

Reproductive Biology of the Andean Shrub *Discaria nana* (Rhamnaceae)

D. Medan

Cátedra de Botánica, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

Received: July 9, 2002; Accepted: November 27, 2002

Abstract: The widely-held view that the frequency of self-compatibility increases at higher elevations has been questioned for communities of the southern Andes. The study of pollination biology of obligate outcrossers with wide altitudinal range may provide clues on how plants can remain reproductively successful in increasingly hostile environments without resorting to selfing. I studied the phenology, pollinator assemblages, breeding system and maternal success of the perennial entomophile outcrosser, *Discaria nana*, across a 1200-m elevation span in the Andes of Mendoza and Neuquén, Argentina (34°–37°S). *D. nana* behaved as self-incompatible throughout the studied gradient. At the higher site proportionally fewer flowers were pollinated, stigmatic loads were smaller and number of visitor species was lower than at low altitude; however, natural fruit set was 3.6 times higher and seed set 3.3 times higher. As a non-exclusive explanation, it is proposed that increased maternal success reflected better pollination quality at the higher site, to which several factors seemed to contribute. At higher altitude *D. nana* a) exhibited mutualisms with a high proportion of the available pollen vectors, b) had a higher rate of specialized pollinators (Lepidoptera, obligate nectar feeders) to unspecialized ones (Diptera, pollen and mixed feeders) in the visitor assemblage which would reduce ineffective (i.e. self) pollination because nectar feeders visited both male and female phase flowers and pollen feeders favoured male phase flowers, c) had an extended floral longevity (especially the female phase), and d) showed an increased floral display (as number of flowers per unit plant area). Differential rewarding through more concentrated nectar may explain the change in assemblage composition. The flowers' reverse herkogamy (i.e. the location of the stigma below the anthers) seemed to enhance the beneficial effects of the abundance of more effective pollinators.

Key words: High-altitude pollination, Rhamnaceae, *Discaria*, self-incompatibility.

Introduction

The environmental conditions in alpine habitats (low temperatures, short growing seasons and strong winds) may reduce the possibilities of cross-pollination and are believed to represent important driving forces in the evolution of self-pollination at high altitudes and high latitudes (Savile, 1972; Bell and Bliss, 1980; Molau, 1993; Sandvik et al., 1999). The validity of the prediction of increased self-compatibility at higher elevations has been questioned for the Andes of Chile (Arroyo et al., 1985), and the relative importance of biotic vs. abiotic pollination in high altitude communities is still debated (Arroyo et al., 1990). A recent community-scale study in the Andes of Argentina (Medan et al., 2002) showed that the prevalence of selfers at higher altitudes does not reflect a generalized pattern of the plant community, but results from two speciose families (Asteraceae and Fabaceae) that show more self-compatibility at high elevations.

The question remains open, of how a great majority of the plant species in these systems can remain reproductively successful in increasingly hostile environments without resorting to selfing. An answer can be found in a comparative study of the pollination biology of outcrossers living at different elevations. Animal-pollinated, obligate outcrossers would be of special interest because such plants have necessarily established functional mutualisms at all altitudes.

In this paper I report on the reproductive biology of a self-incompatible perennial entomophile across a 1200-m elevation gradient in the Andes of Mendoza and Neuquén, Argentina. The focal plant, *Discaria nana* (Clos) Weberb. (Rhamnaceae) is a mat-forming shrub occurring above 2000 m a.s.l. in the Andes of Chile and Argentina, being the only true alpine species in its genus (Tortosa, 1983). The objectives of this work are 1) to analyse the altitudinal and latitudinal variability of *D. nana* pollinator assemblages, 2) to relate the composition of visitor assemblages with the species' reproductive phenology, floral biology and reproductive success, and 3) to further investigate the breeding system of *D. nana*.

Materials and Methods

Plant species

Discaria nana grows above 2000 m a.s.l. in the Andes range between 31°S and 46°20'S (Tortosa, 1995). It consists of richly ramified, often spiny shrubs forming 1–3-cm thick carpets on the ground. Because of long-term vegetative growth through rooting stems, followed by partial senescence of epigeal parts, currently isolated patches may pertain to the same clone (Tortosa et al., 1996). As patches are usually extensive and dispersal distance is probably less than 3 m (Medan and Agesen, 1995), a given patch may consist of a mosaic of genetically different individuals. Since there was no way to distinguish genets in the field, in this work an "individual" was defined as a *D. nana* patch clearly separated from other conspecific patches by bare soil or other vegetation. The area covered by such individuals was $9.4 \pm 14.1 \text{ m}^2$ (mean ± 1 SD, $N = 33$). To minimize the possibility of genet pseudoreplication, individuals separated by at least 15 m were used within a given treatment or comparison.

Flowers are erect, presented 2–3 mm above foliage level, in reduced, uniflorous axillary synflorescences (Tortosa et al., 1996). They are perfect, actinomorphic, with a white floral tube 5–7 mm long and 2 mm in diameter. Four deltoid sepals 1–1.5 mm long and four hooded petals alternate at the rim of the floral tube. The 4 stamens are opposite to the petals, with the anthers located at the same level as the floral tube mouth. A nectariferous disc is near the bottom of the floral tube. The 3(4)-carpellate gynoecium is composed of a semi-inferior, 3(4)-ovulate ovary, style and a terminal, 3(4)-lobular stigma located 1.3–1.5 mm below the anthers. Fruits are 1–3-seeded explosive capsules. Vouchers are kept at Gaspar Suarez Herbarium (BAA 22354 and 24806).

Study sites

The study was carried out in the provinces of Mendoza and Neuquén, Argentina. Site selection aimed at a wide sampling of the variability in phenological responses and pollination interactions. Two sites are representative of the lower part of the species' altitudinal range, and the third site was located in the upper part of this range. The Valle Hermoso (VH) site (Mendoza) was located at 35°04'S, 70°13'30"W at 2190 m a.s.l. Plant cover was low (< 50 cm), developed on fine gravel and sandy loam. The local *D. nana* population consisted of ca. 60 individuals covering a 140-m long strip along the western shoreline of the Valle Hermoso lake. The Copahue (CP) site (Neuquén) was located at 37°49'13"S, 71°06'04"W at 2080 m a.s.l. Vegetation was developed on sandy, rocky soil and usually low (< 30 cm), but isolated treelets of *Nothofagus antarctica* occurred in wind-protected sites. A 350-m transect including 10 individuals of *D. nana* was established. The Laguna Diamante (LD) site (Mendoza) was located at 34°10'S, 69°42'W at 3300 m a.s.l., on the western shore of the Diamante lake. Vegetation was still lower (< 20 cm), sparsely distributed on sandy soil near the shoreline and on rocky inland soils. A population of ca. 150 *D. nana* individuals covering ca. 3700 m² was used. Long-term climatic data for the Mendoza sites are not available. Mendoza sites located at altitudes comparable to those of LD and VH have mean annual temperatures of -1.5°C and 11.2°C , and mean annual precipitations of 700 mm and 900 mm, respectively (Capita-

nelli, 1972). Corresponding data for the CP site are of 10°C and 1200 mm (Movia et al., 1982). Field work was conducted from 3 to 10 January 1994 at VH, from 14 to 20 January 1995 at LD and from 26 to 28 January 2002 at CP. All reproductive parameters were measured at the higher site (LD) and at one or both lower sites (VH and CP), which were considered equivalent as regards altitudinal comparisons. Lack of site reference indicates that the measurement was carried out at all sites.

Reproductive phenology and pollination biology

Dates of start and end of blooming are based on field observations, complemented with data from Tortosa (1983). Sixty-three flower buds and open flowers were dissected at VH and LD under a 50 \times stereomicroscope. Six floral morphological stages were arbitrarily defined according to the position of sepals and petals, status of pollen and stigma presentation and colour of floral parts (Table 1). Floral stages were recorded twice a day for 29 randomly-selected flowers from 8 individuals (VH), and for another set of 20 flowers from 4 individuals (LD). Mean values of flower stage were plotted against time for both sets of flowers. Air temperature and relative humidity were recorded in the period 8:00–22:00 with hand-held instruments for the days of flower monitoring. Stigmatic receptivity was assayed by applying a tiny drop of 40% H₂O₂ on stigmas (Zietsman and Botha, 1992). Nectar was extracted with hand-drawn capillaries and sugar content (as % sucrose equivalents) was determined with a hand refractometer modified for small volumes. A limited number of volume measurements were made using calibrated 1 μl micropipettes. Scent emission was assessed by offering three people small vials containing samples ($N = 15$) of flowers at different stages of anthesis. Flower visitors were observed and sampled across populations at different times of the day and on different days (cumulated time for all sites = 34 h), including videotaping of selected visitor species, in order to record visitor assemblages as completely as possible. Representative insect individuals ($N = 166$) were caught for identification. Sixty-four percent of the visitor taxa could be identified to species or genus. When identifications to species were not available, the insects were morphotyped (in several cases with the assistance of specialists). All collected material is deposited at the author's laboratory or in collections of assisting entomologists. Common species were captured more often than infrequent ones, and difficulty of capture was uniform among species; thus, the number of sampled individuals of a given visitor species was used as a measure of its overall abundance.

Maternal success and breeding system

Proportion of female-stage flowers that received pollen under natural pollination was determined at LD and CP. At LD this was done by direct observation of stigmas at 50 \times ($N = 118$ flowers from 10 individuals). At CP, 38 flowers from 3 individuals were collected, air-dried and transported to the laboratory. Each excised stigma was macerated for 24 h in a drop of 10 NaOH (w:v) on a slide, then a small volume of melted glycerin jelly was added, and the stigma was gently squashed with a coverslip. The number of pollen grains per stigma was determined for the CP flower set, and for a subsample ($N = 70$) of the LD flower set. Fruit set under open pollination was determined at VH and LD by dividing counts of flowers per unit area ($N = 15$ individuals) by counts of fruits per unit area ($N = 14$ in-

Table 1 Flower phenology of *Discaria nana*, based on 63 flowers from several individuals of low- and high-altitude populations

Floral trait	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Floral tube	closed, white	slightly open, white	slightly open, white	slightly open, white	basally reddish	wilting
Petals	connivent	connivent	erect	erect	erect	wilting
Pollen	unavailable	abundant, available	mostly removed	removed	removed	removed
Stigma	dry	dry, not receptive	swollen, receptive	very swollen, receptive	drying, less receptive	wilting
Scent	none	somewhat unpleasant	pleasant	pleasant	none	none
Nectar	none	present	present	present	none	none
Nectary/ovary	green	green	green	green	yellow/reddish	yellow/reddish

dividuals). The number of mature seeds per fruit was determined at CP for a sample of 94 fruits from 3 individuals, and at LD for a sample of 40 fruits from 4 individuals.

To examine the breeding system of *D. nana*, the P:O ratio was determined at VH and LD (N=3 individuals). I also assessed the effect of pollen source on pollen tube growth at the LD site. Four treatments were applied to groups of flower buds in each of two individuals. Treatment 1: Flower buds were emasculated, covered with 1 mm mesh plastic screen until stigmas appeared receptive, pollinated by gently touching each stigma with an anther of a freshly opened flower of the same branch, and left covered for an additional 85 h (N=35 flowers). Treatment 2: As in 1, but each flower received pollen from an individual growing at least 15 m away. As many different donors were used as there were recipient flowers (N=31 flowers). Treatment 3: As in 1, but stigmas were left unpollinated (N=38 flowers). Treatment 4: Flower buds were left uncovered and experienced natural pollination (N=70 flowers). At harvest, flowers were fixed in FAA, transported to the laboratory, soaked for 60 h in 5% NaOH (w:v) at 30°C, cleared in diluted NaClO, mounted in 0.1% decolorized aniline blue, and viewed with a fluorescence microscope to assess pollen tube growth. For each flower I recorded a) the number of pollen grains per stigma, and b) the pistil section (stigma + upper style, mid style, lower style, upper ovary, or ovary bottom) which had been reached by the farthest-growing pollen tubes. Pollen tube growth data were subjected to a survival analysis (Cox and Oakes, 1984), assuming that pollen tubes had already reached their maximal length when the flowers were collected (i.e., pollen tubes were either already arrested or had reached an ovule). Previous work with related species (Medan and D'Ambrogio, 1998; Medan and Basilio, 2001) suggests that the period of 85 h was long enough for pollen tubes to complete growth.

Throughout the text all variables are given as mean \pm 1 SD.

Results

Phenology

Flowering extends from mid-December to the end of January, with a diffuse peak in late December–early January. Developing fruits were found from early January through February.

Within a population, individuals located on north-facing slopes started flowering earlier, apparently because of earlier melting of the snow cover in such places. At all study sites, some individuals failed to bloom. Flowering was non-simultaneous within individuals, i.e. plants usually showed a mosaic pattern of flower buds, open flowers and ripening fruits.

Flowers were active in pollen export and receipt for 3–4 d (low site) to 5–7 d (high site). Temperatures during flowering were lower at the high-altitude site (mean minimal/mean maximal = 3.8°C/21.2°C) than at the low-altitude site (10.0°C/20.2°C); moreover, at the high site temperatures dropped below zero every night.

Flower buds opened in the morning and, during the first day, passed through a male phase (stage 1, Table 1) and might reach the early female phase (stage 2). During the male phase, pollen was accessible to flower visitors through slits left open by the four petals, which were connivent above the stigma. Pollen was usually thoroughly removed by visitors. A somewhat unpleasant, dung-like scent was perceptible, and stigma reacted negatively to the peroxidase test. Nectar was generally present, but many flowers had only minute amounts, or even lacked it (mean volume was 0.06 μ l, N=4; mean sugar concentration was 6 \pm 3.7% at the low site, 17 \pm 1.7% at the high site).

During days 2–3 most flowers passed through the female phase (stages 2–3) and some entered stage 4. Petals became erect, thus exposing the stigma, which was perceptibly swollen, often producing distinct droplets of a clear, adhesive, mucilaginous secretion on its three receptive surfaces. Tests of receptivity were positive, particularly at stage 3. Floral scent (which continued during the night) was now pleasant to humans. On day 4, colour changes in floral parts and a decrease in stigma receptivity indicated the end of active flower life (stages 4–5). On day 5 most floral parts were wilting.

Pollinators

Across all study sites, 51 species of insects belonging to 21 families in five orders were recorded as flower visitors (Table 2). Diptera dominated the visitor assemblage (70.6% of all species), followed by Lepidoptera (19.6%) and less important groups (Coleoptera and Microcoryphia, 9.8%). The composition of the visitor assemblage varied among sites. At the high-

Table 2 Insect visitors to *Discaria nana* flowers. Probable pollinators printed in bold type. Abbreviations of study sites: LD = Laguna Diamante, VH = Valle Hermoso, CP = Copahue

	Order	Family	Species	Collected at			
				LD	VH	CP	
	Coleoptera	Melyridae	unidentified melyrid beetle		×		
	Diptera	Asilidae	unidentified asilid fly sp. 1		×		
			unidentified asilid fly sp. 2		×		
		Bombyliidae	<i>Geron</i> sp.	×			
			<i>Thyridanthrax</i> sp. 1		×		
			<i>Thyridanthrax</i> sp. 2	×	×		
			<i>Thyridanthrax</i> sp. 3	×			
		Calliphoridae	<i>Chlorobrachycoma versicolor</i>	×			
			<i>Compsomyiops fulvicrura</i>		×		
		Chironomidae	unidentified chironomid fly	×			
		Muscidae	ca. <i>Lispe</i> sp.		×		
			ca. <i>Fannia</i> sp.		×		
			unidentified muscid fly		×	×	
		Sarcophagidae	<i>Microcerella rusca</i>		×	×	
			<i>Microcerella</i> sp. 2	×			
		Stratiomyiidae	ca. <i>Odontomyia</i> sp.		×		
		Syrphidae	<i>Carposcalis</i> sp.		×		
			<i>Dolichogyna</i> sp.	×	×	×	
			<i>Palpada meigenii</i>			×	
			<i>Scaeva</i> sp.			×	
			ca. <i>Toxomerus</i> sp.			×	
			<i>Tropidia notata</i>		×		
			<i>Dasybasis chillan</i>		×		
		Tabanidae	<i>Dasybasis</i> sp. 2			×	
		Tachinidae	<i>Ruiziella ca. frontosa</i>		×		
			<i>Spathipalpus philippii</i>		×		
			<i>Trichoceronia ca. thermitana</i>		×		
			<i>Vibrissomyia ca. notata</i>		×		
			unidentified tachinid fly sp. 1			×	
			unidentified tachinid fly sp. 2			×	
			unidentified tachinid fly sp. 3		×		
			unidentified tachinid fly sp. 4		×		
			unidentified tachinid fly sp. 5			×	
			unidentified tachinid fly sp. 6	×	×		
		unidentified tachinid fly sp. 7		×			
		unidentified tachinid fly sp. 8	×		×		
		Tephritidae	unidentified tephritid fly		×		
	Hymenoptera	Vespidae	<i>Hypodinerus</i> sp. 1	×			
				<i>Hypodinerus</i> sp. 2	×		
		Formicidae	unidentified formicid ant		×		
	Lepidoptera	Geometridae	unidentified geometrid moth			×	
		Lycaenidae	<i>Pseudolucia charlotte</i>		×		
		Noctuidae	<i>Agrotis ipsilon</i>	×			
			<i>Peridroma saucia</i>	×			
			<i>Pseudoleucania nobilis</i>	×			
			<i>Pseudoleucania</i> sp. 2	×			
			<i>Tamseuxoa ingoufii</i>	×			
			<i>Issoria lathonioides</i>	×			
			Pieridae	<i>Phulia nymphula</i>	×		
			Satyridae	<i>Chillanella stelligera</i>		×	
	Microcoryphia	Meinertellidae	unidentified meinertellid bristletail	×			
Grand totals	5	21	51				
Totals by site							
Order				4	4	2	
Family				12	12	7	
Species				21	24	13	

altitude site *D. nana* had 21 visitor species, of which four (14.3%) were in common with either or both low-altitude sites. Taken together, the assemblages recorded at the low sites (34 species, of which 7.5% were present both at VH and CP) were more diverse than the high-altitude assemblage. Lepidoptera were more species-rich at high than at low altitude (33.3% vs. 5.9% of the whole assemblage, $\text{Chi}^2 = 7.15$, $p = 0.007$, $\text{df} = 1$) and the reverse was true for Diptera (52.3% vs. 85.3%, $\text{Chi}^2 = 7.09$, $p = 0.007$, $\text{df} = 1$). Among comparatively speciose families (≥ 3 spp.), Muscidae, Syrphidae and Tachinidae were more strongly (or exclusively) represented at low-altitude sites, while Noctuidae were recorded at the high-altitude site only.

Daytime visitation rate was clearly lower in the high- than in low-altitude populations. Comparing two sites where similar sampling effort was made, overall catch size was higher at the low-altitude site (98 individuals at VH vs. 42 at LD).

Diptera and Hymenoptera visitors usually landed on leaves and then visited nearby flowers in sequences of varying durations, foraging for pollen and/or nectar. Among dipteran visitors, Syrphidae and especially Tachinidae were the most species-rich and frequent families. Diurnal Lepidoptera probed for nectar in short sequences and flew among plants more often than the other floral visitors, while Noctuidae tended to stay on a branch, working well after dawn at temperatures around 4°C, even with moderate wind. Among less important groups, jumping bristletails (Meinertellidae) are included as flower visitors because they occurred in high numbers at the LD site. Although no feeding behaviour at flowers was observed, bristletails walked permanently along blooming branches, and consequently contacts with active flowers were very likely.

On the basis of their feeding habit, abundance, mobility and constancy as *D. nana* visitors across study sites, 21 insect species are the probable main pollinators (Table 2). This subset is dominated by Tachinidae (seven species) and Noctuidae (five spp.), and also includes nine species of Bombyliidae, Lycaenidae, Muscidae, Nymphalidae, Pieridae, Sarcophagidae and Syrphidae.

Breeding system and maternal success

Under natural pollination, more female phase flowers received pollen at the low-altitude site (100%) than at the high site (83%). Stigmatic pollen loads were significantly higher at the low- than at the high-altitude site (182.2 ± 182.8 vs. 105.3 ± 95.3 grains, $N = 38$ and 70 , respectively; Mann-Whitney $U = 1025.0$, $p = 0.049$). The P:O ratio was also higher at the low site (9773:1) than at the high site (4938:1).

Pollination experiment at the high-altitude site

Of the 38 emasculated, otherwise unmanipulated flowers, 84.2% received no pollen, and the six flowers that received pollen had very small stigmatic pollen loads (5.5 ± 3.8 grains). The fact that these flowers were excluded from visitors, but exposed for over 4 days to moderate/strong winds (only covered with a 1-mm mesh), indicates that the importance of wind pollination was negligible. Airborne pollen loads were significantly smaller (Mann-Whitney $U = 19.0$, $p < 0.01$) than those delivered by hand pollination or natural pollination ($114.6 \pm$

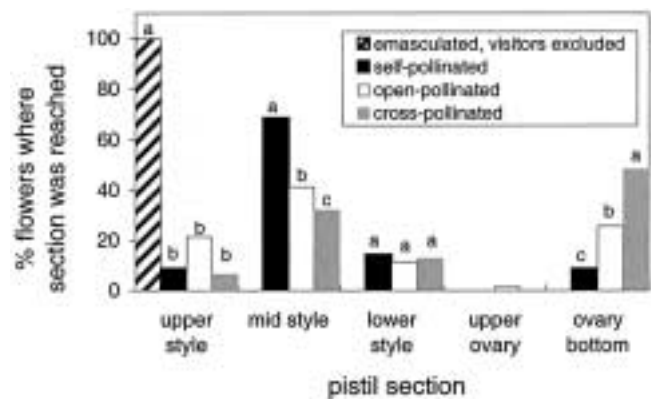


Fig. 1 Pistil section reached by the farthest-growing pollen tubes in flowers of *Discaria nana* under different pollination treatments: self-pollination ($N = 35$ flowers), cross-pollination ($N = 31$ flowers), open pollination ($N = 70$ flowers) and emasculating with visitor exclusion ($N = 6$ flowers). Within a pistil section, different letters indicate significant differences between treatments (pairwise comparisons, Chi^2 , $p < 0.05$). Data from two individuals were pooled.

59.5 grains in the self-pollination treatment, $N = 35$; 106.1 ± 60.9 grains in crossed flowers, $N = 31$; 132.3 ± 137.3 grains in open-pollinated flowers, $N = 108$). The latter three load types were of similar size (Mann-Whitney $U = 1612.0$, $p = 0.75$). Eighty-five hours after manual pollination or exposure of stigmas to natural pollination, the proportion of flowers in which the farthest-growing pollen tubes had reached a given pistil section varied with the pollination treatment and with the section considered (Fig. 1). All pollen tubes of emasculated, insect-excluded flowers ceased growth in the stigma or the upper section of the style. Following the other treatments, pollen tubes grew beyond the upper style, and in some flowers they reached the bottom of the ovary (the level at which the micropyle is located). In most selfed flowers (91.5%), pollen tubes ceased growth without entering the ovary, being mainly arrested at mid-style, where they outnumbered pollen tubes of open- and cross-pollinated flowers. In contrast, in almost half of crossed flowers (48.4%) pollen tubes reached the ovary bottom. A survival analysis showed that overall differences among treatments were highly significant ($\text{Chi}^2_3 = 23.5$; $p < 0.00003$). Moreover, pairwise comparisons indicated significant differences between treatments at upper style, mid-style and ovary bottom (Fig. 1). The per flower number of pollen tubes reaching the micropyle level was higher in crossed flowers than in selfed flowers (13.4 ± 14.5 vs. 3.3 ± 1.5 pollen tubes, respectively) although the difference did not reach significance (Mann-Whitney $U = 10.5$, $p = 0.18$). Open-pollinated flowers had intermediate values (22.8% of these showed pollen tubes reaching ovary bottom, at a rate of 4.7 ± 3.2 pollen tubes per flower).

On average, *D. nana* individuals produced fewer flowers at the low- than at the high-altitude site (11720 ± 3890 vs. 53390 ± 26610 flowers m^{-2}), and natural fruit set was lower at the low- than at the high-altitude site (13.2 vs. 48.2%). Assuming that only half of its surface bore flowers, a typical individual (area = 9.4 m^2) would produce ca. 7250 vs. 120000 flowers in low- and high-altitude populations, respectively. A fruit of *D. nana* contained an average of 0.82 and 2.7 well-developed seeds in the low and high sites, respectively; therefore, medi-

um-sized individuals were expected to disperse ca. 5900–324 000 seeds per year (low and high altitudes, respectively).

Discussion

Variability of pollinator assemblages

Knowledge of the *Discaria nana* pollinator assemblages was hitherto restricted to high-altitude sites (25 species, Arroyo et al., 1982; Medan et al., 2002). This paper adds 30 species from two low-altitude sites. None of the fully identified species occur on both sides of the Andes, but probable trans-Andean species may be found among incompletely identified Sarcophagidae and Tachinidae.

The composition of the visitor assemblages was strongly site-dependent at the species level. The two low-altitude sites had very few species in common, and the number of species shared between either of the low sites and the high-elevation site was likewise very low. Since the low-elevation sites were ca. 300 km apart in a N–S direction, the differences between their assemblages may reflect latitude-associated changes in the overall entomofauna. Other causes might also be involved, since work at the southern site was carried out later in the summer, and the sampling effort was less intense due to the shorter field stay. Unfortunately, no comparable data on other plant species are available for the areas involved.

A comparison between the VH and LD sites, both of which are located at similar latitudes and were sampled in early summer, shows that the number of species of visitors to *D. nana* decreased with altitude by a factor of 0.87 ×, while the proportion of Lepidoptera in the assemblage increased (8.1 ×) and that of Diptera decreased (0.6 ×). It is illustrative to compare these figures with whole community values, which are available for the LD site and for Río Blanco, a site somewhat lower than VH (1900 m) but also in the Andes of Mendoza (Medan et al., 2002). Along the Río Blanco – LD gradient the number of species of visitors decreased with altitude (0.62 ×), while the proportion of butterflies and moths increased (4.7 ×) and that of flies remained unchanged. This suggests that, with increasing altitude, *D. nana* loses mutualists at a lower rate than the community average, and that the resulting assemblage is richer in Lepidoptera and poorer in Diptera than the community average.

At the LD site, *D. nana* had the most generalized visitation pattern in the community, showing interactions with 45.6% of all mutualists available in the system (Medan et al., 2002). The species' ability to establish mutualisms with a) a high proportion of the available pollen vectors, with most available vector types being well represented in its visitor assemblage, and b) an increasing proportion of specialized vectors (Lepidoptera), at the expense of mostly unspecialized vectors (Diptera), may compensate for the decrease in pollination service (see below).

Phenology and altitude

The flowering time reported here for *D. nana* (December to January) is congruent with that found by Arroyo et al. (1981) at Cordón del Cepo (mid-January). Delay in the start of flowering in south-facing locations was also noted and discussed in detail by these authors as a community-scale phenomenon.

In *D. nana*, floral longevity grew from 3.5 d at the VH site to 6 d at the LD site, which is 1110 m higher. The resulting increase of 0.22 d for every 100 m is very close to that found by Blionis et al. (2001) within the genus *Campanula* on Mt. Olympus, Greece (0.2 d for every 100 m of elevation). At community level, Arroyo et al. (1981) and Primack (1985) reported increases in floral longevity with elevation in Chile and New Zealand. Longer flower duration may have adaptive value in *D. nana*, since pollinator activity was much less intense at the higher site. A proportionally longer female phase may have adaptive value too, because in *D. nana* female phase flowers tend to be visited only by nectar feeders, and these are expected to deliver a better pollination service (see below).

Breeding system

The pollen tube growth experiment showed that in over 90% of selfed flower pollen tubes were arrested at the stigma of style. In contrast, pollen tubes reached ovules in almost half the crossed flowers, and the mean number of cross tubes making their way to ovules was four times higher than in selfed flowers. These findings suggest that *D. nana* is mostly, if not wholly, self-incompatible. This is in agreement with pollen:ovule ratios of 4938:1 and 9773:1 (high and low altitude populations, respectively), suggesting obligate xenogamy (Cruden, 1977). The use of the P:O ratio as a predictor of a plant's breeding system has received criticism (Cruden et al., 1996) but it remains widely employed in studies referred to hermaphrodite species (Cruden, 2000; Wyatt et al., 2000; Pías and Guitián, 2001). Self-incompatibility was already known in other *Discaria* species (Primack, 1979; Webb, 1985; Medan, 1991; Medan and Vasellati, 1996). Since both selfing and wind pollination seem to be of little consequence for reproduction, most, if not all, seed set must be due to insect-mediated cross pollination.

Floral display and maternal success

D. nana shows a combination of obligate entomophily, protandry, reverse herkogamy (i.e. the stigma is located below the anthers, Webb and Lloyd, 1986), use of colour and scent as flower advertising, and of pollen and nectar as rewards, which is frequent among Rhamnaceae and was already documented in the genus *Discaria* (Primack, 1979; Webb, 1985; Medan, 1991). Change in scent during flower lifetime was also reported for *Discaria americana* (Medan, 1991). Female phase flowers are also better smelling than male phase ones in *Peltandra virginica* (Patt et al., 1995), a coincidence that could reflect some general, as yet unknown, pattern.

Mass flower presentation coupled with simple flower morphology and easily available rewards seem sufficient to explain the diversity of insect taxa visiting *D. nana*. At the higher site, *D. nana* constituted a "cornucopia" or "keystone" species (Medan et al., 2002), i.e. one that attracts a high proportion of the available animal mutualists and may represent the main nectar and pollen source in the system, thus playing a crucial role in the community (Moldenke and Lincoln, 1979; Memmott, 1999; Ne'eman et al., 2000).

Visitors able to reach nectar are expected to provide better pollination service, because they will visit both male and female phase flowers, while exclusive pollen feeders and mixed fee-

ders will tend to visit male phase flowers only. However, short-tongued flies foraging for pollen might accomplish some intrafloral pollen transfer because of the proximity of anthers to the stigma and the lack of strict separation between the male and female phases. Note that, if visitation rate is low, pollen may remain in anthers when the flower has entered the female phase. Flowers being visited by pollen feeders at this stage are at risk of being self- (i.e. ineffectively) pollinated.

The floral display (as number of flowers per unit plant area) was 4.5 times higher at the high-altitude than at the low-altitude site, but the proportion of pollinated flowers, and the size of stigmatic loads, were lower by factors of 0.87 and 0.57, respectively. The per flower amount of pollination service was thus lower at higher altitude. Surprisingly, natural fruit set was 3.6 times higher, and seed set 3.3 times higher, at high altitude than at low altitude. Several uncontrolled factors may be involved in producing this result. As a non-exclusive explanation, I propose that pollination at the higher site, although quantitatively inferior, was of better quality. For a self-incompatible (SI) plant, better pollination quality means receiving less self pollen and more pollen from conspecifics. Since the degree of relatedness with other conspecifics is important for SI plants, better pollination may also imply receiving pollen from distant and perhaps less related plants. The high-elevation assemblage of *D. nana*, with its higher rate of specialized vs. unspecialized pollinators, is arguably capable of delivering better pollination service: obligate nectar feeders (Lepidoptera) were more abundant, and pollen and mixed feeders (Diptera) less abundant in this assemblage, both of which should reduce ineffective pollination because of the above-discussed differential consequences of each group's feeding behaviour. Moreover, Lepidoptera are intrinsically mobile and may promote cross-pollination among distant individuals. Rewards to nectar feeders were higher at the higher site, since nectar was almost three times as concentrated as in the lower site. This differential rewarding may contribute to explain the change in assemblage composition and, indirectly, the better pollination service.

An independent, morphological aspect of *D. nana* flowers may enhance the beneficial effects of a visitor assemblage richer in nectar feeders. Reverse herkogamy should increase the frequency of cross-pollination in species where nectar feeders are better cross pollinators than pollen feeders. This hypothesis, originally developed by David Lloyd (Medan, 1991), is based on the reasoning that if pollen feeders eat or waste the pollen without redepositing much of it on receptive compatible stigmas, placing stigmas in a tube below the anthers may cause less redeposition from the pollen feeders, but it could still permit adequate redeposition by the nectar feeders with a concomitant increase in cross-pollination and a reduction in the self interference caused by the pollen feeders. The joint occurrence in *D. nana* of smaller pollen loads and higher fruit set at the site where nectar feeders were more abundant, is congruent with Lloyd's hypothesis and apparently provides its first experimental support.

Acknowledgements

L. Aagesen, M. C. Álvarez, L. Frank, R. J. C. León, N. H. Montaldo, G. G. Roitman, and G. Zarlavsky helped me in the field or laboratory. A. Roig-Alsina, F. C. Thompson, J. C. Mariluis, F. Navar-

ro, M. Gentili, A. Bachmann, S. Coscarón, and H. Rizzo identified insect specimens. N. Bartoloni provided statistical advice. Earlier drafts of this paper benefited from comments by Susanne Renner, Juan Arroyo, Norberto H. Montaldo, Juan P. Torretta, Mariano Devoto, P. C. Zietsman and an anonymous reviewer. Financial support of UBACyT and CONICET to D. M. is gratefully acknowledged.

References

- Arroyo, M. T. K., Armesto, J., and Primack, R. B. (1985) Community studies in pollination ecology in the high temperate Andes of central Chile. II. Effects of temperature on visitation rates and pollination possibilities. *Plant Systematics and Evolution* 149, 187–203.
- Arroyo, M. T. K., Armesto, J. J., and Villagrán, C. (1981) Plant phenological patterns in the high Andean cordillera of central Chile. *Journal of Ecology* 69, 205–223.
- Arroyo, M. T. K., Primack, R. B., and Armesto, J. J. (1982) Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. *American Journal of Botany* 69, 82–97.
- Arroyo, M. T. K., Rozzi, R., Squeo, F., and Belmonte, E. (1990) Pollination in tropical and temperate high elevation ecosystems: hypotheses and the Asteraceae as a test case. In Mount Kenya area. Dynamics of a tropical mountain ecosystem. (Winiger, M., Wiesmann, U., and Rheker, J. R., eds.), Proceedings of the International "Workshop on Mount Kenya area: differentiation and Ecology and Socio-Economy of Mount Kenya Area" held at Nanyuki, Kenya, March 5–12, 1989, pp. 21–31.
- Bell, K. L. and Bliss, L. C. (1980) Plant reproduction in a high arctic environment. *Arctic and Alpine Research* 12, 1–10.
- Blionis, G. J., Halley, J. M., and Vokou, D. (2001) Flowering phenology of *Campanula* on Mt Olympos, Greece. *Ecography* 24, 696–706.
- Capitanelli, R. (1972) Geomorfología y clima de la provincia de Mendoza. *Boletín de la Sociedad Argentina de Botánica* 13 (Supl.), 15–48.
- Cox, D. R. and Oakes, D. (1984) Analysis of survival data. New York: Chapman and Hall.
- Cruden, R. W. (1977) Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. *Evolution* 31, 32–46.
- Cruden, R. W. (2000) Pollen grains: why so many? *Plant Systematics and Evolution* 222, 143–165.
- Cruden, R. W., McClain, A. M., and Shrivastava, G. P. (1996) Pollination biology and breeding system of *Alliaria petiolata* (Brassicaceae). *Bulletin of the Torrey Botanical Club* 123, 273–280.
- Medan, D. (1991) Reproductive phenology, pollination biology, and gynoecium development in *Discaria americana* (Rhamnaceae). *New Zealand Journal of Botany* 29, 31–42.
- Medan, D. and Aagesen, L. (1995) Comparative flower and fruit structure in the Colletieae (Rhamnaceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 117, 531–564.
- Medan, D. and Basilio, A. M. (2001) Reproductive biology of *Colletia spinosissima* (Rhamnaceae) in Argentina. *Plant Systematics and Evolution* 229, 79–89.
- Medan, D. and D'Ambrogio, A. C. (1998) Reproductive biology of the andromonoecious shrub *Trevoa quinquenervia* (Rhamnaceae). *Botanical Journal of the Linnean Society* 126, 191–206.
- Medan, D., Montaldo, N. H., Devoto, M., Mantese, A., Vasellati, M. V., Roitman, G. G., and Bartoloni, N. H. (2002) Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic, Antarctic and Alpine Research* 34, 233–241.
- Medan, D. and Vasellati, M. V. (1996) Nonrandom mating in *Discaria americana* (Rhamnaceae). *Plant Systematics and Evolution* 201, 233–241.

- Memmott, J. (1999) The structure of a plant-pollinator food web. *Ecology Letters* 2, 276–280.
- Molau, U. (1993) Relationship between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research* 25, 391–402.
- Moldenke, A. R. and Lincoln, P. G. (1979) Pollination ecology in montane Colorado: a community analysis. *Phytologia* 42, 349–379.
- Movia, C. P., Ower, G. H., and y Pérez, C. E. (1982) Estudio de la vegetación natural de la provincia del Neuquén. Tomo II. Relevamiento. Anexo figuras y fotografías. Provincia del Neuquén: Subsecretaría de Estado de Recursos Naturales.
- Ne'eman, G., Dafni, A., and Potts, S. G. (2000) The effect of fire on flower visitation rate and fruit set in four core-species in east Mediterranean scrubland. *Plant Ecology* 146, 97–104.
- Patt, J. M., French, J. C., Schal, C., Lech, J., and Hartman, T. G. (1995) The pollination biology of tuckahoe, *Peltandra virginica* (Araceae). *American Journal of Botany* 82, 1230–1240.
- Pías, B. and Guitián, P. (2001) Flowering phenology and pollen-to-ovule ratio in coastal dune communities near Eurosiberian-Mediterranean border in the NW Iberian peninsula. *Flora* 196, 475–482.
- Primack, R. B. (1979) Reproductive biology of *Discaria toumatou* (Rhamnaceae). *New Zealand Journal of Botany* 17, 9–13.
- Primack, R. B. (1985) Longevity of individual flowers. *Annual Review of Ecology and Systematics* 16, 15–37.
- Sandvik, S. M., Totland, O., and Nylehn, J. (1999) Breeding system and effects of plant size and flowering time on reproductive success in the alpine herb *Saxifraga stellaris* L. *Arctic, Antarctic and Alpine Research* 31, 196–201.
- Savile, D. B. O. (1972) Arctic adaptations in plants. Canada Department of Agriculture, Monograph 6, 1–81.
- Tortosa, R. D. (1983) El género *Discaria* (Rhamnaceae). *Boletín de la Sociedad Argentina de Botánica* 22, 301–335.
- Tortosa, R. D. (1995) Rhamnaceae. *Flora Fanerogámica Argentina*, Vol. 9. Córdoba: Proflora, pp. 1–18.
- Tortosa, R. D., Agesen, L., and Tourn, G. M. (1996) Morphological studies in the tribe Colletieae (Rhamnaceae): analysis of architecture and inflorescences. *Botanical Journal of the Linnean Society* 122, 353–367.
- Webb, C. J. (1985) Protandry, pollination, and self-incompatibility in *Discaria toumatou*. *New Zealand Journal of Botany* 23, 331–335.
- Webb, C. J. and Lloyd, D. G. (1986) The avoidance of interference between the presentation of pollen and stigma in angiosperms. II. Herkogamy. *New Zealand Journal of Botany* 24, 163–178.
- Wyatt, R., Broyles, S. B., and Lipow, S. R. (2000) Pollen-ovule ratios in milkweeds (Asclepiadaceae): an exception that probes the rule. *Systematic Botany* 25, 171–180.
- Zietsman, P. C. and Botha, F. C. (1992) Flowering of *Ziziphus mucronata* subsp. *mucronata* (Rhamnaceae): anthesis, pollination and protein synthesis. *Botanical Bulletin of the Academia Sinica* 33, 33–42.

D. Medan

Cátedra de Botánica
Facultad de Agronomía
Universidad de Buenos Aires
Av. San Martín 4453
C1417DSE Buenos Aires
Argentina

E-mail: diemedan@mail.retina.ar

Section Editor: G. Gottsberger

