Anther-stigma separation in *Desmodium* species (Papilionoideae-Fabaceae) from Northwestern Argentina

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Abstract

The repeated evolutionary transition from outcrossing to self-fertilization in Angiosperms has been suggested to occur because selfing provides reproductive assurance. This transition is commonly associated with modifications of floral traits, among them herkogamy (the spatial separation between dehiscing anthers and the receptive stigma). Here, we investigate variation in levels of herkogamy in *Desmodium incanum*, *D. pachyrrhizum*, *D. subsericeum* and *D. uncinatum*, to determine the relationship between variation in this floral trait and self-pollen deposition. These species are self-compatible and bee-pollinated, with ornamental potential and may be used for forage given their high nutritional value. We studied natural populations located in Lerma Valley, Salta Province, Argentina. In January and February of 2009, we marked and bagged 10 floral buds from 10 individual plants per species. The flowers were collected before their senescence; anther-stigma separation was measured immediately after collection with digital calipers (0.01 mm) as the distance between the surface of the stigma and the nearest anther. The stigmas were preserved in 70% ethanol. Pollen grains were counted under a microscope (10 x) in the laboratory. There was significant variation in herkogamy levels among species. There was a negative correlation between anther-stigma distance and the number of self pollen grains deposited on the stigma in isolated flowers. The variation in levels of herkogamy may represent a mechanism to ensure the production of some self-fertilized progeny in the absence of pollinators.

Keywords: autonomous self pollination, herkogamy, reproductive assurance, self pollen load.

INTRODUCTION

A predominant pattern in the diversification of flowering plants is the repeated transition from outcrossing to self-pollination (Stebbins, 1974; Barrett et al., 1996). This transition is commonly associated with modifications of floral traits, including developmental adjustments affecting the timing of self-pollination and the spatial separation of pollen and stigma (herkogamy) (Ritland and Ritland, 1989; Fenster et al., 1995; Barrett et al., 1996; Schoen et al., 1996; Fishman and Wyatt, 1999; Motten and Stone, 2000). For self-compatible, non-dichogamous species, herkogamy correlates negatively with autonomous selfing rate (Ennos, 1981; Carr and Fenster, 1994; Elle and Hare, 2002; Chen et al., 2009), self-pollen deposition (Thomson and Stratton, 1985; Parra-Tabla and Bullock,

2005) and marker-based estimates of selfing (Barrett and Shore, 1987; Holtsford and Ellstrand, 1992; Motten and Antonovics, 1992; Brunet and Eckert, 1998; Takebayashi et al., 2006).

Many species that possess herkogamy are also selfincompatible. This has led to the proposal that herkogamy more generally serves to reduce interference between maternal and paternal functions (Webb and Lloyd, 1986). Sexual interference can take several forms, but of particular significance are floral strategies that promote more effective pollen dispersal among plants and reduce male gamete wastage through pollen discounting (Barrett, 2002).

Although in some species individual differences in anther–stigma separation may largely reflect plastic responses to variation in environmental factors (Elle and Hare, 2002; Weinig, 2002), investigations on the genetic basis of variation in herkogamy have generally found moderate to high heritabilities, and a rapid response to artificial selection (Shore and Barrett, 1990; Holtsford and Ellstrand, 1992; Motten and Stone, 2000; Lendvai and Levin, 2003).

Previously, marked variation in autonomous fruit production has been observed in the species *Desmodium incanum, D. pachyrrhizum, D. subsericeum* and *D. uncinatum* (Etcheverry et al., 2008; M. Alemán, pers. commun.). In order to explore the possible effect of stigma-anther separation on fruit production, in the present study we focused on the following questions: 1) Is there variation within and among species of *Desmodium* in anther-stigma-separation (ASD)? and 2) Is there some relationship between ASD and deposition of self pollen? We would expect that self pollen loads decline with increasing herkogamy.

The study of this plant group is of great interest because in contrast to the introduced legumes used as forage, (e.g. *Medicago*), the *Desmodium* species are native to the area, and therefore, are adapted to local conditions.

MATERIALS AND METHODS

The studied species belong to Tribe *Desmodieae*: *Desmodium incanum* D.C., *D. subsericeum* Malme, *D. pachyrrhizum* Vogel, *D. uncinatum* (Jacq.) DC. They are bee-pollinated, self-compatible species, with a mixed breeding system. The flowers present little or no protandry, and autonomous self-fertilization is highly variable (Etcheverry et al., 2008; M. Alemán unpubl. results). All the studied species are of great interest to landscape gardening (Etcheverry et al., 2010) and may also be used for forage given their high nutritional value (Fernández et al., 1988).

This study was carried out in the Lerma Valley within the Salta Province, Argentina. Voucher specimens were deposited in the Museo de Ciencias Naturales, Universidad Nacional de Salta (MCNS). The field site extends from the Yungas seasonal rainforest (Eastern Andes) to the eastwards Chaco dry forest of Argentina (Olson et al., 2001). There is a strong seasonality in the Lerma Valley, with 80 percent of the rains concentrated to the period from November to May. Mean annual precipitation is 662.58 mm, whereas mean annual temperature is 17.55°C (Bianchi and Yáñez, 1992).

In January and February of 2009 we marked and bagged 10 floral buds from 10 individual plants per species. To minimize any possible effect of flower age-related variation in anther–stigma separation, floral buds chosen for measurement were all of the same developmental stage. The flowers were collected before senescence; ASD was measured immediately after collection with digital calipers (0.01 mm) as the distance between the surface of the stigma and the nearest anther. The stigmas were preserved in

70% ethanol. Pollen grains were counted under a microscope (10 x) in the laboratory. As the data did not fit the normality and homoscedasticity assumptions, we used nonparametric analysis. Variation in ASD within and among species was tested using Kruskal-Wallis analysis of variance test (Zar, 1984). The relationships between ASD and self-pollen deposition on stigmas for each species were analyzed by Spearman's correlation analysis (Zar, 1984).

RESULTS

There were no significant differences in anther-stigma distance among individuals within each of the species; *D. incanum*: $H = 4.026$, $P = 0.546$; *D. pachyrrhizum*: $H = 7.73$, $P = 0.102$; *D. subsericeum*: $H = 4.307$, $P = 0.635$; and *D. uncinatum*: $H = 8.33$, $P = 0.687$. All the studied species presented the stigma above the anthers, but there was a significant variation among them. *Desmodium incanum* presented the lowest value of ASD and *D. pachyrrhizum* showed the greatest value, while *D. subsericeum* and *D. uncinatum* presented intermediate values ($H = 26.61$, $P < 0.0001$; Fig. 1).

The number of self pollen grains deposited on stigmas differed among species ($H = 33.25$, $P<0.0001$; Fig. 2). *Desmodium incanum* showed the greatest value in self pollen load and *D. pachyrrhizum* presented the lowest value, but this value did not differ significantly from those obtained for *D. subsericeum* and *D. uncinatum* (Fig. 2). We found significant negative relationships for all *Desmodium* species between ASD and the stigmatic self pollen loads in isolated flowers (Table 1).

DISCUSSION

In the present study, all *Desmodium* species showed herkogamy in which stigmas were located above anthers (approach herkogamy). This floral construction captures less total pollen but exhibits significantly higher outcrossing than reverse herkogamous flowers, where stigmas are located below anthers making them more susceptible to pollinator-mediated intrafloral self-pollination (Webb and Lloyd, 1986).

Our results support the hypothesis that flowers with the lowest ASD deposit more self pollen grains on stigmas, indicating a high level of self pollination even in the absence of flower visitors. This finding is consistent with those reported by Lush (1979) in cultivated lines of *Vigna ungiculata* (Fabaceae), Murcia (1990) in *Ipomoea trichocarpa* (Convolvulaceae) and Parra-Tabla and Bullock (2005) in *Ipomoea wolcottiana*. For self-compatible, non-dichogamous species, variation in the expression of herkogamy is frequently negatively correlated with the potential autonomous selfing rate (Carr and Fenster 1994). In all the studied *Desmodium* species, overlap occurs between female and male phases, allowing autonomous self-pollination (Etcheverry et al., 2008; M. Alemán, pers. commun.). In the absence of pollinators, preliminary results of autonomous selfing (% fruit set) varied as follows: *D. incanum*, 50%; *D. subsericeum*, 35%; *D. pachyrrhizum*, 23% and *D. uncinatum*, 1% (M. Alemán, pers. commun.). Comparing these results with the obtained values of ASD in the present work, we suggest that autonomous selfing decline with increasing herkogamy in the studied species. Similar results were reported by Moeller and Gebber (2005) for *Clarkia xantiana* (Onagraceae). Thus, plants with reduced herkogamy may benefit from having the possibility to self when pollinator visits are scarce (reproductive assurance hypothesis). When opportunities for outcrossing decline, theoretical models predict that reproductive assurance can outweigh the disadvantage of inbreeding depression (Lloyd 1992; Schoen et al., 1996). Field studies reported that selfing mechanisms commonly elevate reproductive success in natural populations (e.g.,

Herlihy and Eckert 2002; Elle and Carney, 2003; Kalisz and Vogler, 2003). However, strong selection against selfed progeny in other animal-pollinated species with mixed mating systems has been reported: e.g. *Decodon verticillatus* (Eckert and Barrett, 1994), *Shorea leprosula* (Lee et al., 2000), *Sagittaria latifolia* (Dorken et al., 2002), *Aquilegia canadensis* (Herlihy and Eckert, 2002), *Daphne laureola* (Medrano et al., 2004).

Given the economical potential of these species, our future research will endeavour to evaluate the phenotypic and genetic covariation of floral characteristics involved directly with the degree of herkogamy and other floral characteristics which could be related to the success of fruit production.

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Table 1. Spearman's correlation coefficients between anther-stigma distance and the number of self pollen deposited on stigmas in *Desmodium* species (Fabaceae-Papilionoideae) from Northwestern Argentina.

Species	Relationships between ASD and the number of self pollen grains deposited on stigmas
Desmodium incanum	r = -0.52, P= 0.02
Desmodium subsericeum	r = -0.57, P= 0.01
Desmodium uncinatum	r = -0.67, P= 0.02
Desmodium pachyrrhizum	r = -0.87, P= 0.01

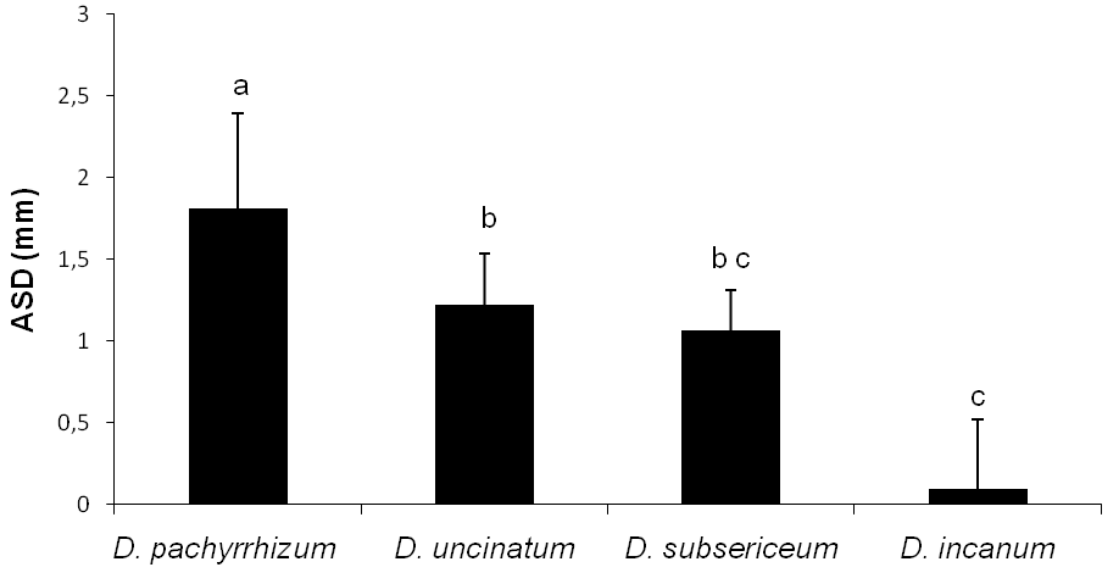

Fig. 1. Anther-stigma distance (mean ± SE) in four *Desmodium* species from Northwestern Argentina. Different letters indicate significant differences ($P < 0.005$).

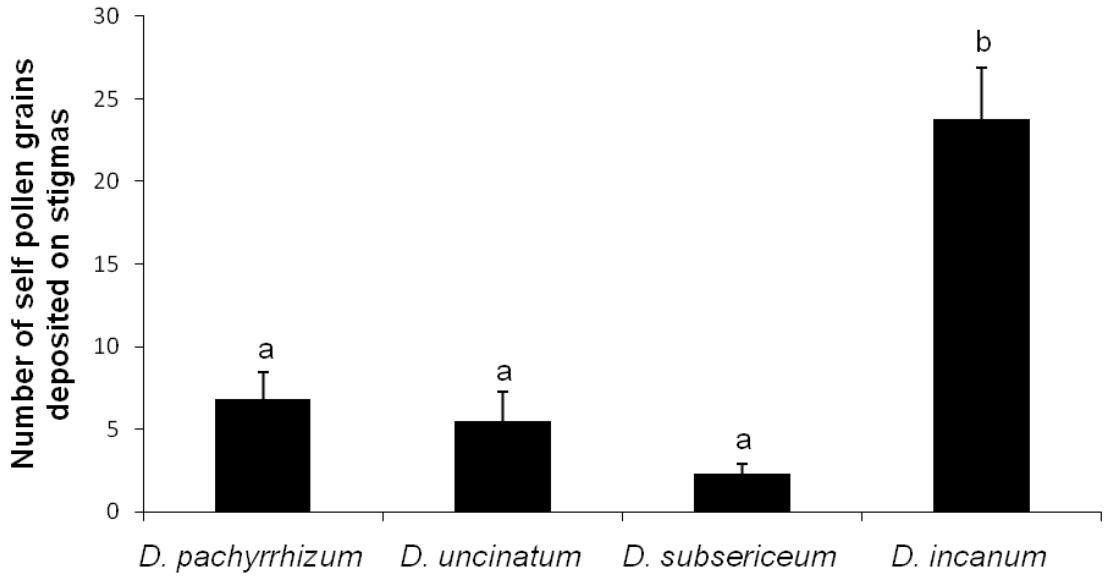

Fig. 2. Number of self pollen grains deposited on the stigmas in flowers of *Desmodium* species from Northwestern Argentina. Different letters indicate significant differences ($P < 0.005$).