

Ambophily, not entomophily: the reproduction of the perennial *Discaria chacaye* (Rhamnaceae: Colletieae) along a rainfall gradient in Patagonia, Argentina

Diego Medan^{1,2} · Mariano Devoto^{1,2}

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Abstract Geographical gradients are useful tools for studying how changing environmental conditions affect communities' structure and function, and any perceived general pattern can be tested by examining the response of different populations of a given species widely distributed along the focal gradient. We studied populations of the perennial, putatively entomophilous plant *Discaria chacaye* located along a strong east–west rainfall gradient in the north-west of Patagonia, Argentina, where a significant community-level eastwards replacement of Diptera by Hymenoptera as main flower visitors takes place. *Discaria chacaye* showed an invariant set of traits (flowers rewarding with nectar and pollen a fly-dominated pollinator assemblage, combined with protandry, incomplete dichogamy and self-incompatibility) which were modulated by site-dependent factors (availability of pollinators, wind strength, and perhaps abundance of seed predators). The interplay between all factors resulted in an increase in fruit set from the wet to the dry end of the gradient. Unexpectedly, wind was found to be an important pollen transporter, while (a) the plant's use of pollinator groups was mostly uncorrelated with their local abundances along

the gradient, and (b) both pre- and postzygotic fecundity losses occurred, mostly attributable to geitonogamy. The finding of ambophily in *D. chacaye* suggests that this pollination strategy could be more frequent than previously suspected along the studied gradient.

Keywords Ambophily · Breeding system · Patagonia · Wind pollination

Introduction

Variation in plant–pollinator interactions has been reported in response to differences in landscape structure (Steffan-Dewenter et al. 2002), habitat fragmentation (Aizen and Feinsinger 1994a, b), and changes in altitude (Arroyo et al. 1982; Malo and Baonza 2002; Medan et al. 2002), latitude (Elberling and Olesen 1999; Ollerton and Cranmer 2002), insularity (Olesen and Jordano 2002), temperature (Martín González et al. 2009) and rainfall (Devoto et al. 2005, 2009). The effect of environmental variables on plant–pollinator systems can be approached by studying geographical gradients (Rech et al. 2016). These have been commonly used as ecological tools for understanding the influence of environmental factors on structure and functioning of terrestrial ecosystems (Vitousek and Matson 1991; Steffen et al. 1999), among other reasons, because geographical environmental variation gives rise to changes in the species composition of interacting guilds (Totland 1993; Medan et al. 2002; Fabbro and Körner 2004; Devoto et al. 2005, 2009). In turn, such changes may give rise to spatial mosaics of interactions which may be a key factor for speciation driven by interactions (Thompson 1998; Johnson and Steiner 1997; Totland 2001).

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✉ Diego Medan
diemedan@agro.uba.ar

¹ Cátedra de Botánica General, Facultad de Agronomía, Universidad de Buenos Aires, Avda. San Martín 4453, C1417DSQ Buenos Aires, Argentina

² Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

In the Patagonian region of South America, from 35° to 55°S, there is a strong east–west rainfall gradient caused by the Andes which impose an important barrier to the wet air masses from the Pacific Ocean (Paruelo et al. 1998a). This gradient is associated with a striking shift in vegetation in less than 150 km in an east–west direction from xeric desert shrubland to grass-shrub steppe, to a low stature tree cover and finally to closed canopy forest (Movia et al. 1982; Paruelo et al. 1998a, b; Austin and Sala 2002). Three previous studies in the region analysed the community-scale effect of this geographical gradient on plant–pollinator interactions. Aizen and Ezcurra (1998) found a significant eastwards (i.e. from the forest towards the steppe environments) decrease in bird pollinated taxa throughout the region and related this pattern to a response of plants to abiotic conditions rather than to an innate scarcity of suitable bird flower visitors. Devoto et al. (2005, 2009) reported a significant eastwards replacement in the flower-visitor assemblage of Diptera by Hymenoptera species. However, very little is known whether this community-level pattern is reflected in the visitor assemblages of particular plant species with a widespread distribution along the gradient. In the single study available to date on this respect, Devoto et al. (2006) found a highly significant increase in nectar concentration in the plant *Embothrium coccineum* towards the drier end of the gradient, a change that nevertheless was uncoupled to a significant turnover in the species' flower-visitor assemblage. It was noted, however, that perhaps because of the high viscosity of nectar, the apparently “best adapted” pollinators (a hummingbird and a tanglewing fly) were absent at the easternmost populations, lending support to Aizen and Ezcurra's (1998) hypothesis. Recently Chalcoff et al. (2012) showed that the absence of the hummingbird at those populations was associated with increased pollen limitation and eventual reproductive failure.

This paper focuses on *Discaria chacaye* (G. Don) Tort. (Rhamnaceae–Colletieae), a woody perennial which shows remarkable ecological plasticity. It is found along the southern Andes, from sea level to 2300 m a.s.l., between central Chile and the Beagle Channel at Tierra del Fuego, ranging in growth habit from carpet-like shrub to medium-sized tree, and being generally related to riparian areas (Tortosa 1983a, b; Moore 1983). At the northern part of its latitudinal distribution (39–41° S), *D. chacaye* is a frequent species along the above-described rainfall gradient, occurring first as a relatively tall tree in the humid *Nothofagus dombeyi* and mesic *Austrocedrus chilensis* forests, then as small tree along streams and nearby permanent or temporarily inundated areas, and finally as low shrub at the dry forest-steppe ecotone. The exploration of its reproductive responses could provide further insight into the ways in which the gradient affects a particular plant

species and, conversely, into the ways in which the species is adapted to the abiotic and biotic conditions along this gradient. According to a previous report, along the gradient *D. chacaye* interacts with a relatively large assemblage of insect flower visitors (Medan et al. 2012), suggesting a pollination-generalist condition.

The focal species' riparian habit makes of direct water availability an unimportant variable along the gradient, so reducing the number of factors possibly influencing its reproductive responses. On the other hand, although *D. chacaye* shows obvious adaptations to entomophily, routine controls to distinguish between insect- and wind-transported pollen were included in the experimental design, partly because wind is a ubiquitous factor in Patagonia (Paruelo et al. 1998a), and also because ambophily (wind pollination combined with insect pollination, Culley et al. 2002) has been previously reported in a related and partly sympatric species, *Ochetophila trinervis* (Medan and Devoto 2005). Although a taxonomically relatively restricted pollination strategy, ambophily is probably more widespread than presently known (Culley et al. 2002; Rech et al. 2016), and new cases continue to be added (see e.g. van Tussenbroek et al. 2016; Custodio et al. 2017). This strategy could provide reproductive assurance, whereas insect pollination can ensure reproductive success in closed, forested habitats, wind pollination may be more important in open sites (Culley et al. 2002).

In this paper, we specifically seek to answer the following questions: (1) which are the reproductive traits of *D. chacaye* with respect to reproductive phenology at the population- and individual floral scales, composition of the pollinator assemblage, pollination strategies, breeding system, and maternal success, and (2) how these traits change along the gradient.

Materials and methods

Focal species

Discaria chacaye is a spiny, leafy plant ranging in height from 0.1 to 15 m (Medan and Tortosa 1976) (Fig. 1a). Flowers are presented in three-flowered cymes grouped in axillary inflorescences, which immediately resume vegetative growth after anthesis (Tortosa et al. 1996). They are pedicellate, hermaphrodite, actinomorphic, 4–5 merous, 4 mm in diameter, and with a 2 mm long × 1.8 mm wide cup-shaped white floral tube. The deltoid sepals and the stamens are alternate, while petals are lacking. Supported by short filaments, anthers are raised 0.5 mm above the opening of the floral tube. An annular-type nectary is at the bottom of the floral tube, around the ovary (Medan and Aagesen 1995). The 3(4)-carpellate gynoeceum is



Fig. 1 *Discaria chacaye*. **a** Habit at the Lago Tromen site, **b** an inflorescence, with phenological stages indicated by numbers at selected flowers (see Table 2 for stage coding). Bar 1 m (a), 5 mm (b). Photo (b) by D. Medan

composed of a semi-inferior, 3(4)-ovulate ovary, a style, and a terminal stigma that reaches anthers' height. Fruits are 1-3(4)-seeded explosive capsules.

Study sites

Research on reproductive biology of *D. chacaye* was carried out between 1995 and 2007 on six populations located in Argentina's provinces of Neuquén and Río Negro (Table 1; Online Resource 1). All field work sites were located at lake shores and stream banks, where *D. chacaye* formed almost pure riparian woods of (0.5) 2–4 m tall treelets. Populations ranged in size from ca. 1000 individuals covering 3 hectares (Lago Tromen) to fifteen individuals growing over 0.1 ha (Estancia San Ramón). Most work concentrated on the three largest populations (Lago Huechulafquen, Lago Tromen and Lago Queñi, hereafter H, T and Q) which were visited up to three times each. Manipulative experiments were done at these populations in 1997, 1998 and 1999 (Table 1). Representative

individuals were documented by depositing herbarium vouchers at the Gaspar Xuarez herbarium (BAA, University of Buenos Aires, Argentina). Climate in the study area is cold temperate, with an annual rainfall of 780–2500 mm (concentrated in the winter months). During experimental work at sites H, T and Q, local weather conditions (temperature, relative humidity and wind speed) were recorded at the same times of the day (every 2 h from 8:00 to 22:00) with hand-held instruments. For further environmental data about the study area see Movia et al. (1982), Roig (1998), Eskuche (1999) and Devoto et al. (2005, 2009).

Reproductive phenology and pollination biology

At the population scale, dates of blooming start and end are based on field observations, collection notes of 47 specimens from the BAA herbarium, and also from the literature (Tortosa 1983a, b; Moore 1983; Medan et al. 2012). Individual flower phenology was studied at populations H and T. Fifty flower buds and open flowers from different individuals were dissected and inspected under a Wild M5 50× stereomicroscope in the field. Additionally, groups of 16–25 flowers from 3 to 5 individuals were separately labelled and monitored from the unopened bud stage until anthesis was completed, in order to define floral morphological stages according to the position of sepals and stamens, and the status of pollen, stigma, nectar and scent presentation. Stigmatic receptivity was assessed with the hydrogen peroxide test (Dafni et al. 2005). Nectar was collected with handmade microcapillaries, and sugar concentration was measured with a hand-held refractometer modified for small volumes (Eclipse model 45–81, Bellingham and Stanley, UK). As proper equipment was unavailable, variation of scent emission during flower lifetime was assessed by offering five people vials with freshly collected flowers in different developmental stages. To compute pollen/ovule ratios, number of pollen grains per flower were calculated by extrapolating haemocytometer counts ($N = 9$ flowers from three individuals of population H) and using three as the number of ovules per flower. Pollen size was measured under 600× magnification ($n = 20$ pollen grains).

Flower visitors were observed and sampled across the six studied populations during daytime and dusk, in order to record the entire visitor community (cumulative time = 75 h). No attempt was made to establish the specific period nor the weather conditions associated with the activity of the different visitor taxa.

An insect was recorded as a pollinator when it was possible to confirm its contact with anthers and stigmas. Four hundred and forty-three insect individuals were caught for identification and permanently mounted according to standard entomological methods. Seventy-two

Table 1 Studied populations of *Discaria chacaye* (ordered by increasing date of first visitation) and types of field work made in them

Population					Work	
Location	Geographical coordinates	Annual precipitation (mm)	Altitude (m a.s.l.)	Approx. no. individuals	Type	Date
Lago Huechulfquen (H)	39°47'55"S	780	900	390	PP	18.12.1995
	71°12'45"W				BS, FB, PP, PS, RS	9–14.12.1997
					BS, FB, PP	12–14.12.1999
					FB	14–15.12.2003
Puerto Canoa	39°45'00" S	1000	900	100	FB, PP	15.12.1997
	71°30'00" W					
Lago Tromen (T)	39°34'15"S	1000	1050	1000	BS, FB, PP, PS, RS	9–16.12.1998
	71°26'30"W				FB	11–12.12.2003
Lago Queñi (Q)	40°09'04"S	2550	820	130	BS, FB, PP, PS, RS	9–17.12.1999
	71°43'10"W					
Villa Trafal	40°40'08"S	1250	890	15	FB, PP	10.12.2001
Estancia San Ramón	41°04'27"S	750	890	30	BS, FB, PP	14–17.12.2007
	71°07'07"W					

Field sites H, T and Q, where manipulative experiments were done (dates in boldtype), were visited again a month later to harvest the experimental material. Precipitation data after Movia et al. (1982)

BS breeding system, FB floral biology, PP population phenology, PS population structure, RS reproductive success

per cent of the visitor taxa were identified to genus or species, in many cases with the assistance of taxonomists (see Acknowledgments). When identification to species was not possible, the specimens were morpho-typed. All collected material is deposited at the Entomological Collection of the General Botany Unit (FAUBA), School of Agronomy, University of Buenos Aires. The proportion of flowers that had received pollen when anthesis was completed was determined in populations H and T by inspecting stigmas of freshly collected flowers at 50× ($N = 1980$ flowers of 24 individuals).

Breeding system and maternal success

To assess the relative effect of wind and insect visitors on fruit set, experiments were conducted involving three treatments that were applied to groups of randomly chosen individuals in the populations H, T and Q ($N = 32$ plants). (1) *Open pollination*: Flower buds were left uncovered and experienced natural pollination ($N = 17,312$ flowers). (2) *Mesh bagging*: Flower buds were covered with 1 mm-mesh bridal veil bags to prevent insect visitation while enabling wind pollination ($N = 13,005$ flowers). (3) *Cloth bagging*: As in 2, but using white muslin bags, excluding both insects and airborne pollen ($N = 14,577$ flowers). Occasional observations indicated that neither mesh bagging nor cloth bagging changed flower phenology, suggesting that bagging did not affect floral viability in either treatment. Experimental branches were collected 30 days

after the start of the experiment, when all flowers had abscised or were developing into fruit. For each population and treatment, per-plant fruit-to-flower ratios were computed.

To measure the relative effect of outcross pollen on fruit set, a hand-pollination treatment was applied to four randomly selected plants of population T. In experimental branches bagged as in treatment (3) above, all open flowers (except flower buds expected to open on the next few days) were removed from inside the closed bags. Two days later, the bags were opened and flowers with abundant stigmatic secretion (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 and pollinated by gently touching them with an anther of a freshly opened flower of the same individual (geitonogamy, $N = 255$ flowers) or of another individual located at least 10 m distant from the receptor individual (xenogamy, $N = 167$ flowers). Afterwards, bags were closed again. Emasculation was not carried out because, given the small flower size, it would have been associated with a high risk of damaging the stigma (see Medan et al. 2013, 2015 for a similar approach). Two days later, a subset of the flowers ($N = 116$, of which 71 corresponded to the geitonogamy treatment and 45 to the xenogamy one) was collected, fixed in FAA, transported to the laboratory, soaked for 48 h in 5% NaOH (w:v) at 30 °C, cleared in diluted NaClO, mounted on 0.1% decolourised aniline blue, and viewed with a fluorescence microscope (Martin

1959). For each flower, the number of pollen grains per stigma was recorded, as well as the number of pollen tubes reaching the bottom of the ovary, i.e. the level at which micropyles of ovules are located. The remaining experimental flowers ($N = 306$) were left inside the bags and were collected 30 d after the start of the experiment, when they either had abscised or were developing into fruit. In the first subset, stigmatic loads and the proportions of flowers with pollen tubes reaching micropyles were compared between treatments. In the second subset, fruit-to-flower ratios were compared. Separately, samples of untreated, open-pollinated flowers were collected and processed for pollen tube observation ($N = 347$ flowers of 24 individuals of populations H and T).

The total number of flowers per plant was estimated for 33 individuals of populations H and T by multiplying the number of flowers in a representative branch by the number of equivalent branches of each individual. The number of fruits per plant was computed using the fruit-to-flower ratios from the open pollination controls of the pollination experiment (see above) supplemented with data from an additional ten individuals of population T ($N = 28$ individuals). Using number of flowers per plant as a proxy for plant size (for the positive relationship between these variables, see Ollerton and Lack 1998 and references therein), the possible dependence of reproductive effort on plant size was explored in populations H and T by relating fruit set rate to plant size ($N = 22$ individuals). Viable seed-to-ovule ratios were computed for samples of fully grown fruits from populations H and T ($N = 864$ fruits from 22 individuals).

Statistics

Samples were described using arithmetical mean \pm 1 S.D. Among-populations differences in the local meteorological data were analysed with Kruskal–Wallis tests followed by Median tests. Assemblages of flower visitors were compared with the Jaccard Similarity Index. Results of the pollination experiments were assessed in the context of a binomial GLMM with site and treatment as fixed effects, and a random intercept associated with each individual plant. To define the binomial response variable, the number of fruits produced was considered as the “number of successes” in the experiment and the number of aborted flowers was considered the “number of failures”. Further, because the interaction between treatment and site was significant (see details in Online Resource 2), a new factor was created from the combination of each level of treatment and site. A GLMM was then run with this new factor and its levels were then compared with multiple pair-wise comparisons using Tukey’s contrasts. The relationship between plant size and fruit set was tested with a Pearson’s

correlation test. Among-population differences in size of stigmatic loads and number of flowers per individual, as well as among-treatment differences in pollen tube performance and fruit set in the hand crossing experiment, were analysed using two-tailed Wilcoxon’s tests. The R (R Development Core Team 2008; version 3.3.3) functions used were “glmer” to compute the GLMMs, “interaction” to construct the synthetic treatment \times site factor, and “glht” to perform the multiple comparisons (corresponding R code is available from the authors upon request).

Results

Reproductive phenology and flower biology

At the population scale, blooming of *D. chacaye* extends from November to March, with a peak during December–January in the north of the species’ range, and a progressive delay in start of blooming with increasing southern latitude. In the populations included in this study, most individuals (70–100%) reached full bloom approaching mid-December. Individuals at different phenophases coexisted, indicating variation among individuals in blooming start date. Mean inter-individual distance varied between 2 m (population H) and 4.25 m (population T). Weather conditions differed slightly but significantly between experimental sites. In comparison, T was the most windy site (wind speed was 6.3 ± 2.7 m/s, vs. 3.0 ± 2.2 in H and 0.6 ± 1.2 in Q; $H = 90.3$, $p < 0.0001$), and also the coldest site (15.4 ± 3.6 °C vs. 16.8 ± 5.6 in Q and 17.9 ± 4.7 in H; extreme values statistically different; $H = 8.5$, $p < 0.015$). Relative humidity at site Q was significantly higher than in H and T ($70.1 \pm 17.7\%$ vs. 62.4 ± 16.9 and 61.7 ± 14.2 , respectively; $H = 7.5$, $p < 0.03$). The diurnal variation (Fig. 2) included opposite peaks of temperature and relative humidity around 14:00–16:00, and a trend to increased wind speeds in the afternoon.

Inflorescences typically displayed 12–16 flowers at the same time (Fig. 1b). Individual flowers started anthesis at different times of the day, and shed and received pollen for 4–5 days. Five distinct floral stages were identified (Table 2). At day 1 (stage 1), sepals were closed but anthers were already dehisced, while stigma and nectary were still dry. No evidence of the occurrence of automatic self-pollination was observed. At day 2 (stages 2 and 3), sepals opened and gradually spread, nectar secretion and pollen removal started, and a mildly faeces-like scent was perceptible. At days 3–5 (stage 4), sepals became first horizontal (i.e. transversal to floral axis) then reflexed, nectar secretion continued, scent became weaker and sweeter, pollen was fully removed, and the stigma became swollen and wet, showing a clear reaction to the peroxidase

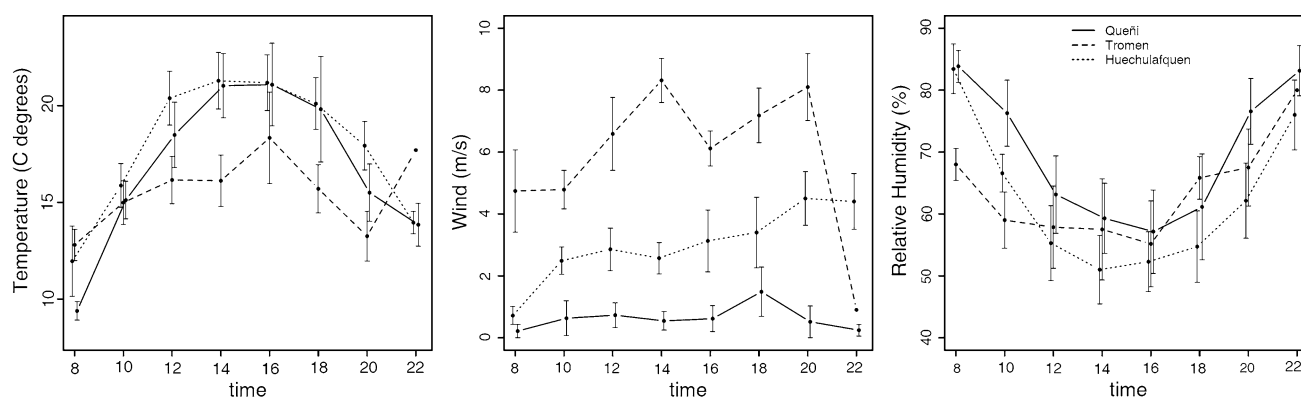


Fig. 2 Diurnal variation of three weather variables at three sites where manipulative experiments on *Discaria chacaye* were carried out. For all variables, sample sizes [*n* datapoints/*n* days] were [55/7] (site H), [44/8] (T), and [56/7] (Q)

Table 2 Individual flower phenology of *Discaria chacaye*

Floral part/ floral stage	Day 1	Day 2		Days 3–5	Day 6 onwards
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Sepals	Closed	±Erect	Spreading	Horizontal to reflexed	Wilting, later dropped
Stamens	Anthers open	Filaments convergent, pollen abundant	Filaments erect, pollen partly removed	Filaments erect, pollen fully removed	Wilting, later dropped
Pistil	Stigma dry, peroxidase test negative	Stigma dry, slightly below anther height, peroxidase test negative	Stigma dry, at anther height, peroxidase test negative	Stigma swollen, wet, slightly over anther height, peroxidase test positive	Stigma and style wilting, later dropped; ovary enlarging
Nectary	Dry	Green, secretion scarce	Yellow, secretion abundant	Yellow, secretion abundant	Dry
Scent	Very faint	Strong	Strong	Mild and somewhat sweeter	None

test. Average nectar concentration varied between 9.7% (H, $n = 4$) and 21.4% (T, $n = 10$), and average nectar volume was 0.5 μ l (T, $n = 20$). Both cross-pollination and intra-plant self-pollination were possible at stage 4. At day 6 and later (stage 5), sepals, floral tube, stamens and style started to wither, nectar secretion and scent production stopped, and visitation ceased, while the ovary enlarged and the pedicel became thicker. Pollen grain diameter averaged 24 μ m, and the pollen/ovule ratio was $28,437 \pm 5573:1$.

Pollinators and pollination service

Visits of insects to flowers occurred between 9:00 am and 10:00 pm, at temperatures above 13° C and with breezes in the range 0–9.8 m/s. One hundred and thirty-one species of insects belonging to forty families of the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera and Thysanoptera were recorded as flower visitors (Online Resource 3). With almost half the total species, Diptera dominated the overall visitor assemblage and also prevailed in the specific assemblages of the most intensively collected field sites (H, 62%; T, 54%, Q, 45%). Hymenoptera, Coleoptera and

Lepidoptera were less represented (20.6, 15.3 and 13.7%, respectively).

Assemblages differed markedly between sites. Seventy-three per cent of the species were collected at only one site, only fourteen species (10.6%) were recorded at three or more sites, and just five species were found at four or more sites. Considering the three best sampled sites, the main orders of visitors changed in importance from the most xeric (H) to the most humid one (Q): Coleoptera and Lepidoptera increased (3–22 and 0–16%, respectively) while Diptera and particularly Hymenoptera decreased (62–45 and 32–16%, respectively). Accordingly, overall similarity decreased with increasing distance between sites (Jaccard Index [H vs. T] = 0.12; [H vs. Q] = 0.08; [T vs. Q] = 0.21).

Breeding system and maternal success

Most open-pollinated flowers received pollen (91.2 and 80.7% in H and T, respectively; the difference was non-significant). In H, stigmatic loads were smaller than in T (45.2 ± 53.6 vs. 89.5 ± 79.6 grains/stigma, respectively;

$W = 8312.5$, $p < 0.0001$; $n = 71$ and 276 flowers, respectively) and pollen tubes reached micropiles in fewer flowers (3.3 vs. 38.0%; $W = 20.0$, $p = 0.02$).

The bagging experiment showed that pollination conditions affected fruit set in two of the three sites (H and T) (Fig. 3; see GLMM details and plot of main effect in the Online Resources 2 and 4, respectively). Fruit set was higher in open-pollinated flowers (18.0 and 11.3% in H and T, respectively) than in flowers covered with cloth bags (1.3 and 0.6%, respectively). Fruit set in mesh-bagged flowers had intermediate values, and differed from fruit set under cloth bagging (Tukey's tests between mesh and cloth, $p < 0.001$ at both H and T) and open pollination (Tukey's tests between mesh and open, $p < 0.001$ at both H and T). Fruit set at population Q was very low and similar between all treatments, although there was a trend towards a difference between open pollination and mesh bagging at this site (Tukey, $p = 0.063$).

Hand-pollination delivered 226.2 ± 81.3 pollen grains to each stigma. In selfed flowers pollen tubes failed to grow farther than the stigma or the upper third of the style, while in crossed flowers they reached the bottom of the ovary in 55.5% of the cases. Compared to manual selfing, manual outcrossing increased fruit set by two orders of magnitude (0.5 vs. 36.0%; $W = 26.0$, $p = 0.028$, $n = 184$, 122 selfed/outcrossed flowers, respectively). Also, outcrossed flowers set more fruit than open-pollinated flowers of the same individuals (36 vs. 11.3%; $W = 26.0$, $p = 0.028$, $n = 6343$, 122 open-pollinated/outcrossed flowers, respectively).

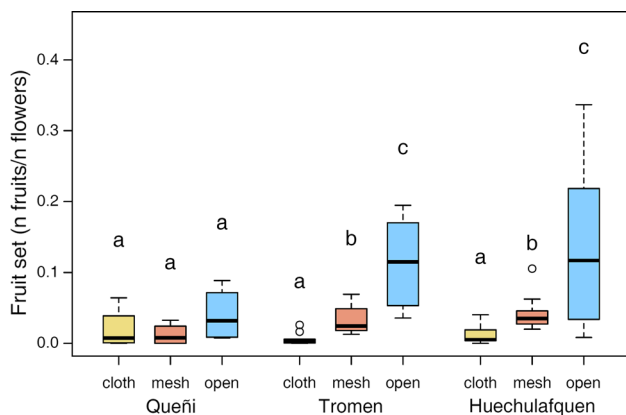


Fig. 3 Per cent fruit set of *Discaria chacaye* under three pollination treatments at three experimental sites. From left to right, sites are arranged following decreasing precipitation. Within each site, treatments with different letters are significantly different (Tukey's post hoc tests, $p < 0.05$). See details of GLMM and post hoc comparisons in Online Resources 2 and 3. Sample sizes [n flowers/ n individuals] for site H: [2198/9] (open pollination), [3836/9] (mesh bagging), and [3597/9] (cloth bagging); for site T: [14,285/9], [8380/8], and [10,020/9]; for site Q: [829/4], [789/4], and [960/4]. For treatment details see "Materials and methods"

Individuals bore more flowers in T than in H ($801,770.6 \pm 705,449.3$ vs. $54,392.3 \pm 72,664.4$ flowers/individual; $W = 122.0$, $p = 0.0003$; $n = 20$ and 13 individuals, respectively). The average number of fruits per individual, calculated as the mean number of flowers/individual multiplied by the fruit set rate under open pollination, was $54,392.3 \times 18.0\% = 9790.6$ fruits per reproductive episode in H, and $801,770.6 \times 11.3\% = 90,600$ fruits per reproductive episode in T. At site H, fruit set increased with plant size (Pearson's $r = 0.99$, $p = 0.012$, $n = 3$ individuals) while at site T these variables were uncorrelated ($r = -0.2$, $p = 0.4$, $n = 19$). On average, fruits had 1.0 ± 0.4 viable seeds in H and 1.0 ± 0.5 viable seeds in T (difference was nonsignificant; $n = 142$ and 786 fruits, respectively). Thus, annual seed output per individual was expected to be grossly equivalent to the number of matured fruits per individual.

Discussion

Pollinator assemblages of *Discaria chacaye* in the context of the rainfall gradient

Although *D. chacaye*'s assemblage was dominated by flies, the importance of these as potential pollinators decreased towards the wet end of the gradient, against the gradient's general increase in diversity of this insect group. Bees are best represented at the gradient's dry end, this time in accordance with the general trend. This adds a new example of bees as important pollinators at the easternmost sites of the gradient, even for definitely non-melittophilous plants, as previously reported for the hummingbird/fly pollinated *Embothrium coccineum* (Devoto et al. 2006).

Ambophily in *Discaria chacaye*

Flowers excluded from insect visits and airborne pollen set significantly less fruits than open-pollinated flowers did, and this difference was statistically significant at two of the three populations (Fig. 3). Therefore, exogamous pollen was important in maximizing fruit set. Mesh bagging (i.e. only wind-carried pollen could reach stigmas) produced intermediate values, and open pollination (both wind- and insect-carried pollen could reach stigmas) produced the highest fruit set. This indicates that both wind and insects play a role in the pollination of this species.

Anemophily seems to make an important contribution to reproduction in this species, as suggested by the facts that (a) fruit set was lowest at the least windy and most humid population (Q), and (b) stigmatic pollen loads of open-pollinated flowers were largest at the driest and most windy

population (T). The environmental conditions were unfavourable for anemophily at population Q, where mean wind speed was 0.6 m/s (see also Fig. 2), well below the low end of the speed ranges recorded for anemophiles growing in forests (1–10 m/s; Whitehead 1969), and the annual rainfall was 2550 mm (Table 1), a value hardly compatible with the low humidity conditions typically associated with wind pollination (Culley et al. 2002). On the other hand, mean wind speed at populations H and T (3.0 and 6.3 m/s, respectively; see also Fig. 2) was well within the above-mentioned wind speed range. Low humidity around noon and growing wind speed in the afternoon suggest that conditions for anemophily at these sites are most favourable in the interval 12:00–20:00.

As usual in ambophiles (Culley et al. 2002), morphological traits of *D. chacaye* are not typically anemophilous. Pollen is not powdery, but tends to aggregate in clumps as in most entomophiles; hence, a relatively high wind speed would be particularly effective in dislodging pollen from anthers. On the other hand, mass flowering and some flower traits favour wind pollination, including anthers exposed beyond the mouth of the flower tube, and lack of petals, which facilitates pollen removal (unlike most species of the tribe where anthers are protected by antheropposite, often hood-like petals). Moreover, the pollen-ovule ratio is relatively high (above 28,000:1) and pollen grain diameter is comparatively small (24 μm). A value of 36.2 μm , reported by Heusser (1971) for a population of southern Chile (under the name *Discaria discolor*), suggests that pollen size is subject to variation; hence, this trait deserves further study. Clearly, additional experimental effort is needed to unveil how much pollination is effected by each agent, including sampling of airborne pollen, inspection of stigmatic loads in bagged flowers, and investigating pollen loads on insects.

Anemophily has evolved repeatedly within basically entomophilous lineages in the Angiosperms (Culley et al. 2002; Rech et al. 2014). Wind pollination has very few antecedents in the otherwise entomophilous family Rhamnaceae. Exceptions were reported in *Rhamnus alaternus* (Aronne and Wilcock 1995), *Ziziphus mucronata* (Zietsman 1990), and *Ochetophila trinervis* (as *Discaria trinervis*, Medan and Devoto 2005). Plants showing ambophily show strong diversity in life history and reproductive traits, including a few examples of perennials with hermaphroditic flowers like *D. chacaye*. This species' habitat seems consistent with the evolution of wind pollination, since much of its range falls within Patagonia, an ecoregion characterized by persistent westerly winds with average speeds of 0.4–7.5 m/s, and maxima occurring between September and January (Paruelo et al. 1998a), a period that includes the species' flowering time. Interestingly, the simultaneous presence of ambophily in *D.*

chacaye and in the partly sympatric *Ochetophila trinervis* helps to explain the presence of hybrids between both species, until now attributed solely to shared pollinators (Medan et al. 2012).

Ambophily may provide reproductive assurance (Culley et al. 2002). In this regard it is illustrative to compare the ambophilous *D. chacaye* with the exclusively biotically pollinated tree *Embothrium coccineum*, which shares the rainfall gradient with the former almost exactly (Chalcoff et al. 2012). As hummingbirds and other pollinators, including passerine birds and nemestrinid flies, are replaced by bees towards the dry eastern range limit, pollen limitation increases and reproduction of *E. coccineum* eventually fails. A similar predominance of pollinating bees at the eastern end of *D. chacaye*'s latitudinal range is not associated with reduced reproductive success, which in part may be due to wind pollination.

Breeding system and reproductive success in the gradient's context

The pollination experiment showed that if both insect- and wind-borne pollen fails to reach stigmas, fruit set drops to very low values at all sites, indicating that neither automatic selfing nor apomixis are important in *D. chacaye*. In addition, hand pollinations and analysis of pollen tube behaviour demonstrated that access of outcross pollen tubes to ovules is what triggers fruit initiation, also suggesting that some mechanism of self-incompatibility operates in this species, as previously shown for several members of the Colletieae (Medan et al. 2013; Medan and Torretta 2015; Medan et al. 2015). While these basic traits were invariant along the gradient, factors affecting maternal success seemed to act differently among populations.

Pollen quantity

Reproductive output at sites H and T was not primarily limited by pollen quantity, since depending on site (a) between 80.7 and 91.2% of open-pollinated flowers received stigmatic loads, and (b) these loads consisted on average of 45.2 to 89.5 pollen grains each, which is numerically sufficient to deliver between 15 and 30 pollen tubes to each ovule, i.e. much more than necessary for producing full seed and fruit set. Since no data on stigmatic loads are available for site Q, the possibility of quantitative pollen limitation at that site cannot be ruled out. Interplant pollen transport at Q probably relied only on insects, because weather conditions (wind speed was extremely low and ambient humidity was high) were unfavourable for wind pollination. Also, the small size of the population may have reduced its attractiveness to insects, negatively affecting reproduction.

Pollen quality

Since average fruit set under open pollination was 18% or less, other factors apart from pollen quantity (e.g. low pollen quality, insufficient resources, postzygotic losses, or some combination thereof) constrained maternal success. Hand cross-pollination led to dramatic increases of fruit set, pointing out to the presence of self-pollen in stigmatic loads as the most probable cause of low pollination quality. In *D. chacaye*, selfing may occur both within flower because of incomplete dichogamy, and between flowers of the same individual (geitonogamy) because of the simultaneous anthesis of many flowers at the inflorescence, branch and plant levels. Since geitonogamy tends to increase with plant size (Robertson 1992; Karron et al. 2004, and references therein), intra-plant pollination was probably more intense at site T, where average individuals had ca. 15 times more flowers than at site H. Thus, the large stigmatic pollen loads recorded at T may have included mostly self, i.e. ineffective pollen.

Resources

Plant size and rate of fruit set were positively related at population H, suggesting that resources limit fruit production in younger individuals, while this restriction diminishes with age. It is generally accepted that younger plants may proportionally allocate more resources to growth and less to reproduction (Weiner 2004). No relationship size-fruit set rate was perceived at site T, where average individuals were probably above a critical size beyond which no size-related resource limitation occurs.

Postzygotic losses

During the evaluation of the pollination experiment, insect-damaged seeds and fruits were observed in the material harvested at sites T and Q. While these observations could not be systematized and therefore were excluded from “Results”, the impression remains that owing to predation by insects, and especially at site Q, more seeds and fruits were initiated and reached maturity than those that appeared in the final counts. This phenomenon could have affected both controls and bagged treatments, because oviposition could occur early in the flower development, i.e. before experimental branches were bagged. Weevils (Curculionidae-Curculionini) are probably involved here, given their feeding habits and taxonomic diversity at the study area (Morrone and Roig-Juñent 1995). Clearly, additional research is called for here to measure the relative importance of postzygotic losses as a limiting factor of maternal success.

Reproduction of *Discaria chacaye* as seen in the tribal context

Flowers of *D. chacaye* showed several traits shared by all members of Colletieae, including the tribe-dominant white floral colour (pure red flowers are exceptional in this group, see Medan and Montaldo 2005), but its faeces-smelling floral scent, which recalls that of the aromatic organic compound indole, is uncommon in the tribe. Similar fetid scents were reported in *D. americana* (Medan 1991) and *Adolphia infesta* (Medan et al. 2015), two species sharing with *D. chacaye* a fly-dominated flower-visiting assemblage. In *D. americana*, in male-stage (first-day) flowers the smell was stronger but not so offensive as in female-stage (fourth-day) flowers, in contrast to the change from fetid to sweet observed in *D. chacaye* for the same stages. In *Adolphia infesta*, the floral smell was milder and uniform during the two stages. Changes in floral scent during anthesis are known to occur both during the diurnal course of scent emission and along flower development (Wright and Schiestl 2009; Weiss et al. 2016, and references therein), in some cases effectively influencing pollinator behaviour. While no obvious differential visitation pattern was observed in male-phase as compared to female-phase flowers of *D. chacaye*, its existence cannot be ruled out without additional, objective-specific field studies, coupled with research on the volatiles that constitute the flower scent.

In *D. chacaye* pollen and nectar were offered as rewards for visitors (the usual tribal trait; see Medan et al. (2013) for the alternative high-pollen–low-nectar reward strategy found in a few members of the tribe). On a per-flower basis, the amount of nectar offered to visitors was small, but it could be reached even by insect species with short proboscides, because the flower tube is relatively short. Since only specialized flower morphologies tend to restrict visitor arrays (Nilsson 1988; Moré et al. 2007; Bloch and Erhardt 2008), the high diversity of the flower-visiting insect assemblage of *D. chacaye* was expected.

Limitations of the study

Logistic reasons prevented us from working at all sites during the same reproductive season; therefore, inter-annual variation in biotic and abiotic variables may have distorted actual between-site differences in the reproductive behaviour of *D. chacaye*. Also, since this study explored only part of the species’ geographical and ecological amplitude, traits as (for instance) the composition of the visitor assemblage are expected to show wider variability than here reported. On the other hand, several facts contributed to the quality of the dataset, namely (a) the temporal window during which experimental work

took place was the shortest possible (three consecutive years), (b) field sites were included in a narrow range, thus minimizing latitude- and altitude-associated changes in temperature, (c) the riparian habit of the studied populations precluded possible inter-annual changes in phenology due to fluctuating water availability, and (d) repeated visits to two of the field sites confirmed *D. chacaye*'s inter-annual phenological regularity.

Conclusions

At any place, a plant's reproductive behaviour can be viewed as the final result of two groups of factors: phylogenetically constrained traits, and site-specific biotic and abiotic variables that modulate the expression of the first group. In the particular case of *D. chacaye* in the north-west Patagonian rainfall gradient, the first set included flowers adapted to nectar- and pollen-rewarded, fly-biased entomophily, combined with protandry, incomplete dichogamy and self-incompatibility, while the second set included site-dependent pollinator availability, wind strength, and perhaps abundance of seed predators. The interplay of all factors resulted in per-flower increase in the species' maternal reproductive output from the wet to the dry end of the gradient. Unexpectedly, wind was found to be an important pollen transporter, the plant's use of pollinator groups was mostly uncorrelated with their local abundances along the gradient, and both pre- and postzygotic fecundity losses occurred, mostly attributable to geitonogamy.

The finding of ambophily in *D. chacaye* points out to wind as a factor that might have a non-random variation along the north-west Patagonian rainfall gradient and which, if incorporated to the current pollinator-availability model, could increase its predictive value. We hypothesize that given its ubiquity and local strength, wind can play a significant role in the transport of pollen of other not-obviously anemophilous plants found along the gradient. More case studies are clearly called for.

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Compliance ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Location of study sites in north-west Patagonia, Argentina.

Online Resource 2. Details of the binomial GLMM model and multiple post hoc comparisons.

Online Resource 3. Identity of insect visitors to *Discaria chacaye* flowers.

Online Resource 4. Plot of the main effect (extracted from a GLMM, see "Materials and methods") of pollination treatment on fruit set.

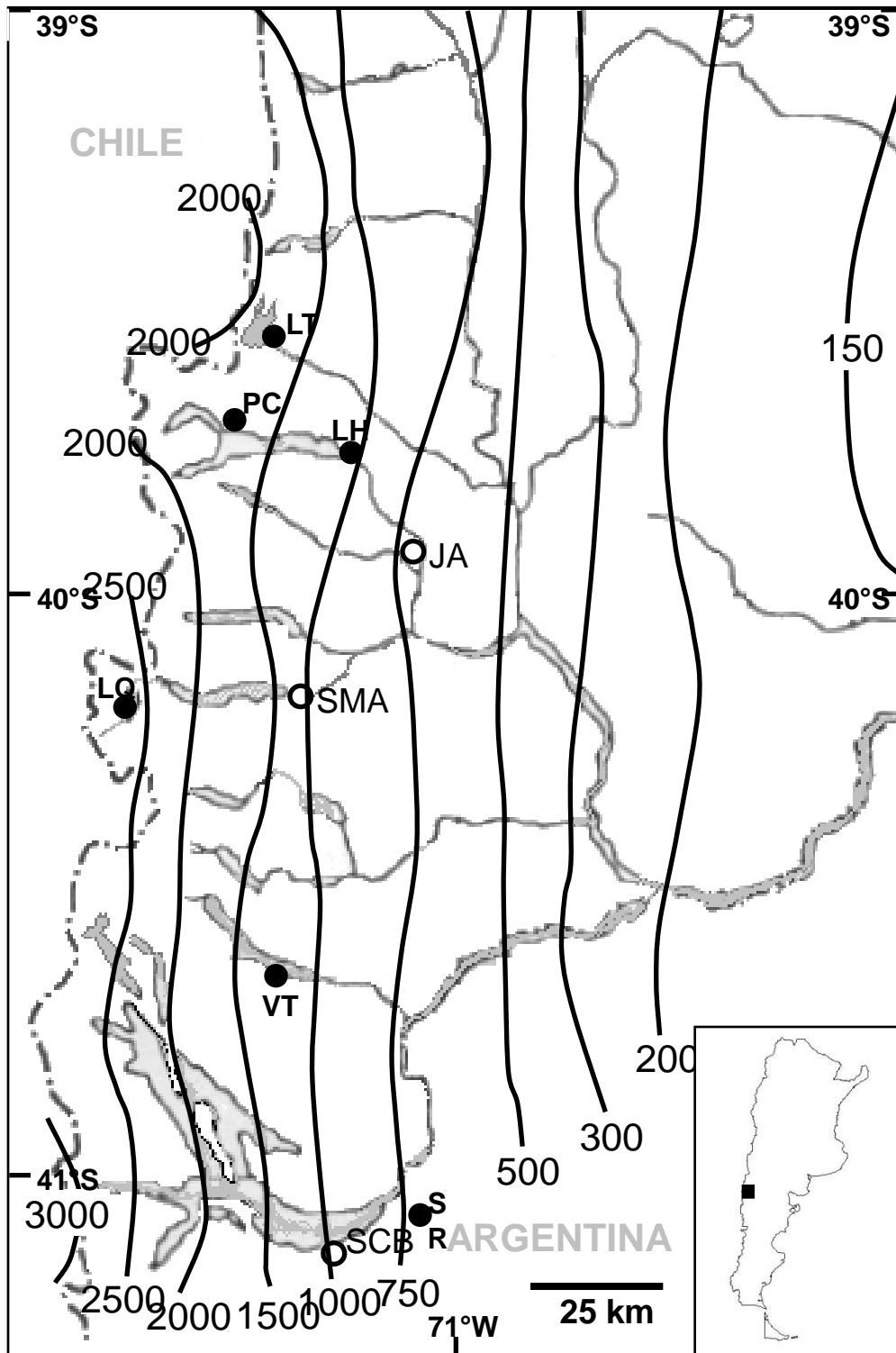
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Online Resource 1. Ambophily, not entomophily: the reproduction of the perennial *Discaria chacaye* (Rhamnaceae: Colletieae) along a rainfall gradient in Patagonia, Argentina. Plant Systematics and Evolution. Diego Medan^{1,2} and Mariano Devoto^{1,2}. ¹ Universidad de Buenos Aires, Facultad de Agronomía. Cátedra de Botánica General, Avda. San Martín 4453, C1417DSQ Buenos Aires, Argentina. ² Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Buenos Aires, Argentina. E-mail of corresponding author: diemedan@agro.uba.ar

Location of study sites in north-west Patagonia, Argentina. LH, Lago Huechulafquen; LQ, Lago Queñi; LT, Lago Tromen; PC, Puerto Canoa; SR, Estancia San Ramón; VT, Villa Traful. The main three cities in the region are also indicated: Junín de los Andes (JA), San Carlos de Bariloche (SCB), and San Martín de los Andes (SMA). Main hydrographical features are also included. Isohyets are redrawn from Movia et al. (1982).



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Details of the binomial GLMM model (and multiple post-hoc comparisons) used to analyze the effect of pollination treatment on reproductive output.

	Estimate	Std. Error	z value	<i>p</i>
(Intercept)	-41.782	0.4718	-8.855	2e-06
treatmentmesh	-0.4260	0.4743	-0.898	0.36910
treatmentopen	0.7389	0.3511	2.105	0.03533
siteT	-11.358	0.5468	-2.077	0.03778
siteH	-0.4474	0.5529	-0.809	0.41847
treatmentmesh:siteT	22.615	0.4970	4.550	5.36e-06
treatmentopen:siteT	24.092	0.3772	6.388	1.68e-10
treatmentmesh:siteH	15.248	0.5050	3.019	0.00253
treatmentopen:siteH	20.838	0.3877	5.375	7.65e-08

Post hoc multiple comparisons

site	treatments compared	Estimate	Std. Error	z value	<i>p</i>
H	mesh v cloth	109.859	0.17346	6.333	<0.001
	open v cloth	282.259	0.16422	17.188	<0.001
	open v mesh	172.400	0.10762	16.020	<0.001
Q	mesh v cloth	-0.42639	0.47435	-0.899	0.98792
	open v cloth	0.73866	0.35105	2.104	0.37423
	open v mesh	116.505	0.40589	2.870	0.06379
T	mesh v cloth	183.546	0.14833	12.374	<0.001
	open v cloth	314.818	0.13783	22.841	<0.001
	open v mesh	131.272	0.06766	19.400	<0.001

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Identity of insect visitors to *Discaria chacaye* flowers. From left to right sites are arranged by decreasing geographical longitude. Totals by taxonomic category and site appear at the bottom line. Abbreviations: C: Coleoptera, D: Diptera, H: Hymenoptera, L: Lepidoptera, T: Thysanoptera.

Order	Family	Species	Lago Queñi	Puerto Canoa	Lago Tromen	Villa Traful	Lago Huechulafquen	Estancia San Ramón
C	Buprestidae	unidentified	x		x			
C	Buprestidae	unidentified			x			
C	Cantharidae	unidentified	x	x	x			
C	Carabidae	unidentified		x				
C	Cerambycidae	<i>Callideriphus laetus</i> Blanchard	x		x	x	x	
C	Cerambycidae	<i>Calydon submetallicum</i> Blanchard	x					
C	Chrysomelidae	unidentified sp. A	x			x		
C	Chrysomelidae	unidentified sp. B				x		
C	Chrysomelidae	unidentified sp. C	x	x				
C	Chrysomelidae	unidentified sp. D	x					
C	Chrysomelidae	unidentified sp. E	x					
C	Chrysomelidae	unidentified sp. F	x			x		
C	Curculionidae	unidentified Apioninae sp.			x			
C	Meloidae	unidentified sp.			x			
C	Melyridae	unidentified sp. A	x	x	x			
C	Melyridae	unidentified sp. B	x					
C	Melyridae	unidentified sp. C			x			

C	Melyridae	unidentified sp. D		x			
C	Mordellidae	unidentified sp.		x			
C	unidentified	unidentified sp.					x
D	Acroceridae	<i>Megalybus pictus</i> Philippi	x	x			
D	Anthomyiidae	<i>Calythea</i> sp.		x			
D	Bibionidae	unidentified sp.					x
D	Bombyliidae	<i>Thyridanthrax</i> sp. A					x
D	Bombyliidae	<i>Thyridanthrax</i> sp. B					x
D	Bombyliidae	<i>Thyridanthrax</i> sp. C		x			
D	Calliphoridae	<i>Calliphora vicina</i> Robineau-Desvoidy					x
D	Calliphoridae	<i>Chlorobrachycoma versicolor</i> Bigot					x
D	Calliphoridae	<i>Compsomyiops fulvicrura</i> Robineau-Desvoidy	x	x	x		x
D	Calliphoridae	<i>Phaenicia sericata</i> (Meigen)		x			
D	Empididae	<i>Empis fulvicollis</i> Collin		x	x		
D	Lauxaniidae	unidentified sp.	x	x	x		x
D	Muscidae	<i>Helina</i> sp. A		x			
D	Muscidae	<i>Helina</i> sp. B		x			
D	Muscidae	<i>Palpibracus</i> sp.					x
D	Muscidae	unidentified sp.		x			
D	Nemestrinidae	<i>Trichophthalma</i> cf. <i>porteri</i> Stuardo		x			
D	Nemestrinidae	<i>Trichophthalma philippii</i> Rondani	x				
D	Sarcophagidae	<i>Macronychia (Moschusa)</i> sp.					x
D	Sarcophagidae	<i>Microcerella spinigena</i> (Rondani)		x			
D	Sarcophagidae	<i>Sarcophagula</i> sp.		x			
D	Sarcophagidae	<i>Tricharaea brevicornis</i> (Wiedemann)		x			
D	Syrphidae	<i>Allograpta hortensis</i> Philippi	x	x	x		x
D	Syrphidae	<i>Allograpta pulchra</i> Shannon	x				x
D	Syrphidae	<i>Aneriophora aureorufa</i> Philippi		x			
D	Syrphidae	<i>Carposcalis fenestrata</i> Macquart	x	x	x		

D	Syrphidae	<i>Eristalis croceimaculata</i> Jacobs		x	
D	Syrphidae	<i>Eristalis tenax</i> Linnaeus			x
D	Syrphidae	<i>Hemixylota varipes</i> Shannon	x	x	
D	Syrphidae	<i>Macrometopia atra</i> Philippi	x	x	
D	Syrphidae	<i>Myolepta luctuosa</i> Bigot	x		
D	Syrphidae	<i>Notiocheilosia nitescens</i> Shannon & Aubertin		x	
D	Syrphidae	<i>Odyneromyia odyneroides</i> Philippi	x		
D	Syrphidae	<i>Odyneromyia valdiviformis</i> Shannon & Aubertin	x		
D	Syrphidae	<i>Orthonevra quadristriata</i> Shannon & Aubertin			x
D	Syrphidae	<i>Palpada chilena</i> Rondani		x	x
D	Syrphidae	<i>Palpada elegans</i> Blanchard	x		
D	Syrphidae	<i>Scaeva melanostoma</i> Macquart		x	x
D	Syrphidae	<i>Scaeva occidentalis</i> Shannon		x	
D	Syrphidae	<i>Sterphus coeruleus</i> Rondani		x	
D	Syrphidae	<i>Stilbosoma cyaneum</i> Philippi	x	x	x
D	Syrphidae	<i>Syrphus octomaculatus</i> Walker	x	x	
D	Syrphidae	<i>Syrphus</i> sp.		x	
D	Syrphidae	<i>Toxomerus calceolatus</i> Macquart	x	x	x
D	Syrphidae	<i>Toxomerus vertebratus</i> Rondani			x
D	Syrphidae	unidentified sp.			x
D	Syrphidae	<i>Valdiviomyia camrasi</i> Sedman		x	
D	Syrphidae	<i>Valdiviomyia</i> sp.	x		
D	Tabanidae	<i>Scaptia (Pseudoscione)</i> sp.	x		
D	Tabanidae	<i>Scaptia</i> cf. <i>australis</i> Philippi	x	x	
D	Tachinidae	<i>Deopalpus</i> sp. A		x	
D	Tachinidae	<i>Deopalpus</i> sp. B	x	x	x
D	Tachinidae	<i>Patelloa</i> cf. <i>tanumeana</i> (Townsend)			x
D	Tachinidae	<i>Peleteria filipalpis</i> (Rondani)	x		

D	Tachinidae	<i>Prosopochaeta cf. anomala</i> Aldrich			x		x
D	Tachinidae	<i>Steatosoma cf. rufiventris</i> Aldrich					x
D	Tachinidae	unidentified sp. A			x		
D	Tachinidae	unidentified sp. B			x		
D	Tachinidae	unidentified sp. C					x
D	Tachinidae	unidentified sp. D					x
D	Tachinidae	unidentified sp. E					x
D	Tachinidae	unidentified sp. F			x		
D	Tachinidae	unidentified sp. G					x
D	Therevidae	unidentified sp.	x				
D	unidentified	unidentified					x
H	Andrenidae	<i>Calliopsis trifasciata</i> Spinola					x
H	Apidae	<i>Apis mellifera</i> Linnaeus					x
H	Apidae	<i>Bombus dahlbomii</i> Guérin-Ménéville	x				
H	Colletidae	<i>Cadeguala albopilosa</i> (Spinola)	x				x
H	Colletidae	<i>Cadeguala occidentalis</i> (Haliday)					x
H	Colletidae	<i>Colletes seminitidus</i> Spinola			x		x
H	Colletidae	<i>Colletes</i> sp.					x
H	Halictidae	<i>Caenohalictus opaciceps</i> (Friese)					x
H	Halictidae	<i>Corynura (Callistochlora)</i> sp.					x
H	Halictidae	<i>Corynura (Corynura)</i> sp. A	x	x	x		
H	Halictidae	<i>Corynura (Corynura)</i> sp. B				x	
H	Halictidae	<i>Corynura (Corynura)</i> sp. C		x	x		x
H	Halictidae	<i>Corynura chilensis</i> (Spinola)					x
H	Halictidae	<i>Corynura corinogaster</i> (Spinola)					x
H	Halictidae	<i>Corynura prothysteres</i> (Vachal)	x	x	x		
H	Halictidae	<i>Corynura rubella</i> (Haliday)			x		
H	Halictidae	<i>Halicillius reticulatus</i> González-Vaquero			x	x	
H	Halictidae	<i>Halicillius</i> sp.	x				

H	Halictidae	<i>Ruizanthedella mutabilis</i> (Spinola)	x	x	x		x	x
H	Ichneumonidae	unidentified sp.					x	
H	Megachilidae	<i>Megachile santacruzensis</i> Durante & Abrahamovich			x			
H	Pompilidae	<i>Pepsis caridei</i> Brèthes	x					
H	Pompilidae	<i>Pepsis</i> sp. A						x
H	Pompilidae	<i>Pepsis</i> sp. B						x
H	Vespidae	<i>Hypodynerus</i> sp. A			x			x
H	Vespidae	<i>Hypodynerus</i> sp. B	x		x			
H	Vespidae	<i>Vespula germanica</i> (Linnaeus)			x			
L	Geometridae	<i>Hagnagora mesenata</i> (Felder & Rogenhofer)	x				x	
L	Geometridae	<i>Oratha significata</i> Walker			x			
L	Geometridae	<i>Psilaspilates signistriata</i> (Butler)	x					
L	Geometridae	<i>Psilaspilates</i> sp.			x			
L	Geometridae	<i>Rheumaptera exacta</i> (Butler)			x			
L	Geometridae	unidentified sp. A	x					
L	Geometridae	unidentified sp. B			x			
L	Geometridae	unidentified sp. C			x			
L	Hesperiidae	<i>Butleria quilla</i> Evans	x					
L	Lycaenidae	<i>Strymon bicolor</i> (Philippi)			x			
L	Noctuidae	<i>Peridroma saucia</i> (Hübner)			x			
L	Nymphalidae	<i>Argyrophorus</i> sp.	x					
L	Nymphalidae	<i>Issoria cytheris</i> (Drury)			x			
L	Nymphalidae	<i>Nelia humilis</i> (Felder)					x	
L	Nymphalidae	<i>Vanessa braziliensis</i> Moore	x					
L	Nymphalidae	<i>Vanessa carye</i> Hübner	x					
L	Pieridae	<i>Colias vauthierii</i> Guérin-Méneville	x					
L	Pieridae	<i>Tatochila theodice</i> (Boisduval)			x			
T	unidentified	unidentified sp.			x			x

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49

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Online Resource 4. Ambophily, not entomophily: the reproduction of the perennial *Discaria chacaye* (Rhamnaceae: Colletieae) along a rainfall gradient in Patagonia, Argentina. Plant Systematics and Evolution. Diego Medan^{1,2} and Mariano Devoto^{1,2}. ¹ Universidad de Buenos Aires, Facultad de Agronomía. Cátedra de Botánica General, Avda. San Martín 4453, C1417DSQ Buenos Aires, Argentina. ² Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Buenos Aires, Argentina. E-mail of corresponding author: diemedan@agro.uba.ar

Plot of the main effect (extracted from a GLMM, see Methods) of pollination treatment on the proportion of fruits produced in three populations of *Discaria chacaye* in NW Patagonia. From left to right sites are arranged following decreasing precipitation.

