not available, the insects were morphotyped (in several cases with the assistance of specialists, see "Acknowledgements"). All collected material was deposited at the authors' laboratory or in collections of the assisting entomologists. The number of collected individuals was used as a primary measure of species abundance. Eleven 10-min visitor censuses were conducted at population 3 on two consecutive days in December 2010. Each census involved a different plant individual and included between 60 and 180 open flowers per individual.

Size of pollen loads was assessed on caught specimens under 50× magnification and classified as abundant (pollen masses easily visible), present (few scattered grains) or nil (no grains). Composition of the pollen load was studied in one representative individual per visitor species. Pollen was extracted by rubbing the specimen (head, prothorax and legs) with a small piece of safranin-stained glycerine jelly, which was melted on a slide, covered, and microscopically inspected.

Breeding system and maternal success

The proportion of anthetic flowers that received pollen under natural pollination and the size of stigmatic loads was determined for populations 1 ($n = 121$ flowers from 8 randomly selected individuals) and 3 ($n = 67$ flowers from the 10 individuals used in the pollen tube performance experiment, see below). Styles were collected, air-dried, and transported to the laboratory. Once excised from the style, each stigma was macerated for 24 h in a drop of 10 %NaOH (w:v) on a slide. Then a small volume of melted safranin-stained glycerin jelly was added, and the stigma was gently squashed with a coverslip, and inspected microscopically.

To assess the effect of wind and insect visitors on fruit set, an experiment was conducted at populations 1, 2, and 3 in January 2009. Three treatments were applied to groups of three individuals per population. *Treatment 1*: Flower buds were left uncovered and experienced natural pollination ($n = 1{,}206$ flowers). *Treatment 2:* Flower buds were covered with 1 mm-mesh bridal veil bags, to prevent insect visits while enabling wind pollination ($n = 973$ flowers). *Treatment 3:* As in 2, but cloth bags were used, excluding both insects and airborne pollen ($n = 772$ flowers). Experimental branches were collected 30 days after the start of the experiment, when all flowers had abscised or developed a fruit, fruit-to-flower ratios were computed, and analyzed with Kruskal–Wallis and Median tests. Also, seed-to-ovule ratios were computed for a subset of full-grown fruit under treatment 1 ($n = 37$).

To assess the effect of pollen origin (self vs. outcross) on pollen tube performance, a second experiment was conducted on population 3 in December 2010–January 2011. Three treatments were applied to a group of ten individuals. *Treatment 1*: All flowers (except flower buds expected to open on the next day) were removed. Branches were left uncovered and experienced natural pollination ($n = 236$ flowers). *Treatment 2:* As in 1, but branches were covered with cloth bags ($n = 229$ flowers). Experimental flowers in treatments 1 and 2 were left unmanipulated until harvest. *Treatment 3:* As in 2, but bags were reopened 2 days later, and flowers with lightly swollen stigmas (considered as an indication of stigmatic receptivity) were selected for manipulation. With the aid of a 10× hand lens, stigmas were visually controlled for lack of self pollen, then pollinated by gently touching them with an anther of a freshly opened flower of a designated co-specific individual (mean distance between pollen donor and pollen receptor = 53.3 m). Pedicels of pollinated flowers were marked with fluorescent dye. Later, unpollinated flowers were removed, and bags were closed ($n = 77$ flowers). Nine days after pollination, flowers from all treatments 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. In 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. Data were analyzed with Kruskal–Wallis tests.

Results

Phenology and flower biology

Across populations, blooming extended from late November to mid January. Within populations, individuals at different phenophases coexisted (e.g., plants opening their first flowers and plants already ripening fruit) suggesting that there is considerable inter-individual diversity as to blooming start date. Subject to plant size, individuals bore from tens to hundreds of flowers. No sterile individuals were observed. Nearly half of the individuals bore fruit pedestals from the previous year's reproductive episode. Interindividual distance was 2–3 m.

Flowers were active in pollen export and receipt for 3–4 days (Fig. 1a, b, f, g; stages 1–2 in Table 3). Each flower produced ca. 47,600 pollen grains. From day 1 onwards, pollen was increasingly accessible to flower visitors because of centrifugal movement of petals, and at stage 3, pollen was completely removed (Fig. 1c). Because of style elongation, in stage 2 the stigma emerged among the anthers and it also became slightly swollen (Fig. 1g). No stigmatic secretion was observed, and peroxidase tests were negative at all stages. In the transition from stage 1 to stage 2, automatic or visitor-facilitated deposition of self-pollen on stigma was possible. Nectar covered the nectary as a thin film, but it was rarely accumulated in amounts extractable with microcapillaries. After a systematic search (10 flowers per individual from 3 to 5 individuals of each population) nectar could be extracted from only one individual in each of populations 1, 3, and 4 (sugar concentrations 14, 19 and 25 %, respectively; mean volume = 0.3 μl). Flowers in stages 1–2 produced a faint but pleasant scent and received insect visits. Visits occurred between 10:00 am–04:00 pm, with temperatures around 15 C or a little higher. In postanthetic stages (stages 3–5, Table 3 and Fig. 1c–e, h–j) the pedicel thickened and curved upwards and the ovary enlarged, both elevating the style and inflating the lower flower tube. During stages 3–4 the flower tube still contributed to overall color display, but at stage 5 the tube's upper half split longitudinally, while perianth parts, stamens and style gradually wilted and dropped.

Pollinators and pollination service

Twenty-seven species of insects belonging to 15 families of Coleoptera, Diptera and Hymenoptera were recorded as flower visitors (Table 4). Amounting to two-thirds of all species, Diptera dominated the visitor assemblage. Large hoverflies (like *Scaeva* and *Eristalis* spp.), tachinids, sarcophagids, large bombyliids, and most hymenopterans (except ants) moved from one plant to another relatively often while foraging for pollen and/or nectar. All other visitors tended to remain on the same plant for long periods. While present in all populations, *Camponotus* ants were abundant on some individuals but absolutely absent from others. No aggressive interactions between ants and other flower visitors were observed. Two unidentified butterfly species were frequent at the experimental sites (D. Medan, pers. observation), but they never visited *K. weddellianus*. Although systematic observations were not carried out for this purpose, no particular preferences for whitish- or reddish-flowered individuals were observed across visitors.

Most species carried modest *K. weddellianus* pollen loads, and a few carried no pollen at all (Table 4). Abundant loads were observed only on apid and colletid bees, and on tiphiid wasps. In most cases (15 out of 21 visitor species inspected microscopically), loads were pure *K. weddellianus* pollen. Mixed loads generally included only traces of heterospecific pollen. The exception was one individual of *Myzinum* sp., whose pollen load included ca. 40 % of an unidentified pollen.

Only four visitor species were recorded in the censuses carried out at population 3 (*Scaeva melanostoma*, accumulated visits = 19; *Apis mellifera*, 14; *Allograpta exotica*, 7; *Camponotus* sp., 3) indicating that many visitor taxa were uncommon. Mean visitation rate inferred from the censuses was 0.004 visits per flower per minute. Assuming constant visitation rates, five hours a day of insect activity and a duration of anthesis of 3–4 days, a typical flower of population 3 would receive 3.7–4.9 visits over its lifetime, i.e., ca. 1.2 visits per day.

Virtually all open-pollinated flowers received pollen at populations 1 and 3 (99.1 and 100 % of flowers,

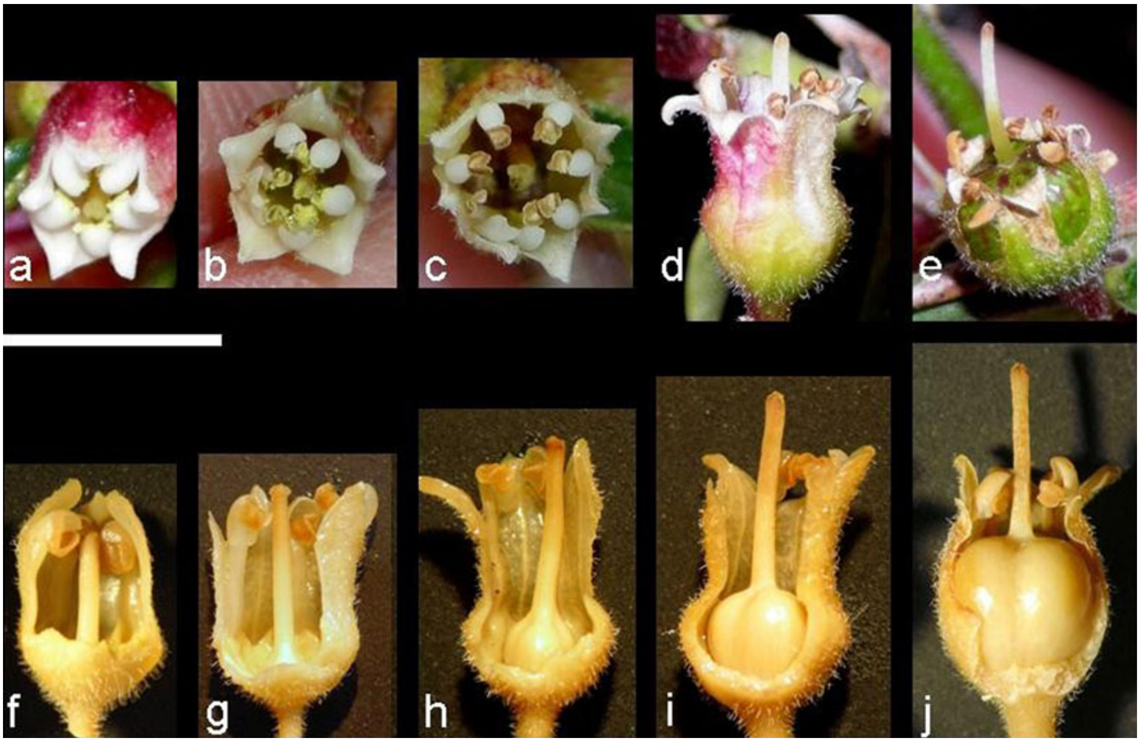

Fig. 1 Development of the flower of *Kentrothamnus weddellianus* shown in fresh, complete objects (**a**–**e**) and fixed, partially dissected objects corresponding to the same stages (**f**–**j**). For stage definitions see Table 3. *Bar* = 5 mm. Photos by D. Medan

respectively). The size of stigmatic loads varied significantly among individuals from both populations (Kruskal–Wallis tests; $H = 19$ and $p = 0.0069$ for population 1; $H = 22.2$ and $p = 0.008$ for population 3; $n = 121$ flowers from 8 individuals, and 67 flowers from 10 individuals, respectively). Loads were higher at population 3 than at population 1 (612.2 ± 244.9 vs. 187.6 ± 48.3 pollen grains per stigma; Kruskal–Wallis $H = 12.6$, $p = 0.0001$). Heterospecific pollen was rarely seen in stigmatic loads (estimated as <0.1 % of all pollen).

Breeding system and maternal success

The bagging experiment showed that pollination conditions affected fruit set (Table 5; Kruskal–Wallis $H = 14.1$, p value = 0.049). Mean fruit set, which was 7.4 % in control branches, dropped to 2.6 % under cloth bagging and to 0.3 % under mesh bagging. While fruit set did not significantly differ between bagging treatments, extreme treatments (control vs. mesh-bagging) statistically differed in two of the three populations, indicating that insect visits partly explained the fruit set of control plants. Fruit set was occasionally high under bagging (6.6 % under cloth bagging in population 3) (Table 5), suggesting some potential for selfing. The low fruit set under mesh bagging suggests that wind pollination was unimportant, because wind was always present at the experimental sites and plants were comparatively close to each other (2–3 m). Mean seed number per fruit in a subset of full-grown fruits was 1.6 ($n = 37$).

Most flowers used in the hand-pollination experiment received pollen grains (93–99 %), and the size of stigmatic loads was similar across treatments (Table 6). Pollen identity (self vs. outcross) significantly affected pollen tube performance. In out-crossed flowers, pollen tubes grew farther down the pistil and penetrated more ovules per flower than in self-pollinated flowers (bagging treatment). The lack of significant differences in pollen tube performance between hand-crossed flowers and open-pollinated flowers suggests that the latter received substantial amounts of outcross pollen through insect visits. Actually, pollen tubes penetrated ovules more often in open-pollinated flowers than in hand-crossed flowers (Table 6). Pollen tube performance in hand-crossed flowers was not correlated to the distance between pollen receptor and pollen donor (data not shown).

Discussion

Shared reproductive traits of Colletieae

Kentrothamnus weddellianus shares several reproductive traits with all species of the tribe Colletieae studied up to

Table 4 Identity, abundance and pollen loads of insect visitors to *Kentrothamnus weddellianus* flowers

Order	Family	Species	Population				Pollen load size/ quality
			1	2	3	4–7	
C	Staphylinidae	Unidentified sp. 1	*x*		*x*		n.a.
D	Anthomyiidae	Unidentified sp. 2	1				N
D	Anthomyiidae	Unidentified sp. 3	2				P (1), N (1)
D	Bombyliidae	*Geron* sp.			1		N
D	Bombyliidae	***Villa*** **sp. 1**	1				P
D	Bombyliidae	***Villa*** **sp. 2**	2				P*
D	Calliphoridae	***Paralucilia pseudolyrcea*** **(Mello)**	2		*x*		P*
D	Muscidae	Coenosiini sp.	2				n.a.
D	Sarcophagidae	***Microcerella*** **sp.**	3				P
D	Sarcophagidae	unidentified sp. 4	1				P*
D	Syrphidae	***Allograpta exotica*** **(Wiedemann)**			3		P
D	Syrphidae	*Eristalis* sp.	*x*				n.a.
D	Syrphidae	***Platycheirus punctulatus*** **(Wulp)**	2		*x*		P
D	Syrphidae	*Platycheirus saltanus* (Enderlein)	1				P
D	Syrphidae	*Pseudoscaeva* sp.	1				N
D	Syrphidae	***Scaeva melanostoma*** **Macquart**	5		2		P
D	Tachinidae	unidentified sp. 5	1		1		N (1), P (1)
D	Tachinidae	**unidentified sp. 6**	2				P
D	unidentified	unidentified sp. 7	1				P
H	Apidae	***Apis mellifera*** **Linnaeus**	2		2		A
H	Apidae	***Doeringiella*** **sp.**	1				A*
H	Colletidae	***Colletes*** **sp.**	1				A
H	Colletidae	***Lonchopria*** **sp.**	3		*x*		A
H	Formicidae	*Camponotus* sp.	8	*x*	5	*x*	P (2), N (11)
H	Halictidae	***Caenohalictus*** **sp.**	3				P*
H	Halictidae	***Lasioglossum*** **sp.**			1		P
H	Tiphiidae	***Myzinum*** **sp.**	4				A**

Putative pollinators appear in boldtype. Figures indicate number of collected individuals. '*x*' indicates that the visitor was observed but not collected. When pollen load varied in size among cospecific visitors, the number of individuals in each size category is specified in parentheses

A abundant, *C* Coleoptera, *D* Diptera, *H* Hymenoptera, *N* nil, *n.a.* not available for analysis, *P* present, * isolated, ** abundant non-*Kentrothamnus* grains in the pollen load

now. In all these taxa (see Table 1), individuals display many small, relatively short-lived zoophilous flowers 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. This set of characteristics is invariant from sea level (e.g., in *D. americana*) to high altitudes (*Ochetophila nana* and *K. weddellianus*) and may be considered a reproductive fingerprint of Colletieae. Intergeneric and interspecific differences do exist in flower phenology, shape, color, rewards, and composition of the visitor assemblages.

Distinctive traits of *K. weddellianus*

Advertising

Attraction of flower visitors seems to be explained by colour display (due to flower aggregation) rather than by scent emission, which (at least to humans) was weaker than usual among Colletieae. Interindividual variation in flower color in the gamut white-red is not uncommon among Colletieae (Medan and Aagesen 1995) and it was previously documented for *K. weddellianus* (Johnston 1973). This color variation was not reflected in differential attractiveness to flower visitors and had apparently no impact on reproductive performance.

Reward strategy

Pollen was produced in high amounts, being the main flower reward. The pollen output of *K. weddellianus* (47,600 grains per flower) equals that of *Ochetophila trinervis* (47,910 grains per flower, Medan and Devoto 2005), which is the highest figure known in the Colletieae. *O. trinervis* is an ambophilous species and part of its pollen is devoted to pollen-demanding wind pollination, but this is not the case in *K. weddellianus*, where all pollen remains in the flowers until its removal by flower visitors. Nectar was secreted in minute quantities and apparently not by all individuals. The secondary role of nectar as a reward is consistent with the lack of obligate nectar feeders (like Lepidoptera) in the visitor assemblage. Scarce nectar secretion, high pollen production, and lack of obligate nectar-feeding visitors is an infrequent combination among Colletieae, hitherto known only in the *Trevoa-Retanilla* clade, which is sister to the remaining part of the tribe (Aagesen et al. 2005, Fig. 2).

Table 5 Percent fruit set of *Kentrothamnus weddellianus* at three different populations under three pollination treatments

Population	Treatment		
	Open pollination	Mesh bagging	Cloth bagging
1	4.0 ± 0.4^{a}	0.00 ± 0.00^{b}	1.0 ± 0.1^{ab}
n flowers/n individuals	501/3	124/3	192/3
2	4.4 ± 5.0^{a}	0.37 ± 1.0^{a}	0.27 ± 0.5^{a}
n flowers/n individuals	320/3	367/3	218/3
3	14.0 ± 0.5^{a}	0.46 ± 0.4^{b}	6.6 ± 0.5^{ab}
n flowers/n individuals	385/3	432/3	362/3

Values are mean ± SD. Kruskal–Wallis test for the whole experiment: $H = 14.16$, $p = 0.049$. Significant differences in between-treatment comparisons are indicated by different exponents (Median tests, $p < 0.05$). Within-treatment comparisons revealed no significant differences

Three species, *T. quinquenervia* (Medan and D'Ambrogio 1998), *R. ephedra,* and *R. stricta* (Medan and Arce 1999) share such high pollen–low nectar reward strategy (HPLN) in the matorral of central Chile, where the Mediterranean-type climate imposes very dry conditions during flowering. Such conditions may have caused low nectar secretion (Medan and D'Ambrogio 1998), because excessive nectar concentration and excessive nectar viscosity may negatively influence the feeding behavior of visitors (Plowright 1985; Corbet 1990). Flower visitors of *K. weddellianus* are mainly active at noon, when air humidity is low even during the rainy season in the Puna, when flowering of *Kentrothamnus* occurs. In January, mean relative humidity at 2 p.m. drops to ca. 40 % in the area of experimental sites 1–3 (Ruthsatz 1977). Plants experience severe water deficits in the Puna during the entire year (Ruthsatz 1977); thus, both low soil water availability and high atmospheric water demand could concur in explaining low nectar secretion in *K. weddellianus*. It is worth noting that HPLN is unrelated to high mountain habitats: the mentioned species, *Trevoa* and *Retanilla,* live at a relatively low altitude (1,000 m a.s.l.), and alpine populations of *Ochetophila nana* (3,300 m a.s.l.) regularly offer nectar and are visited by several butterflies and moths. The stigmatic pollen loads observed in open-pollinated flowers (in the range of 200–600 grains/stigma) apparently consisted of loosely adhered grains, since after fixation in liquid, open-pollinated flowers retained much smaller loads (ca. 60 grains per stigma; Table 6). Excess pollen could easily be detached

Table 6 Performance of pollen tubes of *Kentrothamnus weddellianus* under three pollination treatments

Variable	Treatment			Kruskal–Wallis test	
	Open pollination	Bagging	Bagging and hand-crossing	*H*	*P* value
No. pollen grains per stigma	57.5 ± 21.7	46.7 ± 21.8	45.4 ± 34.5	2.4	0.310
Depth of pistil reached by farthest-growing pollen tubes (range = 1–5)	3.3 ± 1.1^{a}	1.4 ± 1.3^{b}	2.6 ± 1.7^{a}	6.2	0.044
Nr. pollen tubes reaching ovary bottom per flower	2.6 ± 2.0^{a}	1.7 ± 4.7^{b}	1.6 ± 1.7^{a}	7.6	0.022
Nr. penetrated ovules per flower (maximum = 3)	0.6 ± 0.3^{a}	0.15 ± 0.3^{b}	0.6 ± 0.9^{a}	8.8	0.012
Percent flowers with penetrated ovule(s)	30.9 ± 17.7^{a}	15.9 ± 37.6^{b}	7.4 ± 19.6^{b}	15.9	0.003

For the codification of pollen tube advancement in the pistil, see "Materials and methods". Values are mean ± SD. Significant differences in between-treatment comparisons are indicated by different exponents by pollen-feeding visitors, in which case the stigma would act as a secondary pollen presenter (Yeo 1993).

Floral phenology

K. weddellianus lacks a strict temporal and/or spatial separation of the functions of pollen delivery and pollen receipt; thus, stigmatic pollen loads of open-pollinated flowers are expected to include both self and non-self pollen. We suggest that homogamy became established in the *Kentrothamnus* lineage because it allows both pollen export and receipt in a single pollinator visit, which is adaptive in an unpredictable pollination environment. As a group, desert species show the lowest level of dichogamy in the Angiosperms (Bertin and Newman 1993). Homogamy was previously reported in *Colletia*, a genus dominated by lowland species (Table 1). The possible role of homogamy as a condition favorable to reproduction at high elevations should be further explored since one *Colletia* species (*C. spinosissima*) reaches alpine altitudes in the Andes of Perú (4,000 m a.s.l., Tortosa 1989). The fixation of homogamy increases pollen-stigma interference (Lloyd and Webb 1986; Bertin and Newman 1993); in *Kentrothamnus*, this negative effect is apparently counterbalanced by the acceptance of self-pollen through partial self-compatibility, as discussed below.

Stigma type

All known Colletieae have wet stigmas with more or less conspicuous secretion. Therefore, the dry stigma of *Kentrothamnus* was an unexpected finding for this tribe—although not for the family. Dry papillate stigmas like that of *K. weddellianus* have been reported for several genera of Rhamnaceae (Heslop-Harrison and Shivanna 1977). Since the same environmental factors limiting nectar secretion in *K. weddellianus* should also influence stigmatic secretion, a dry stigma in this species is perhaps not particularly surprising. Moreover, a role of the stigma in presentation of pollen to flower visitors would be hindered by the possession of a sticky surface.

Pollinators

K. weddellianus has a visitor assemblage of intermediate diversity. Figures in the Colletieae range from 6 species in *Colletia ulicina* (Medan and Montaldo 2005) and 126 species in *Discaria chacaye* (Medan et al. 2012). The absence of Lepidoptera in the *K. weddellianus* assemblage is not surprising, given the scarcity of floral nectar. The discriminative visitation pattern of *Camponotus* ants and their possible role as nectar thieves should be studied further. 15 species (including eight large-bodied syrphid, bombyliid and muscoid flies, six bees, and one myzinid wasp) are proposed here as effective pollinators of *K. weddellianus*. The distribution, size, and composition of stigmatic loads suggest that all open-pollinated flowers received co-specific pollen, even if some individuals were favored with larger loads. Since stigmatic loads were probably always a mix of self-deposited and non-self pollen, and performance of self pollen was inferior to that of non-self pollen (as discussed below) pollination quality among plants might have varied more considerably than did quantity.

Breeding system and maternal success

Control flowers (open pollinated) set a significantly higher proportion of fruit than flowers of bagged plants within each population but the species exhibits a mixed mating system as cloth bagged flowers in population 3 produced a higher proportion of fruits than open pollinated flowers in populations 1 and 2. Greater success of fruit set under outcrossing may be due to superior pollen tube growth in these flowers as tubes in control and hand cross-pollinated flowers grew further down the style and penetrated more ovules per ovary than in selfed flowers of the bagged treatment. However, hand cross-pollinated flowers had less than one-third of the ovaries with pollen tubes penetrating the ovules as open pollinated flowers and this may be due to the style having only a single dose of pollen from a possibly incompatible neighbor or to the stigma being unreceptive at the time of pollination. Mixed mating is not

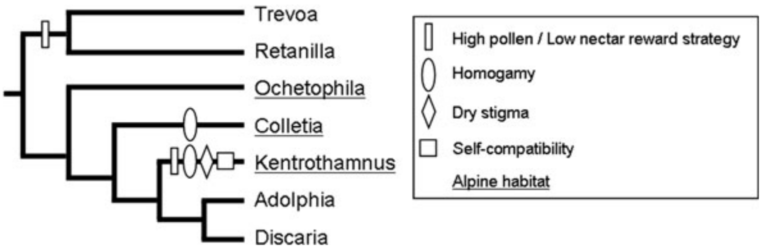

Fig. 2 Simplified consensus tree of the tribe Colletieae (redrawn from Fig. 3 of Aagesen et al. 2005). Selected reproductive traits were mapped onto the tree. Genera that include at least one species that reach alpine altitudes were underlined. Note that *Ochetophila* and *Colletia* include both alpine and lowland species and that some species of Colletieae are still reproductively unknown (see Table 1)

recorded in other Colletieae species but is found in other alpine taxa (Ladd and Arroyo 2009). Fruit and seed set in *K. weddellianus* is within the range of other Colletieae species. It is slightly higher than in *Trevoa quinquenervia* (Medan and D'Ambrogio 1998) but much lower than in *Ochetophila nana* (Medan 2003) and would result in an annual output of 120 seeds/1,000 flowers.

K. weddellianus and high-altitude reproduction in the tribe Colletieae

A set of four traits, including the HPLN reward strategy, homogamy, dry stigma, and partial self-compatibility, seem to be central for *K. weddellianus* to accomplish sexual reproduction in the high-altitude conditions of the Puna. The virtual loss of a floral reward (nectar) and the ensuing impoverishment of the pollinator assemblage are balanced by the increase of a complementary reward (pollen), the ability to take advantage of unique floral visits through homogamy, and the partial acceptance of self pollen. The *Kentrothamnus* 'solution' to reproduction at high altitudes is entirely different from that of the related *Ochetophila nana*, a species showing the trait combination usually found in low-altitude Colletieae, including nectar and pollen as rewards, dichogamy, herkogamy, and self-incompatibility (Fig. 2).

The existence of such different strategies in taxonomically related lineages helps to understand why high-altitude plant reproduction does not globally converge into a single pattern. The case of Colletieae suggests that adaptation to reproduction at high altitudes is context-dependent and can be achieved through different pathways; hence, the final results may or may not strongly diverge from the patterns found in lowland species of each lineage. More case studies still appear to be necessary before regularities can be discerned within the panorama of high-altitude plant reproduction.

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References

Aagesen L, Medan D, Kellermann J, Hilger HH (2005) Phylogeny of the tribe Colletieae (Rhamnaceae)—a sensitivity analysis of the plastid region trnL–trnF combined with morphology. Pl Syst Evol 250:197–214

Anderson GJ, Bernardello G, Lopez P, Stuessy TF, Crawford DJ (2000) Dioecy and wind pollination in Pernettya rigida (Ericaceae) of the Juan Fernandez Islands. Bot J Linn Soc 132:121–141

Arroyo MTK, Squeo FA (1987) Experimental detection of anemophily in *Pernettya mucronata* (Ericaceae) in western Patagonia, Chile. Bot Jahrb Syst 108:537–546

Arroyo MTK, Primack RB, Armesto JJ (1982) Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. Amer J Bot 69:82–97

Basilio AM, Medan D (2001) Pollinator assemblages of *Colletia spinosissima* (Rhamnaceae): composition, behavior, and specificity. Phyton (Buenos Aires) 2001:129–139

Bertin IR, Newman CM (1993) Dichogamy in angiosperms. Bot Rev 59:112–152

Cabrera AL, Willink A (1973) Biogeografía de América Latina. Organización de los Estados Americanos, Washington DC

Corbet SA (1990) Pollination and the weather. Israel J Bot 39:13–30

Dafni A, Motte Maués M (1998) A rapid and simple procedure to determine stigma receptivity. Sex Plant Reprod 11:177–180

D'Ambrogio A, Medan D (1993) Comportamiento reproductivo de *Colletia paradoxa* (Rhamnaceae). Darwiniana 32:1–14

Duan YW, Zhang TF, Liu JQ (2007) Interannual fluctuations in floral longevity, pollinator visitation and pollination limitation of an alpine plant (*Gentiana straminea* Maxim., Gentianaceae) at two altitudes in the Qinghai-Tibetan Plateau. Pl Syst Evol 267:255–265

Fabbro T, Körner C (2004) Altitudinal differences in flower traits and reproductive allocation. Flora 199:70–81

Fagua JC, Gonzalez VH (2007) Growth rates, reproductive phenology, and pollination ecology of *Espeletia grandiflora* (Asteraceae), a giant Andean caulescent rosette. Plant Biol 9:127–135

García-Camacho R, Totland Ø (2009) Pollen limitation in the alpine: a meta-analysis. Arctic Antarctic Alpine Res 41:103–111

Heslop-Harrison Y, Shivanna KR (1977) The receptive surface of the angiosperm stigma. Ann Bot 41:1233–1258

Johnston MC (1973) Revision of *Kentrothamnus* (Rhamnaceae). J Arnold Arboretum 54:471–473

Kellermann J, Medan D, Aagesen L, Hilger HH (2005) Rehabilitation of the South American genus *Ochetophila* Poepp. ex Endl. (Rhamnaceae: Colletieae). N Z J Bot 43:865–869

Körner C (1999) Alpine plant life. Springer, Berlin

Kühn I, Bierman SM, Durka W, Klotz S (2006) Relating geographical variation in pollination types to environmental and spatial factors using novel statistical methods. New Phytol 172:127–139

Ladd PG, Arroyo MTK (2009) Comparisons of breeding systems between two sympatric species, *Nastanthus spathulatus* (Calyceraceae) and *Rhodophiala rhodolirion* (Amaryllidaceae), in the high Andes of central Chile. Pl Species Biol 24:2–10

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437

Larson BMH, Barrett SCH (2000) A comparative analysis of pollen limitation in flowering plants. Biol J Linn Soc 69:503–520

Linder HP (1998) Morphology and evolution of wind pollination. In: Owens SJ, Rudall PJ (eds) Reproductive biology. Royal Botanic Gardens, Kew, pp 123–135

Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. N Z J Bot 24:135–162

Martínez Carretero E (1995) La Puna argentina: delimitación general y división en distritos florísticos. Bol Soc Argent Bot 31:27–40

Medan D (1991) Reproductive phenology, pollination biology, and gynoecium development in *Discaria americana* (Rhamnaceae). N Z J Bot 29:31–42

Medan D (1993) Breeding system and maternal success of a perennial hermaphrodite, *Discaria americana* (Rhamnaceae). N Z J Bot 31:175–184

Medan D (2003) Reproductive biology of of the Andean shrub *Discaria nana* (Rhamnaceae). Plant Biol 5:94–102

Medan D, Aagesen L (1995) Comparative flower and fruit structure in the Colletieae (Rhamnaceae). Bot Jahrb Syst 117:531–564

Medan D, Arce ME (1999) Reproductive biology of the Andean-disjunct genus *Retanilla* (Rhamnaceae). Plant Syst Evol 218:281–298

Medan D, Basilio AM (2001) Reproductive biology of *Colletia spinosissima* (Rhamnaceae) in Argentina. Plant Syst Evol 229:79–89

Medan D, D'Ambrogio AC (1998) Reproductive biology of the andromonoecious shrub *Trevoa quinquenervia* (Rhamnaceae). Bot J Linn Soc 126:191–206

Medan D, Devoto M (2005) Reproductive ecology of a perennial outcrosser with a naturally dissected distribution. Plant Syst Evol 254:173–184

Medan D, Montaldo NH (2005) Ornithophily in the Rhamnaceae: the pollination of the Chilean endemic *Colletia ulicina*. Flora 200:339–344

Medan D, Torretta JP (2006) Evolución de rasgos reproductivos en *Colletia* (Rhamnaceae: Colletieae). Resúmenes, 22 Reunión Argentina de Ecología, Córdoba

Medan D, Tortosa RD (1981) Nódulos actinomicorrícicos en especies argentinas de los géneros *Kentrothamnus*, *Trevoa* (Rhamnaceae) y *Coriaria* Coriariaceae). Bol Soc Argent Bot 20:71–81

Medan D, Vasellati MV (1996) Nonrandom mating in *Discaria americana* (Rhamnaceae). Plant Syst Evol 201:233–241 [corrigenda: Plant Syst Evol 203:1–2, 1997]

Medan D, Basilio AM, Devoto M (1999) Biología reproductiva de *Discaria chacaye* (Rhamnaceae: Colletieae) en el Parque Nacional Lanín, Neuquén. Resúmenes, 19 Reunión Argentina de Ecología, Tucumán

Medan D, Montaldo NH, Devoto M, Mantese A, Vasellati V, Roitman GG, Bartoloni NH (2002) Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. Arctic Antarctic Alpine Res 34:233–241

Medan D, Arbetman M, Chaia EE, Premoli AC (2012) Interspecific and intergeneric hybridization in South-American *Discaria* and *Ochetophila* (Rhamnaceae-Colletieae). Plant Syst Evol. doi: 10.1007/s00606-012-0646-0

Muñoz AA, Arroyo MTK (2006) Pollen limitation and spatial variation of reproductive success in the insect pollinated shrub *Chuquiraga oppositifolia* (Asteraceae) in the Chilean Andes. Arctic Antarctic Alpine Res 38:608–613

Plowright RC (1985) Corolla depth and nectar concentration: and experimental study. Can J Bot 65:1011–1013

Primack RB (1979) Reproductive biology of *Discaria toumatou* (Rhamnaceae). N Z J Bot 17:9–13

Ramos-Jiliberto R, Domínguez D, Espinoza C, López G, Valdovinos FS, Bustamante RO, Medel R (2010) Topological change of Andean plant–pollinator networks along an altitudinal gradient. Ecol Complexity 7:86–90

Rathcke BJ (2003) Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). Amer J Bot 90:1328–1332

Ruthsatz B (1977) Pflanzengesellschaften und ihre Lebensbedingungen in den Andinen Halbwüsten Nordwest-Argentiniens. Dissertationes Botanicae 39:1–168

Steinacher G, Wagner J (2010) Flower longevity and duration of pistil receptivity in high mountain plants. Flora 205:376–387

Thiel-Egenter C, Gugerli F, Alvarez N, Brodbeck S et al (2009) Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians. Global Ecol Biogeography 18:78–87

Torres-Díaz C, Gómez-González S, Stotz GC, Torres-Morales P, Paredes B, Pérez-Millaqueo M, Gianoli E (2011) Extremely long-lived stigmas allow extended cross-pollination opportunities in a high Andean plant. PLoS ONE 6:e19497

Tortosa RD (1989) El género *Colletia* (Rhamnaceae). Parodiana 5:279–332

Tortosa RD, Aagesen L, Tourn GM (1996) Morphological studies in the tribe Colletieae (Rhamnaceae): analysis of architecture and inflorescences. Bot J Linn Soc 122:353–367

Totland Ø, Sottocornola M (2001) Pollen limitation of reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. Amer J Bot 88:1011–1015

Webb CJ (1985) Protandry, pollination, and self-incompatibility in *Discaria toumatou*. N Z J Bot 23:331–335

Yeo PF (1993) Secondary pollen presentation. Springer, Wien