Pl. Syst. Evol. 254: 173–184 (2005)
DOI 10.1007/s00606-005-0335-3

**Plant Systematics
and Evolution**
Printed in Austria

Reproductive ecology of a perennial outcrosser with a naturally dissected distribution

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Received June 1, 2004; accepted April 11, 2005

Abstract. We surveyed four populations of contrasting size (two isolated and two large stands) of a woody outcrossing species, *Discaria trinervis* (Rhamnaceae), which has a naturally dissected distribution. Our main goal was to investigate the floral biology, breeding system and pollination mechanism of the species, which in turn may help to explain the mechanisms underlying the survival of the isolated populations. *Discaria trinervis* is both wind- and insect-pollinated, showing interactions with a large fraction of the available flower visitors at both stand sizes. In the larger populations, stigmatic loads were larger, more pollen tubes reached the ovules, and fruit and seed set were higher; however, the reproductive output in the smaller populations (over 2000 seeds per plant per year) seemed adequate for population persistence. Human disturbance (e.g. destruction of trees for wood extraction) may put these smaller populations at risk through loss of incompatibility alleles. The results suggest that plant species with naturally dissected distributions may provide clues about which reproductive mechanisms ensure survival under fragmentation.

Key words: Ambophily, *Discaria*, outcrosser pollination, population size, Rhamnaceae, fragmentation.

Introduction

Many plant species show naturally fragmented distributions because of the spatial arrangement of their preferred habitats (Coates 2000, Wolf and Harrison 2001), i.e. not because of recent destruction of intervening areas they formerly lived in. Naturally dissected habitat may result from the particular distribution of the specific soil type to which a plant is closely adapted (Wolf and Harrison 2001), or may derive from the fragmentation of an originally large distribution area due to sustained, long-term environmental change along geologic time (Coates 2000). It seems desirable to understand how particular species are presently adapted to their naturally fragmented distributions, because the mechanisms implied have successfully overcome the test of time. In this respect, obligate outcrossers would be of special interest because such plants necessarily attain adequate interplant pollen dispersal (animal-mediated or otherwise) at all patch sizes.

In this paper, we report on the reproductive biology of a self-incompatible plant at contrasting population sizes. *Discaria trinervis*, a woody perennial, occurs in the riparian vegetation along rivers flowing eastward from the Andes into the Patagonian steppe in Argentina. Upstream populations usually constitute continuous riparian forests, while downstream populations (and those occurring at the boundaries of the species range) tend to be isolated stands of few individuals. Objectives of this work are 1) to investigate the floral biology, breeding system, pollination mechanisms and reproductive success of *D. trinervis* and 2) to provide a preliminary analysis of how the latter two reproductive attributes vary as related to local population sizes.

Material and methods

Plant species. *Discaria trinervis* (Hook. et Arn.) Reiche (Rhamnaceae) is a 2–8 m tall shrub or small tree that grows below 2500 m a.s.l at both western (Chilean) and eastern (Argentinean) slopes of the Andes between 31°S and 48°S, often as a riparian element along creeks and rivers flowing into the Patagonian steppe (Tortosa 1983, Roig et al. 1988, Schmelter 1994). A nitrogen-fixing symbiotic system occurs at the roots (Medan and Tortosa 1976) and the species is a valuable forage resource in some parts of its range (Boelcke 1957). Flowers are presented horizontally ± at 45° angle in 2-3-flowered cymes grouped in 2-16-flowered axillary, leafy synflorescences that proliferate after anthesis. Two such synflorescences are opposed at each node of flowering branches (Tortosa et al. 1996). Flowers are creamy white, perfect, actinomorphic, 4-5-merous, 4 mm in diameter, with a 1 mm long × 1.5 mm wide floral tube. The deltoid sepals and the hooded petals alternate at the rim of the floral tube. The stamens are opposite to the petals, with the anthers raised 0.7 mm above the level of the floral tube mouth. A nectariferous disc is near the bottom of the floral tube. The tri-carpellate gynoecium is composed by a semi-inferior, 3-ovulate ovary, style, and a terminal, trilobular stigma located 0.5 mm below the anthers. Fruits are 1-3-seeded explosive capsules. Voucher specimens are kept at Gaspar Xuarez Herbarium (BAA 23644).

Study sites. Four populations in the provinces of Mendoza and Neuquén, Argentina were studied between 1996 and 2001 (see Table 1 for a summary

Table 1. Location and characteristics of the *Discaria trinervis* populations used in the study

Field work site (province)	Río Blanco (Mendoza)	Río Malleo (Neuquén)	Confluencia Traful (Neuquén)	La Lipela (Neuquén)
Coordinates	33°00′00″ S, 69°17′00″ W	39°36′39″ S, 71°21′17″ W	40°43′27″ S, 71°05′50″ W	40°48′54″ S, 71°06′25″ W
Elevation (m a.s.l.)	1900	860	800	750
Date of field work	Dec. 1996 and Jan. 1997	Dec. 1998 and Jan. 1999	December 2001	Dec. 2000 and Jan. 2001
Daytime temperatures during flowering (°C)				
mean maximal (absolute maximal)	22.5 (28.4)	19.2 (27.5)	25.2 (33.5)	24.6 (25.6)
mean minimal (absolute minimal)	13.3 (7.0)	13.0 (10.0)	13.4 (9.1)	12.0 (7.8)
Plant height (m)	0.7–5	4–5	2–3	5–6
Population size (number of individuals)	19	> 100	ca. 15	> 100
Mean distance between individuals (m)	5.7	ca. 4	ca. 4	ca. 4

of site and population features). Two of these were small (< 20 individuals) and isolated (at least 2–3 km from the nearest cospecific stands) [Río Blanco (RB) and Confluencia Traful (CT)], and two others were part of continuous riparian forests growing along rivers or creeks [Río Malleo (RM) and La Lipela (LL)]. All sites supported some degree of grazing by cattle, although reproductive plants did not appear negatively affected by grazing. Logistical reasons forced us to study each population on a different year. Reproductive parameters were measured at one or both small populations, and at one or both large populations. 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. Twenty-five flower buds and open flowers were dissected under a 50x 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 2). Floral stages were recorded once or twice a day for 20 randomly-selected flowers from 4 individuals (RB), and mean values of flower stage were plotted against time. Air temperature was recorded in the period 8:00–22:00 with a hand-held instrument for the days of flower monitoring. Stigmatic receptivity was assayed by applying on stigmas a tiny drop of 40% H_2O_2 (Zietsman and Botha 1992). Nectar amount was too small to obtain reliable measures of volume and sugar concentration. Scent emission was assessed by offering three people small vials containing samples of flowers (n = 10) in different stages of anthesis. Flower visitors were observed and sampled across populations at different times of the day and on different days (cumulated time = 17 h) in order to record visitor profiles as completely as possible. One-hundred and sixty-five insect individuals were caught for identification. The number of sampled individuals of a given visitor species was used as a primary measure of species abundance. Forty-seven percent of the visitor taxa were identified to species or genus. When identifications to species were not available the insects were morphotyped (in several cases with the assistance of specialists). All collected material is deposited at the authors' laboratory or in collections of assisting entomologists. As a measure of the degree of relatedness among visitor assem-

Table 2. Flower phenology of *Discaria trinervis*, based on 20 flowers from four individuals

Floral trait	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Sepals	closed	slightly open	erect	wide open	horizontal	wilting
Petals	connivent	erect, partially concealing anthers	erect, partially concealing anthers	somewhat reflexed, exposing anthers	somewhat reflexed, exposing anthers	wilting
Pollen	unavailable	hardly available in spite of open anthers	easily available	mostly removed	removed	removed
Stigma	dry	dry, not receptive	dry, not receptive	swollen, receptive	swollen, receptive	wilting
Scent and nectar	none	present	present	present	present	none
Nectary / ovary	both green	both green	both green	both green	turning yellow / reddish	yellow / reddish

blages of different sites, we calculated a similarity index I = [2D / (A + B)], where D = number of visitor species shared between two sites, and A, B the number of species in each of these sites. Ten-minute visitor censuses were conducted at RB (n = 10), LL (3), and CT (2). Each census included at least 400 open flowers. Each visitor was assigned to one of the following categories: small- or medium-sized syrphid fly, large-bodied syrphid fly, muscoid fly, apoid bee, and other groups. We assessed the role of *D. trinervis* in the plant-pollinator network comparing published data for a small population (RB) (Medan et al. 2002) and data for a large population (LL) gathered using the same procedures as Medan et al. (2002).

Breeding system and maternal success. Number of flowers per flowering node and flowering branch was determined at RB (n = 360 nodes from three flowering branches of each of 12 individuals). The counts of flowers per branch were extrapolated to estimate total number of flowers per plant, and were related to corresponding counts of fruits per branch to calculate fruit set. Number of flowers in male or female stage per flowering node was determined at RB (n = 90 nodes from 9 individuals). The proportion of female-stage flowers that received pollen under natural pollination was determined by microscopic examination at RB (n = 140 flowers from 9 individuals) and LL (n = 118 flowers from 5 individuals). 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 count the number of pollen grains per stigma. Additionally, we recorded which of the pistil sections (stigma + upper style, mid style, lower style, upper ovary, or ovary bottom) had been reached by the farthest-growing pollen tubes. The number of mature seeds per fruit was determined at RB (n = 55 fruits from 6 individuals) and LL (n = 60 fruits from 6 individuals).

The pollen-ovule ratio of *D. trinervis* is 15,970 (Medan et al. 2002). The effect of pollination conditions on fruit set was assessed at RB, RM and LL. Three treatments were applied to groups of flower buds in each of 3 to12 individuals per site. *Treatment 1*: Flower buds were covered with cloth bags, excluding both insects and airborne pollen, until all flowers had abscised or developed a fruit (n = 5,677 flowers). *Treatment 2*: As in 1, but 1 mm-mesh bridal veil was used instead of cloth bags, to prevent insect visits while enabling wind pollination (n = 11,325 flowers). *Treatment 3*: Flower buds were left uncovered and experienced natural pollination (n = 45,146 flowers). Because of vandalism all but one replication of treatment 1 were lost at LL. To permit statistical analysis this datapoint was replaced with a small dataset (n = 4) of identical mean and very low variance. A few hand self- and cross-pollinations (n = 44, number was limited by technical difficulties derived from the small flower size) were conducted at RB. Flower buds were emasculated, covered with fine mesh cloth bags and periodically inspected until stigmas appeared receptive, then pollinated by gently touching each stigma with an anther of a freshly opened flower of the same branch or from another individual, and rebagged until harvest. Throughout the results, measures are given as arithmetical mean ± SE.

Results

Phenology and flower biology. Flowering of individual trees lasted ca. one month and was concentrated in December, but in particular years or populations blooming could start up to three weeks earlier or later. Individuals flowering out-of-phase were observed in every population. By the end of January, all trees throughout the species' range were developing fruits or dispersing seeds.

A typical flowering node produced 9.29 ± 0.6 flowers over the blooming season. At bloom peak, 5.03 ± 0.5 flowers per node were simultaneously open. Flowers were active in pollen export and receipt for six days. Flower buds opened in the morning and passed through a *male phase* (stages 1 and 2 in the Table 2) during days 1–2, sometimes reaching an early female phase (stage 3). During the male phase, pollen was increasingly accessible to flower visitors, and at end of the male phase it was usually thoroughly removed. A pleasant scent was perceptible (day and night), and stigma reacted negatively to the peroxidase test during this male phase. Nectar was generally present in minute amounts (estimated at 0.1–0.2 µl/flower). During days

4–6 flowers went through the *female phase* (stages 3–4, Table 2). Sepals became horizontal and petals exposed the empty anthers. Each stigma swelled and became covered by a clear, adhesive, mucilaginous secretion on its three receptive surfaces. Tests of stigmatic receptivity were positive, particularly at stage 4. Floral scent and nectar secretion were still present in female stages. On day seven most floral parts were wilting.

Pollinators and pollination service. Sixty-one species of insects belonging to 25 families in five orders were recorded as flower visitors (Table 3). Diptera dominated the visitor assemblage (47.5% of all species), followed by Hymenoptera (26.2%), Coleoptera (18.0%), with less important groups (Lepidoptera and Thysanoptera) together adding 8.3%. At the species level, the composition of the visitor assemblage differed strongly among sites (Table 3). Seventy-eight percent of the visiting species were exclusive to a particular site. The RB and LL assemblages were more similar (similarity index = 0.4) than either was to the CT assemblage (similarity index = 0.15 and 0.17 for the RB-CT and LL-CT comparisons, respectively). No clear relationship was evident between population size and either composition of the visitor assemblage at the order level, or species richness.

Small- and medium-bodied hoverflies, muscoid flies, beetles and thrips tended to remain for long times on the same plant. Non-apoid Hymenoptera and Lepidoptera were more mobile, but comparatively rare as visitors. Geometrid moths actively visited flowers after dusk at RB, but observations at the other sites detected only minor nocturnal visitor activity. Large Diptera (bombyliid flies, and larger hoverflies like *Copestylum aricia* and *Eristalis tenax*), and apoid Hymenoptera (bees belonging to Andrenidae, Colletidae and Halictidae) moved between plants comparatively often while foraging for pollen and/or nectar.

Only a quarter of all visitor species appeared in the censuses, indicating that many visitors were uncommon. Mean daytime visitation rate was higher in the small plant populations (RB: 6.9 visits per census, CT: 6.0 visits / census) than at one large population (LL: 1.5 visits / census). The difference was significant for the RB-LL comparison (Mann-Whitney $U = 2$, $P = 0.028$). Most (40–50%) visits were by medium- to small-bodied syrphid flies, except at LL, where bees constituted the leading group (50% of all visits). Muscoid flies were the second largest category at all sites (20–35%). The number of captured individuals (Table 3) corresponding to each visitor category was largely proportional to that category's share in the visitation rate, suggesting that censuses adequately reflected visitation patterns.

Assuming constant visitation rates and five hours a day of insect activity, flowers would receive between ca. 4–5 visits (RB and CT) and ca. 1 visit (LL) over a flower's lifetime. However, only a fraction of these visits (0.1x, 0.5x, and 0.3x; RB, CT, and LL, respectively) would correspond to the more mobile visitors (larger Diptera and apoid Hymenoptera).

Pollination was quantitatively superior at the larger population. Under natural pollination, 92.1% of female-phase flowers received pollen at LL, and 71.8% of flowers received pollen at RB (Mann-Whitney $U = 8$, $P = 0.053$). Stigmatic pollen loads were significantly larger at LL than at RB (55.5 ± 13.3 vs. 19.6 ± 3.6 grains/stigma; Mann-Whitney $U = 6$, $P = 0.028$). As measured by the performance of pollen tubes, the quality of pollination also seemed better in the larger population. At LL, pollen tubes were arrested at mid-style or in higher regions of the pistil in 68.7 ± 5.9% of the flowers, while at RB that proportion was significantly higher (92.4 ± 4.1%; Mann-Whitney $U = 3.5$, $P = 0.011$). Likewise, more flowers had pollen tubes reaching the micropyle level in the larger population (31.3 ± 5.9% vs. 7.5 ± 4.1% of the flowers, LL and RB, respectively; Mann-Whitney $U = 3.5$, $P = 0.011$).

Breeding system and maternal success. The pollination experiment (Table 4) showed that fruit set was significantly affected by the pollination treatment in both large populat

Table 3. Insect visitors to *Discaria trinervis* flowers. Abbreviations: **C** Coleoptera, **CT** Confluencia Traful, **D** Diptera, **H** Hymenoptera, **L** Lepidoptera, **LL** La Lipela, **RB** Río Blanco, **T** Thysanoptera. Figures indicate number of collected individuals

Order	Family	Species	RB	CT	LL
C	Cerambycidae	unidentified sp.			1
C	Coccinelidae	unidentified sp. 1			1
C	Coccinelidae	unidentified sp. 2			1
C	Coccinelidae	unidentified sp. 3		1	
C	Coccinelidae	unidentified sp. 4	2		
C	Coccinelidae	unidentified sp. 5	1		
C	Coccinelidae	unidentified sp. 6	1		
C	Dasytidae	unidentified sp.			1
C	Mordellidae	unidentified sp. 1	2		
C	Mordellidae	unidentified sp. 2		3	
C	Mordellidae	unidentified sp. 3		1	
D	Anthomyiidae	*Calythea* sp.	3		1
D	Bibionidae	unidentified sp. 1	2		
D	Bibionidae	unidentified sp. 2	2		
D	Bibionidae	unidentified sp. 3	1		
D	Bombyliidae	*Lyophlaeba* sp.		1	3
D	Bombyliidae	*Thyridanthrax* sp.	1	1	
D	Bombyliidae	unidentified sp.		1	
D	Calliphoridae	*Compsomyiops fulvicrura* (Robineau-Desvoidy)	1		3
D	Calliphoridae	*Sarconesiopsis magellanica* (Le Guillou)	1		
D	Lauxaniidae	unidentified sp. 1			2
D	Lauxaniidae	unidentified sp. 2	4		1
D	Lauxaniidae	unidentified sp. 3	1		
D	Sarcophagidae	*Microcerella rusca* (Hall)	2		
D	Sarcophagidae	unidentified sp.	2		
D	Syrphidae	*Alipumilio* sp.	1		
D	Syrphidae	*Allograpta pulchra* Shannon	9	2	3
D	Syrphidae	*Carposcalis punctulata* Wulp	4		1
D	Syrphidae	*Carposcalis saltana* Enderlein	2		1
D	Syrphidae	*Copestylum aricia* Curran	1		
D	Syrphidae	*Eristalis tenax* L.	1		
D	Syrphidae	*Ocyptamus meridionalis* Fluke	2		
D	Syrphidae	*Scaeva occidentalis* Shannon	3		2
D	Tabanidae	*Scaptia australis* Phil.		1	
D	Tachinidae	unidentified sp. 1	5		
D	Tachinidae	unidentified sp. 2			1
D	Tachinidae	unidentified sp. 3			1
D	Tachinidae	unidentified sp. 4		1	
D	Tachinidae	unidentified sp. 5		1	
D	Tachinidae	unidentified sp. 6		1	
H	Andrenidae	*Calliopsis trifasciata* Spinola		1	2
H	Andrenidae	*Callonychium mandibulare* (Friese)		1	
H	Chrysididae	*Chrysis* sp.		1	
H	Chrysididae	*Pleurochrysis bruchi* (Brèthes)		1	

Table 3 (continued)

	Order	Family	Species	RB	CT	LL
	H	Colletidae	*Chilicola* sp.		1	
	H	Halictidae	*Augochloropsis* sp.	1		
	H	Halictidae	*Caenohalictus* sp.	2		1
	H	Halictidae	*Corynura prothysteres* (Vachal)			4
	H	Halictidae	*Dialictus* sp.			1
	H	Halictidae	*Ruizantheda mutabilis* Spinola		1	
	H	Halictidae	*Ruizantheda proxima* (Spinola)			1
	H	Sphecidae	*Cerceris* sp.			1
	H	Tiphiidae	*Myzinum* sp.	1		
	H	Vespidae	*Hypodynerus* sp. 1	1		1
	H	Vespidae	*Hypodynerus* sp. 2		1	
	H	Vespidae	*Hypodynerus* sp. 3		1	
	L	Geometridae	unidentified sp.	4		
	L	Lycaenidae	*Strymon bubastus* (Stoll)		1	
	L	Noctuidae	unidentified sp.			1
	L	Nymphalidae	*Issoria lathonioides* (Blanchard)		1	
	T	unidentified	unidentified thrips sp.	2	1	1
Grand totals	5	25	61			
Totals by site						
Order				5	5	5
Family				15	14	14
Species				30	22	24

ions (LL and RM; Median test, $P < 0.036$) but not in a small population (RB; Median test, $P = 0.35$). Comparisons among sites revealed significant differences for the mesh-bagging and control treatments (Median tests, $P < 0.017$) but not for the cloth-bagging treatment (Median test, $P = 0.54$).

Fruit set under cloth bagging was very low (among-site range: 0.44–3.27%) suggesting that cross pollination is necessary for substantial fruit set. We strongly assume that many bagged flowers, if not all, received self pollen because thrips were not completely excluded by cloth bagging (unpublished obs.). The same results indicate that apomixis, if present, was unimportant in this species. In comparison to the insect-accessible controls, mesh bagging reduced fruit set by half or more at all sites, but the decrease was nonsignificant (Mann-Whitney U tests, $P > 0.05$). Fruit set of

Table 4. Fruit set (percent) of *Discaria trinervis* at three different sites under three pollination treatments. Values are means ± S.E. Significant differences (Median tests, $p < 0.05$) are indicated by different lowercase exponents (within-site comparisons) and different uppercase exponents (within-treatment comparisons). Abbreviations: LL La Lipela, RB Río Blanco, RM Río Malleo

Site	Control	Mesh bagging	Cloth bagging
RB	1.82 ± 0.7^{aC}	0.43 ± 0.2^{aB}	0.44 ± 0.4^{aA}
n flowers / n individuals	*32833 / 12*	*2214 / 6*	*1046 / 5*
RM	9.82 ± 4.2^{aB}	4.38 ± 1.5^{aA}	0.59 ± 0.3^{bA}
n flowers / n individuals	*3798 / 10*	*3741 / 10*	*3161 / 8*
LL	37.75 ± 11.8^{aA}	10.58 ± 2.8^{aA}	3.27 ± 0.0009^{bA}
n flowers / n individuals	*8515 / 6*	*5640 / 3*	*1470 / 1*

mesh-bagged flowers was significantly higher than that of cloth-bagged flowers in the two large populations (LL and RM; Median tests, $P < 0.033$). Thus, results indicate that airborne pollen played an important role in the pollination of *D. trinervis,* at least in large populations, and suggest that insect-transported pollen had a supplementary effect on fruit set.

Only one out of 14 hand-selfed flowers set fruit at RB and only three (out of 30) did from those that were cross-pollinated. Although seven different individuals acted as pollen donors and/or pollen receivers in the cross pollinations, fruits were produced from reciprocal crossing between just two individuals.

On average, mature *D. trinervis* individuals produced 88,507 ± 29,071 flowers. Natural fruit set ranged from 1.8% at RB to 37.7% at LL (Table 3). Seed set was significantly higher in the larger population (1.45 ± 0.1 seeds per fruit at RB vs. 2.36 ± 0.08 at LL; Tukey t-test, $P < 0.0001$, df = 113).

Discussion

Is *D. trinervis* insect- or wind-pollinated? D. trinervis displays an array of adaptations for insect pollination, including protandry, colour and scent advertising, and pollen and nectar rewards. These characteristics are common among Rhamnaceae, as previously documented in the genus *Discaria* (Primack 1979; Webb 1985; Medan 1991, 2003). Mass flowering, coupled with simple flower morphology and easily accessible rewards, is consistent with the observed diversity of the visitor assemblage. Irrespective of population size, *D. trinervis* had interactions with a considerable proportion of the potential pollinators available in the community (20.6% at RB, Medan et al. 2002; 19.2% at LL, Devoto et al. 2005). Thus, among-site differences in assemblage composition may simply reflect changing entomofaunas across the species' range. Assemblage composition was unrelated to population size, since two differently-sized populations (RB and LL) had similar assemblages, and other two (CT and LL) had dissimilar assemblages.

Visitors searching for nectar are expected to provide better pollination service, because they will visit both male- and female-phase flowers, while exclusive pollen-feeders and mixed-feeders will tend to visit male-phase flowers only. Visitors foraging for pollen might accomplish some intrafloral pollen transfer, given 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 available for intrafloral selfing may remain in anthers when the flower has entered the female phase. Flowers being visited by pollen-feeders at this stage may therefore be self- (i.e. ineffectively) pollinated.

Flowers exposed to airborne pollen and to insects set on average twice the fruits as mesh-bagged flowers did. Although this difference was not statistically significant, it seems reasonable to conclude that insects play an important part in pollination. We suggest that insects supplemented pollen transport by wind; however, additional experimental effort is needed to show how much pollination is effected by each agent.

The detection of wind pollination in *Discaria trinervis* is a significant finding, because there seem to exist only two previous reports of this pollination mode in the Rhamnaceae (Aronne and Wilcock 1995, Zietsman 1990). In addition, experimental testing of anemophyly has given consistently negative results in other species of *Discaria* and in three closely related genera of the uniformly entomophilous tribe Colletieae, where *Discaria* belongs (D'Ambrogio and Medan 1993; Medan 1991, 2003; Medan and Arce 1999; Medan and Basilio 2001; Medan and D'Ambrogio 1998), all which underlines the uniqueness of anemophily in *D. trinervis*. Plants showing ambophily (the combination of biotic and wind pollination, Culley et al. 2002) show strong diversity in life history and reproductive traits, including a few examples of perennials with hermaphroditic flowers like *D. trinervis*

(Zietsman 1990, Gómez and Zamora 1996, Norman et al. 1997, Mahy et al. 1998).

Anemophily has evolved repeatedly within basically entomophilous lineages in the Angiosperms (Culley et al. 2002). The pollen of ambophilous plants tends to be smaller than that of biotically pollinated ones, having sizes in the range 10–50 µm. Thus it is interesting that the pollen of *D. trinervis* is 22 µm in diameter (Heusser 1971), which is near the lower end of the *Discaria* pollen size range [which runs from 20 µm in *D. articulata* (D. Medan unpublished) to 36.2 µm in *D. chacaye* (Heusser 1971)]. Pollen of the ambophilous *Rhamnus alaternus* is 21 µm in diameter (Aronne and Wilcock 1995).

The species' habitat seems consistent with the evolution of wind pollination, since much of the range of *D. trinervis* is included in Patagonia, an ecoregion characterized by strong and persistent westerly winds. Maximum wind speeds occur between September and January (Paruelo et al. 1998), a period that includes the flowering time of *D. trinervis*. Many riparian woods of this species are oriented in a West-East direction, thus at least leeward between-plant pollen transport should be common.

Breeding system. Results of the bagging experiment are consistent with the high pollen-ovule ratio of *D. trinervis* (15970 ± 2621, Medan et al. 2002) in suggesting self-incompatibility in this species. The use of the pollen-ovule ratio as a predictor of a plant's breeding system has received criticism (Charnov 1979, 1982; Cruden et al. 1996) but it remains widely employed in studies of hermaphrodite species (Cruden 2000, Wyatt et al. 2000, Pías and Guitián 2001). Self-incompatibility was already known in *Discaria* (Primack 1979, Webb 1985, Medan 1991, Medan and Vasellati 1996) and other Rhamnaceae (*Colletia*: D'Ambrogio and Medan 1993, Medan and Basilio, 2001; *Frangula*: Medan 1994; *Retanilla*: Medan and Arce 1999; *Ziziphus*: Galil and Zeroni 1967, Mehrotra and Gupta 1985).

We also obtained limited evidence for cross incompatibility in *D. trinervis*. Nonrandom mating has already been documented in *Discaria* (Medan and Vasellati 1996) and in the related genus *Colletia* (Medan and Basilio 2001). Cross-incompatibility was also reported in *Ziziphus celata*, an endangered Rhamnaceae of Florida (Weekley and Race 2001, Weekley et al. 2002). Weekley and Race (2001) indicated that a reduction in the number of S incompatibility alleles in small populations of *Z. celata* probably explained the authors' failure at obtaining fruits in at least three quarters of hand crossings. It is likely that a similar mechanism underlies the lower reproductive success of *D. trinervis* at small populations.

Does *D. trinervis* reproduce better in larger than in smaller stands? To extract general conclusions as to whether differences exist between large and small populations is tempting. However, we can only provide a preliminary analysis given that two main concerns could reduce the significance of our results on this respect. First, interannual variation within populations is not accounted for in our sampling procedures, as each population was surveyed on a different year. Thus, it is possible that differences between the populations might be at least partly due to differences in the pollinator assemblages between years. It has been demonstrated that variation in pollinator communities can be very significant between years (see Williams et al. 2001 for a recent review on bees). Second, the data on flower visitor and fruit set have been recorded for only three populations and consequently some of our comparisons are based on a relatively small sample size. However, we are confident on the general validity of our conclusions regarding reproductive features of *D. trinervis* which are unaffected by population size, such as floral biology, breeding system and pollination mechanisms.

Four reproductive variables (size of stigmatic loads, proportion of pollen tubes that reached micropylar level, fruit set, and seed set) indicate that *D. trinervis* reproduced more successfully in the larger populations. The lower performance of small stands seems to conflict with the higher visitation rate experienced by flowers in these populations; however, the advantage over larger populations was lost when the analysis was narrowed to the most mobile insects (the ones expected to effect most between-plant pollen transport, which is what really matters for an outcrosser species. An average load in the smaller population of ca. 20 pollen grains per stigma, likely sufficient for a 3-ovulated pistil, suggests poorer pollen quality as the probable cause for lowered reproductive success in the smaller stands. Note that even an intense interplant pollen transport could fail to ensure effective pollination if, as suggested by the crossing experiment, in the smaller populations a reduced number of individuals were mutually compatible.

Conservation. The seed production of *D. trinervis* seems sufficient for the species' persistence even in small stands, where an average individual (which has many years of life expectancy, Roig et al. 1988) can produce over 2000 seeds annually. Consistently high generalization coupled with wind pollination seems to account for the necessary interplant pollen transfer even in those marginal populations. Thus, in this seed-dependent species the risk of reproductive failure associated to self-incompatibility seems compensated (Bond 1994, but see also Aizen et al. 2002) by a ubiquitous, dependable abiotic force supplemented by a diverse assemblage of animal mutualists. However, genetic impoverishment could put stands formed by very few individuals at risk of extinction where human disturbance (e.g. via random destruction of trees for wood extraction, Schmelter 1994) drives incompatibility alleles below some (still unknown) critical threshold.

In the last years there has been much research on effects of man-induced fragmentation of natural habitats, particularly on ecosystem sustainability (Morgan 1999, Cunningham 2000, Gross 2001, and references therein). Identifying traits associated to survival in small stands is important because the abundance of these characteristics in a given community might reduce its vulnerability to fragmentation. This study suggests that species with naturally dissected habitats may offer useful clues for understanding reproductive success under fragmentation stress. Although our work did not address all reproductive stages, it detected a suite of reproductive traits that primarily characterizes *D. trinervis* as a fragmentation-tolerant plant.

A. Basilio, V. Medan, N.H. Montaldo and G. Zarlavsky helped us in the field or laboratory. A. Roig-Alsina, F. Navarro and F.C. Thompson identified insect specimens. N. Bartoloni provided statistical advice. Earlier drafts of this paper benefited from comments by N.H. Montaldo and three anonymous reviewers. Financial support of UBACyT and CONICET to D.M. is gratefully acknowledged.

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