

## Pollination and breeding system in *Adesmia bijuga* Phil. (Fabaceae), a critically endangered species in Central Chile

### Polinización y sistema reproductivo en *Adesmia bijuga* Phil. (Fabaceae), una especie en peligro crítico en Chile central

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#### ABSTRACT

*Adesmia bijuga* Phil. is an endemic shrub in central Chile, classified as a critically endangered species of plant. After more than 100 years since its original description by R. A. Philippi (1884), the species was rediscovered exclusively in two locations of the Maule Region. One of them, Fundo San Pedro-Las Cañas, is close to extinction with a low number of shrubs, occurring in a disturbed habitat due to plantations of *Pinus radiata*. This study examined the pollination biology and breeding system of *A. bijuga* in order to explain their small number of shrubs. We estimated composition of pollinator species attracted to flowers and visitation rates. In order to describe its breeding system in *A. bijuga* we used manipulated treatments to estimate autonomous self-pollination, level of reproductive assurance and pollen limitation. The results of this study indicate that only pollinators from the order Hymenoptera visit *A. bijuga*. The pollinator assemblage did not show temporary variation during the flowering season, with *Bombus dahlbomii* as its most frequent visitor. *A. bijuga* showed a mixed mating system that involves both cross- and self- pollination. The fruit set ranges from 20% to 60% in the absence or presence of pollinators, respectively. This selfing ability would provide reproductive assurance when pollinators fail. The taxon showed ability of asexual formation of seeds without the occurrence of fertilization by agamospermy. Supplemental outcross pollen did not increase female reproductive success, suggesting that the species is not pollen limited. All these factors may help to explain how this species has been able to survive over time in a small and isolated population. Finally, this work discusses how the results of our study can be applied to future *A. bijuga* conservation measures.

**KEYWORDS:** Critically endangered species, *Adesmia bijuga*, pollination, breeding system, visitation rate.

#### RESUMEN

*Adesmia bijuga* Phil. es una especie arbustiva endémica de Chile central, clasificada como especie en peligro crítico. Después de más de 100 años desde su descripción original por R.A. Philippi (1884), la especie fue redescubierta exclusivamente en dos localidades de la Región del Maule. Una de ellas, en el Fundo San Pedro-Las Cañas, se encuentra cercana a la extinción, en un hábitat perturbado debido a plantaciones de *Pinus radiata*. Este estudio examinó la biología de la polinización y el sistema reproductivo de *A. bijuga*, que podrían explicar su restringido número de plantas. Se estimó la composición de polinizadores atraídos por las flores y la tasa de visita. Con el objetivo de caracterizar el sistema reproductivo en *A. bijuga* se realizaron tratamientos manipulativos para cuantificar la capacidad de autofertilización, el nivel de aseguramiento reproductivo y la limitación de polen. Los resultados de este estudio indican que *A. bijuga* es visitada solamente por polinizadores que pertenecen al orden himenóptera. El ensamble de polinizadores no varió temporalmente durante el periodo de floración, siendo *Bombus dahlbomii* el visitador más frecuente. *A. bijuga* presentó un sistema reproductivo mixto, con polinización cruzada y autopolinización. El rango de fructificación varió entre 20% a 60% en ausencia y presencia de polinizadores, respectivamente. Esta capacidad de autopolinización podría proveer de aseguramiento reproductivo cuando los polinizadores fallan. Tratamientos con suplemento de polen no incrementaron el éxito reproductivo, sugiriendo que la especie no se encuentra limitada de polen. El taxon mostró capacidad de formar semillas asexualmente sin la ocurrencia de fertilización (agamosperma). Todos estos factores podrían ayudar a explicar cómo esta especie es capaz de persistir en el tiempo en una población pequeña y aislada. Finalmente, este trabajo discute cómo los resultados de este estudio pueden ser aplicados en futuras medidas para la conservación de *A. bijuga*.

**PALABRAS CLAVE:** Especie en peligro crítico, *Adesmia bijuga*, polinización, sistema reproductivo, tasa de visita.

## INTRODUCTION

Understanding the ecology of critically endangered species is crucial both in comprehending the causes of their conservation status as well as formulating appropriate management measures. Nevertheless, insufficient appreciation of the ecology of a particular species is repeatedly cited as a shortcoming in the management of threatened and endangered plants (Schemske *et al.* 1994, Tear *et al.* 1995). Critically endangered species are at the sharp end of today's global extinction crisis. These are species judged most likely to become extinct in the immediate future unless conservation efforts are made (Rossetto *et al.* 1997). Causes of rarity and critical endangerment are diverse, ranging from direct human destruction of wild ecosystems to processes such as recent evolutionary origin or reproductive failure of relict species under changed environmental circumstances (Fiedler & Ahouse 1992, Pate & Hopper 1993). For example, several comparisons of rare-common species pairs have demonstrated that reproduction and recruitment are often particularly low in rare species (Münzbergová 2005, Young *et al.* 2007). The need to gather information on the basic biology of rare species is vital to both species- and community-level conservation efforts (Saunders & Sedonia 2006).

Pollination is a fundamental aspect of plant reproduction, and pollination by animals is largely considered a co-adaptive process in which plants evolve traits to attract certain pollinators, whereby pollinators then evolve traits to better exploit floral resources of particular plants, with the occurring natural selection mediated by that pollinator (Faegri & Van der Pijl 1980, Heinrich 1983). For natural selection to occur, the pollinator must first benefit from the plants fitness, increasing for example their reproductive success (Gómez & Zamora 2006). Plant reproductive success often depends on pollination biology (including the frequency and identity of floral visitors), capability of autonomous self-pollination, and/or the magnitude of pollen limitation (Banks 1980, Mehrhoff 1983, Burd 1994, Gaston & Kunin 1997, Knight *et al.* 2005, Lavergne *et al.* 2005, Rymer *et al.* 2005, Aizen & Harder 2007). Autogamy provides reproductive assurance and can benefit alien as well as narrowly distributed plants. It is easier for autogamous, introduced plant species to invade new territories than those that require cross-pollination through animal pollinators (Baker 1955, Richardson *et al.* 2000, Van Kleunen & Johnson 2007). Likewise, autogamous, plant species with reduced habitat distribution are less likely to experience failed or low reproductive output when they occur at low densities (Banks 1980, Clampitt 1987, Kaye 1999). Numerous studies have recognized that mating strategies play a critical role in the survival of small plant populations (Gargano *et al.* 2009).

*Adesmia bijuga* is a perennial plant forming a shrub

(reaching 2 m in height) with hermaphrodite flowers (Hahn & Gómez 2008). After more than 100 years since the original description by R. A. Philippi (1884), the species was rediscovered in two localities of the Maule Region, Chile: Fundo San Pedro-Las Cañas (Constitución) and Huelón (Curepto) (Figs. 1a-1b), both populations which are separated by approximately 55 km. The population in Fundo San Pedro-Las Cañas (Fig. 1c) is in a disturbed habitat, growing mainly underneath *Pinus radiata* D. Don plantations, and sclerophyllous vegetation such as *Baccharis rhomboidalis* J. Remy subsp. *truncata* (Phil.) F. H. Hellw., *Adesmia elegans* Clos, *Ugni molinae* Turcz., *Ribes punctatum* Ruiz et Pav. and *Escallonia pulverulenta* Pers. (Hahn & Gómez 2008). Based on demographic characteristics, *A. bijuga* was classified as a critically endangered plant (Gómez *et al.* 2009). Studies in pollination biology and breeding systems of *A. bijuga* are unknown, even less so given that the pollination ecology may be contributing to their small habitat distribution. Since plant mating systems affect population genetic diversity (Hamrick & Godt 1989) and potentially population survival, developing an effective management plan for plants with reduced habitat distribution, should be the first step to understanding the reproductive biology of the taxon (Hamrick *et al.* 1991). Indeed, the lack of data on species biology is repeatedly cited as a shortcoming of threatened and endangered species recovery plans (Schemske *et al.* 1994, Clark *et al.* 2002). In this context, we examined the natural history of the pollination biology and breeding systems of *A. bijuga* (Fabaceae). We used observational and experimental approaches to address three main questions: (i) Is *Adesmia bijuga* visited by pollinators?, (ii) Is *A. bijuga* capable of autonomous selfing?, and (iii) Does selfing provide reproductive assurance when pollinators fail?

## METHODS

### STUDY SITE AND NATURAL HISTORY

The study was conducted from December 2010 to January 2011 at the Fundo San Pedro (Maule Region; lat. 35°29'31"S, long. 72°22'47"W), located about 20 km southeast of Constitución, Chile (Fig. 1). In this area, the climate is of a Mediterranean type with rainfall concentrated in the winter season (Di Castri & Hajek 1976). The population of *A. bijuga* grows primarily underneath sclerophyllous vegetation. However, about 20 years ago, this habitat was disturbed by *Pinus radiata*. After that, *A. bijuga* grows in clearings between two *Pinus* plantations (Fig. 2a). This population is composed by approximately 300 shrubs (juveniles and adults) with only 33 (11%) being observed as flowering shrubs. The hermaphrodite flowers are yellow with brownish lines, composed by five petals with butterfly-shaped papilionaceous (Fig. 2b). Flowers are 1.48-1 ± 0.03 (± SE) cm long with 10 staminoids. The indehiscent fruit

(legumes) are 0.5-1.4 cm long, covered with numerous hairs and with 1-4 seeds per legume (Fig. 2c). The flowering season is from December through January, while fructification is from January through February. At the study site, 40.9 % of the *A. bijuga* flowers presented some level of herbivory by larvae (9 out of 22 flowers), due to the absence of reproductive structures (ovary, pistil and staminoids).

POLLINATOR VISITATION

We recorded the identity and visitation rate of diurnal insects in 33 flowering shrubs. On each focal flower, we observed pollinator visits during 30 min, recording the species and number of visits. We defined a visit as the contact between a pollinator and the sexual structure of the flower. The observations of pollinator activity were performed between 1100 and 1700 h by two trained observers. We spent a total of 14,400 min of observation

during a total period of 4 days, once a week from December to January. Only during the first-day of observation were unknown pollinators captured and identified in the laboratory. Visitation rates were calculated as the number of visits per open flower per hour. Probability of visits were estimated as  $P_v = 1 - 1/e^x$ , where x is the visitation rate for each plant (Cavieres *et al.* 1998). To evaluate temporal variation during the flowering season, we statistically compared visitation rates between weeks using repeated measures ANOVA. Additionally, we calculated pollinator diversity using the Shannon–Wiener  $H'$  index. In order to accomplish this, we estimated 95% confidence intervals for each index by randomizing samples in 500 bootstrap replications in EstimateS (Colwell 2009). We compared pollinator diversity between weeks using the *t*-test with Bonferroni's adjustments (Sokal & Rohlf 1995).

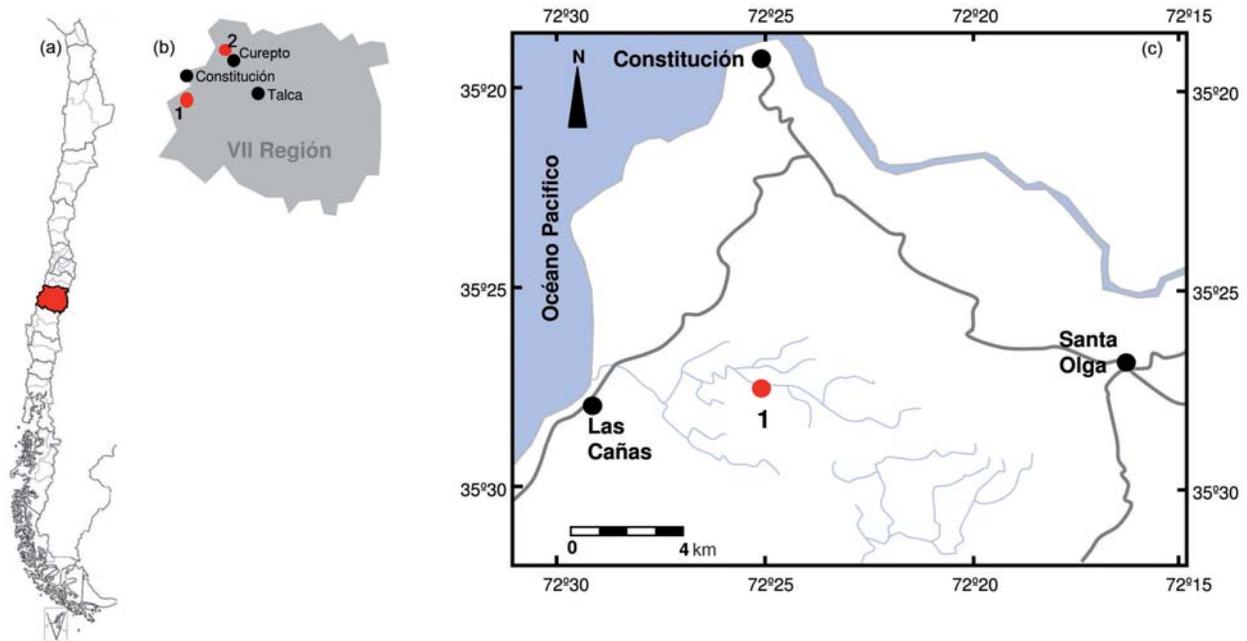


FIGURE 1. Geographic location of *Adesmia bijuga* populations. (a) Map of Chile characterizing the Maule Region. (b) Close-up of the Maule Region with two locations of *A. bijuga*, 1) Population Fundo San Pedro-Las Cañas, Constitución and 2) Population Huelón, Curepto. (c) Close-up to the exact location of the study population (Fundo San Pedro-Las Cañas).

FIGURA 1. Ubicación geográfica de las poblaciones donde se encuentra *Adesmia bijuga*. (a) Mapa de Chile caracterizando la VII Región del Maule. (b) Acercamiento de la VII Región del Maule con las dos únicas localidades en que se ha encontrado *A. bijuga*, 1) Población del Fundo San Pedro-Las Cañas, Constitución and 2) Población de Huelón, Curepto. (c) Acercamiento para mostrar con exactitud la ubicación geográfica de la población en estudio (Fundo San Pedro-Las Cañas).

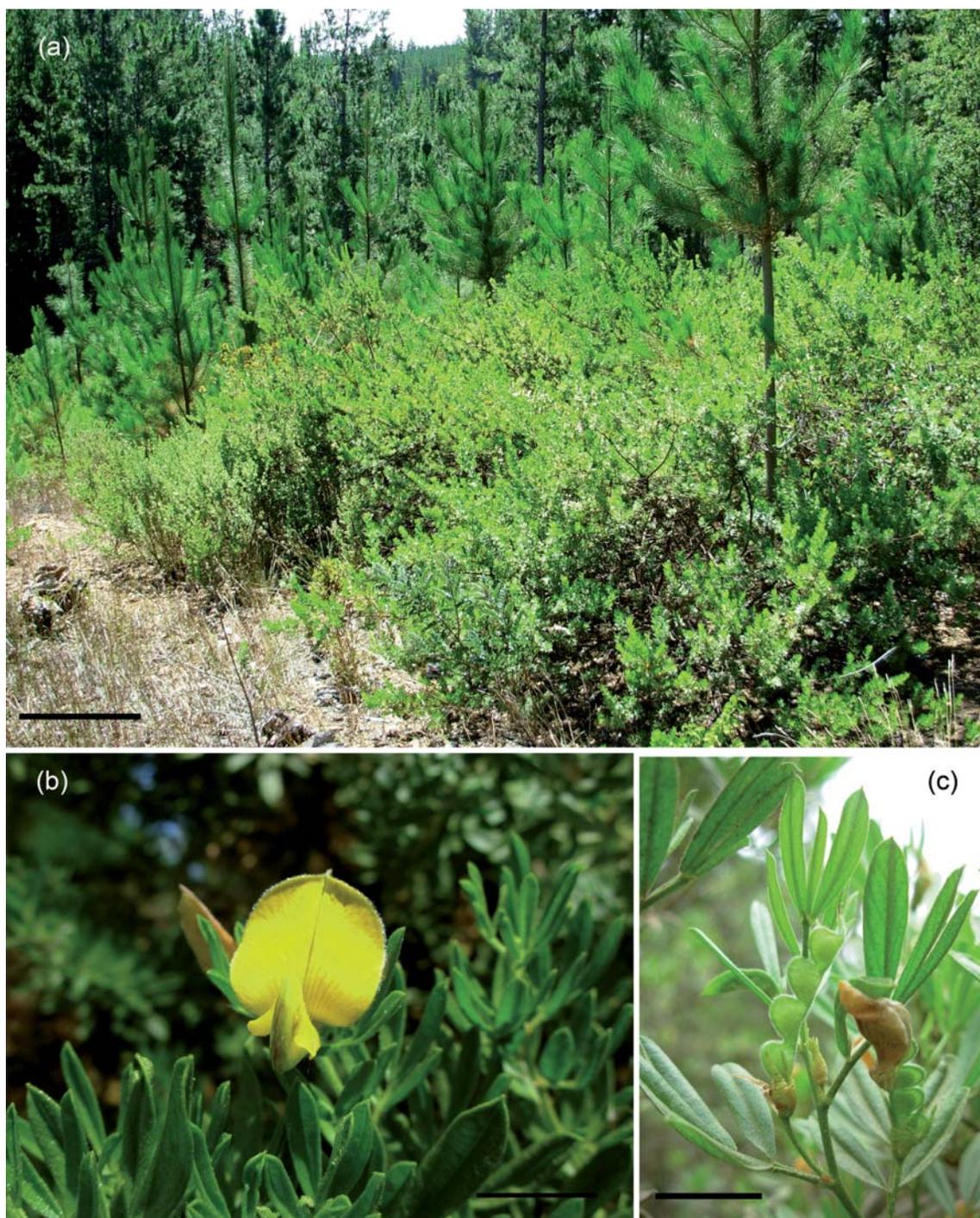


FIGURE 2. *Adesmia bijuga* from Fundo San Pedro (Maule Region). (a) *A. bijuga* grows mainly underneath sclerophyllous vegetation that includes *Pinus radiata* plantation and sclerophyllous shrubs; scale bar= 1 m. (b) Yellow hermaphrodite flowers, composed of five petals; scale bar= 1 cm. (c) The indehiscent fruit (legumes) with 1-4 seeds per legume; scale bar= 1 cm.

FIGURA 2. *Adesmia bijuga* del Fundo San Pedro (región del Maule). (a) *A. bijuga* creciendo bajo la vegetación que incluye plantación *Pinus radiata* y arbustos esclerófilos, escala =1 m. (b) Flores amarillas hermafroditas compuestas por cinco pétalos; escala = 1 cm. (c) El fruto indehiscente (lomento) con 1-4 semillas por lomento; escala = 1 cm.

BREEDING SYSTEM

In order to evaluate the dependence upon pollinator agents for effective fertilization, we evaluated the breeding system of *A. bijuga* at the study site. For this, we tagged 20 plants and set up five treatments on each one, and they were tested for: (1) Autogamy, estimating the capacity for automatic self-pollination, in which buds were bagged throughout their flowering period without emasculation; (2) Geitonogamy, estimating genetic self-incompatibility by hand self-pollination. One bud per plant was immediately bagged after emasculation, during anthesis the flowers were pollinated with pollen from the same individual previously isolated in the bud stage. The pollinated flower was re-bagged until fruit formation; (3) Agamospermy, estimating the capacity of asexual formation of seeds without the occurrence of fertilization, in which buds were immediately bagged after emasculation; (4) Manual cross-pollination (Xenogamy), in which emasculated flowers in anthesis were pollinated with pollen from another plant at least 10 m away and then bagged; and (5) open-pollinated flowers (Control), in which flowers were exposed to the natural agents of pollination. All emasculation treatments were conducted before anther dehiscence and the stigma was rubbed with a brush as well as the control treatment. From a total of 100 flowers, we quantified the percentage of flowers that set fruit four weeks after anthesis. Fruit sets were estimated as mature fruits per flower (legume formation). Additionally, we estimated fruit size (legume length), and number of seeds per fruit (N° of seed per legume) for each treatment. We compared fruit set among treatments using G-test with Bonferroni's adjustments (Sokal & Rohlf 1995). Comparisons of fruit size and number of seeds per fruit were made using one-way ANOVA with *a posteriori* Tukey-HSD test (Sokal & Rohlf 1995). We examined whether the levels of fruit set exhibited

a relationship to fruit size and seeds per fruit, to do this we used Spearman correlation analyses. All statistical analyses were performed using STATISTICA (data analysis software system) version 7 (StatSoft, Inc. 2004, www.statsoft.com). In addition, we estimated an index of self-incompatibility (ISI) using the formula: ISI = N° seed geitonogamy/ N° seed xenogamy (Ruiz & Arroyo 1978). An index of automatic self-pollination (IAS) was obtained using the formula: IAS= % fruit set by autogamy / % fruit set by geitonogamy (Ruiz & Arroyo 1978).

RESULTS

POLLINATOR VISITATION

We recorded a total of 688 visits to 33 focal plants of *A. bijuga*. The assemblage of diurnal visitors consisted of five taxa (Table I), all of them hymenopterans species from the Apidae family. In terms of frequency, the most important visitor was the endemic *Bombus dahlbomii* (88.2%), visiting *A. bijuga* plants between 8 to 46 times more often than other species (Table I). Most of the visitors (10.7%) were introduced species including *Bombus terrestris* (4.6%), *Bombus ruderatus* (4.4%), and *Apis mellifera* (0.7%). The remaining 2% of visits were made by the native species, *Manuelia gayatina*. During the study period visitation rates and pollinator diversity did not differ between weeks of observation (ANOVA  $F=0.05$ ,  $P=0.98$ ; Table II), indicating no temporal variation in diurnal visitor assemblages during their flowering season (December to January). This result suggests that *A. bijuga* attracted a variety of potential pollinators that probably play an important role on the dispersion and amount of pollen deposited on the stigmas.

TABLE I. Pollinator visitation rate (visits flower<sup>-1</sup> h<sup>-1</sup>) mean ± SE and probability of visits of pollinator on *Adesmia bijuga* during their flowering season (December to January).

TABLA I. Tasa de visita de los polinizadores (visita flor<sup>-1</sup> h<sup>-1</sup>) promedio ± EE y probabilidad de visita de los polinizadores en *Adesmia bijuga* durante su periodo de floración (diciembre a enero).

POLLINATOR SPECIES	VISITATION RATE (Visits flower <sup>-1</sup> h <sup>-1</sup> )	PROBABILITY OF VISITS (Pv)
<i>Apis mellifera</i>	0.02 ± 0.03	0.02
<i>Manuelia gayatina</i>	0.05 ± 0.09	0.05
<i>Bombus ruderatus</i>	0.12 ± 0.09	0.11
<i>Bombus terrestris</i>	0.12 ± 0.04	0.11
<i>Bombus dahlbomii</i>	2.31 ± 0.49	0.90

BREEDING SYSTEM

*A. bijuga* Fundo San Pedro showed a mixed mating system that involves both cross- and self-pollination. The fruit set ranges from 20% to 60% in the absence or presence of pollinators, respectively (Table II). In addition, plants were self-compatible (ISI=1.13) and partially autogamous (IAS=0.55).

Fruit-set among treatments was statistically different ( $G = 10.04$ ,  $df = 4$ ,  $P = 0.04$ ; Table III). Paired comparisons showed significant differences in the percentage of fruit sets between Control flowers and the other treatments, reaching 60% of fructification in untreated flowers. Treatments with flowers excluded from the pollinators set showed no statistical differences between them; autogamously treatments showed capability for automatic self-pollination in 25% of the sampled flowers, while geitonogamously

selfed flowers showed genetic self-compatibility in 45% of the sampled flowers. Additionally, the capability of the asexual seed formation without the occurrence of fertilization (agamospermy) was found in only 20% of the sampled flowers, and there were no statistical differences when compared to flowers from which pollinators had been excluded (autogamy and geitonogamy, Table III). Conversely, supplemental outcross pollen treatment (xenogamy) did not significantly increase female reproductive success in *A. bijuga* compared to the Control flowers, this treatment reached only 25% of the sampled flowers, and did not differ from other treatments excluded from pollinators (Table III). Overall, these results indicate that *A. bijuga* showed a mixed mating system, however pollinators play an important role as inter- and intra-plant pollen vectors increasing fruit set percentages of sampled flowers.

TABLE II. Comparisons of pollinator visitation during studied period (four weeks) on *Adesmia bijuga*. Visitation rate (visits flower<sup>-1</sup> h<sup>-1</sup>) mean ± SE, and diversity ( $H'$  mean ± SD).

TABLA II. Comparación de la visita de polinizadores durante el periodo de estudio (cuatro semanas) en *Adesmia bijuga*. Tasa de visita de los polinizadores (visita flor<sup>-1</sup> h<sup>-1</sup>) promedio ± EE y diversidad ( $H'$  promedio ± DS).

PERIOD OF POLLINATORS OBSERVATIONS	VISITATION RATE (visits flower <sup>-1</sup> h <sup>-1</sup> )	POLLINATOR DIVERSITY ( $H'$ mean ± SD)
1 <sup>st</sup> week	0.6 ± 0.53 <sup>a</sup>	0.43 ± 0.14 <sup>a</sup>
2 <sup>nd</sup> week	0.6 ± 0.47 <sup>a</sup>	0.46 ± 0.10 <sup>a</sup>
3 <sup>rd</sup> week	0.5 ± 0.44 <sup>a</sup>	0.48 ± 0.05 <sup>a</sup>
4 <sup>th</sup> week	0.5 ± 0.45 <sup>a</sup>	0.48 ± 0.00 <sup>a</sup>

Different letters show significant differences ( $P < 0.001$ ). / Letras diferentes indican diferencias significativas ( $P < 0,001$ ).

TABLE III. Fruit-set, legume length and number of seed from different treatment of breeding system on *Adesmia bijuga* (mean ± SE).

TABLA III. Fructificación, tamaño del lomento y número de semillas de los diferentes tratamientos del sistema reproductivo en *Adesmia bijuga* (promedio ± EE).

TREATMENT	N° OF PLANTS PER TREATMENT	FRUIT-SET (%)	LEGUME LENGTH (cm)	N° OF SEED PER LEGUME
Autogamy	20	25 <sup>a</sup>	0.8±0.2 <sup>ab</sup>	1.6±0.1 <sup>ab</sup>
Geitonogamy	20	45 <sup>ab</sup>	0.8±0.3 <sup>ab</sup>	1.8±0.1 <sup>ab</sup>
Agamospermy	20	20 <sup>a</sup>	1.2 ±0.1 <sup>a</sup>	2.8±0.1 <sup>a</sup>
Xenogamy	20	25 <sup>a</sup>	0.8±0.2 <sup>ab</sup>	1.6±0.1 <sup>ab</sup>
Control	20	60 <sup>b</sup>	0.7±0.2 <sup>b</sup>	1.5±0.1 <sup>b</sup>

Different letters show significant differences ( $P < 0.001$ ). / Letras diferentes indican diferencias significativas ( $P < 0,001$ ).

The fruit size (legume length) and number of seeds per legume differed among treatments ( $F=3.50$ ,  $df=4$ ,  $P=0.018$  and  $F=2.72$ ,  $df=4$ ,  $P=0.047$ , respectively). In both cases, *A posteriori* comparisons revealed significant differences between Control flowers and agamospermy treatment (Table III). We found a positive correlation between legume length and number of seeds per legume ( $r = 0.98$ ,  $P = 0.002$ ). By contrast, non-significant associations were found between fruit set percentage and legume length ( $r = -0.64$ ,  $P = 0.242$ ), and between fruit set percentage and number of seeds by legume ( $r = -0.51$ ,  $P = 0.382$ ). These results suggest that longer legumes produce a higher number of seed in *A. bijuga* from Fundo San Pedro.

## DISCUSSION

Results from this study indicate that the critically endangered species, *A. bijuga*, from Fundo San Pedro, is visited by pollinators from the order hymenopterans. The pollinator assemblage did not show temporal variation during the flowering season, with *Bombus dahlbomii* being the most important visitor. *A. bijuga* has a mixed mating system that involves both cross- and self-pollination. Cross pollination was characterized due to a higher visitation rate from *Bombus dahlbomii*; whereas self-pollination was observed in their capacity for being self-compatible and being relatively autogamous. This selfing ability would provide reproductive assurance when the pollinator fails to visit the flower. In this population the species showed ability for asexual formation of seeds, without the occurrence of fertilization by agamospermy. Supplemental outcross pollen did not increase female reproductive success, indicating that female reproductive success is not pollen limited. All these factors may help to explain how this species has been able to survive over time in a small and isolated population.

Under natural conditions the *A. bijuga* populations exist in an already fragmented system, and the introduced bees *Apis mellifera*, *Bombus ruderatus* and *Bombus terrestris* were among the main flower visitors. However, the endemic *Bombus dahlbomii* was 9 times more likely to visit *A. bijuga* than introduced species. Fragmentation and introduced pollinators are known threats to the successful pollination of native plants (Goulson 2003, Tschardtke & Brandl 2004). In principle, habitat fragmentation is expected to affect plant population genetic diversity due to endogamy and genetic drift, both reducing the adaptive capability of the species to environmental changes (Aguilar *et al.* 2009). Nevertheless, habitat fragmentation influences plant-pollinator interactions by reducing the richness of pollinators, or by decreasing efficient pollinator abundance (e.g. Aizen & Feinsinger 1994, Rathcke & Jules 1993, Steffan-Dewenter & Tschardtke 1999, Cane 2001, Aguilar 2005). In both cases, the plants populations experience reduced fitness due

to unsuccessful pollination, with lower fructification and seed formation (e.g. Aguilar & Galetto 2004). Even though it is difficult to speculate on the possible impact of either of these processes on the examined *A. bijuga* populations without prior knowledge of the pollination conditions upon their establishment, it is likely that hymenopterans are the visitors with the greatest ability for affecting pollination in *A. bijuga*, with the native *Bombus dahlbomii* as one of the most important visitors. Moreover, pollinator introduction can displace native species (Morales *et al.* 2009), especially when the new pollinator is more of a generalist than its native counterpart. For example, *Apis mellifera* and *Bombus terrestris* are the most commonly introduced species due to human activities, and both are considered generalist pollinators that can reach higher abundance (see Morales *et al.* 2009 for a review). With *A. bijuga*, introduced species showed lower frequency of visits than *Bombus dahlbomii*, but future studies should address the effect of pollinator abundance, efficiency, and their consequences on *A. bijuga* reproductive success.

The species *A. bijuga* has several characteristics suggesting a mixed mating system of outbreeding and self-fertilization. Versatility in mode of reproduction has been described for other *Adesmia* legume species (Tedesco *et al.* 2000), and probably explains their maintenance over time. For example, in very variable pollination environments, such as the Atacama Desert, the mixed mating system keeps the population of *A. argyrophylla* alive (González & Pérez 2010). Numerous studies demonstrated a mixed mating system in species with reduced habitat which probably helps to explain how the species are able to survive over time in small and isolated populations (Hill *et al.* 2008, Gargano *et al.* 2009, Shi *et al.* 2010, Powell *et al.* 2011). In this context, selfing ability and autogamous capacity provide reproductive assurance, producing enable seed production when pollinators or mates are scarce or unavailable, and outcross pollen is insufficient for full fertilization of ovules. In our studied population, selfing occurred by automatic self-pollination (autogamy, 25% of fructification), and between flowers of the same plant (geitonogamy 45% of fructification). In this scenario, only autogamy would provide reproductive assurance, the geitonogamy which can be due to common bee movement within-inflorescence of *A. bijuga* or lower flowers display for pollination is not expected to provide reproductive assurance because it involves both pollen and seed discounting (Lloyd 1992, Lloyd & Schoen 1992, Herlihy & Eckert 2002). Geitonogamy will result in greater inbreeding depression (Holsinger & Thomson 1994, Harder & Barrett 1995), with lower seed set and fitness when compared to populations receiving large amounts of outcross pollen (Jennersten 1988, Lamont *et al.* 1993, Agren 1996, Fischer & Matthies 1998). Inbreeding depression may, in turn, have serious negative consequences for population survival

(Oostermeijer 2000), as reduction in genetic diversity and fitness over time (Ellstrand & Elam 1993). Numerous studies have suggested that geitonogamy reduces female reproductive success due to post-fertilization abortion (Finner & Morgan 2003). Due to its widespread and largely deleterious nature, geitonogamy has been hypothesized as a major selective force in plant reproductive ecology (Wyatt & Broyles 1994). Even though we found a higher rate of geitonogamy in *A. bijuga*, future studies about inbreeding depression by loss of genetic variability and fitness are needed in order to be able to explore the data on seed quality/viability and early developmental phases, which are critical in estimating successful seedling establishment population.

The lack of pollen limitation may be attributed to three different factors. First, the application of a low-diversity pollen grains, as we found low number of flowering *A. bijuga* shrubs (11%) in the studied population, the number of pollen donors was limited, second, a high probability of flowers of visitation by *Bombus dahlbomii* (0.9) under natural condition suggests that supplemental pollination should not increase female reproductive output. Third, the ability of *A. bijuga* to self-fertilize probably affects pollen limitation. Burd (1994) detected a correlation between plant mating systems and pollen limitation, showing that self-compatible species are not pollen-limited, whereas self-incompatible species are commonly pollen limited.

Finally, flower herbivory is another factor which probably affects *A. bijuga* populations. We found large numbers of floral buds with destroyed reproductive structures (ovary, pistil and staminoids), due to the presence of insect larvae. Numerous studies have demonstrated that flower herbivory can have a direct and immediate impact on plant attractiveness when florivores destroy primary reproductive tissues such as anthers, pistils, or ovaries (Kirk 1987, Maron *et al.* 2002, Wise & Cummins 2002, Althoff *et al.* 2005). Future studies should evaluate the impact of florivory on *A. bijuga* female reproductive success.

Overall, our work provides initial insight into the pollination biology and breeding system of the critically endangered species *A. bijuga*, helping to identify possible biological reasons for the limited number of plants of this species. Nevertheless, this is only a first step towards conserving the remaining population of *A. bijuga*. Their continuous habitat fragmentation and the introduction of alien species implies the need for futures studies to obtain information about pollinators (diversity, abundance, and efficiency), and their consequences on reproductive success. Furthermore, *A. bijuga* conservation probably involves studies in seed dispersion, herbivory, successful seedling establishment, species reintroduction and habitat management.

## ACKNOWLEDGEMENTS

We thank M. Murúa, S. Cuartas and A. Conley for help and comments. Financial support was obtained from the following grants: DI from Universidad de Talca, PSD-66, and VID I 09/07-2. The authors also thank two anonymous reviewers for suggestions and criticisms that greatly improved the manuscript.

## REFERENCES

- AGREN, J. 1996. Population size, pollinator limitation, and seed set in self-incompatible herb *Lythrum salicaria*. *Ecology* 77(6): 1779-1790.
- AGUILAR, R. 2005. Efectos de la fragmentación del hábitat sobre el éxito reproductivo de especies nativas del Bosque Chaqueño Serrano de Córdoba. Tesis Doctoral. Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina. 169 pp.
- AGUILAR, R. & L. GALETTO. 2004. Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae). *Oecologia* 138: 513-520.
- AGUILAR, R., L. ASHWORTH, L. CAGNOLO, M. JAUSORO, M. QUESADA & L. GALETTO. 2009. Dinámica de interacciones mutualistas y antagonistas en ambientes fragmentados. En: R. Medel, M. Aizen & R. Zamora (eds.), *Ecología y Evolución de interacciones planta-animal*, pp. 199-230. Editorial Universitaria, Santiago, Chile.
- AIZEN, M.A. & L.D. HARDER. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88(2): 271-281.
- AIZEN, M.A. & P. FEINSINGER. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology* 75: 330-351.
- ALTHOFF, D.M., K.A. SEGRAVES & O. PELLMYR. 2005. COMMUNITY CONTEXT OF AN OBLIGATE MUTUALISM: POLLINATOR AND FLORIVORE EFFECTS ON *Yucca filamentosa*. *Ecology* 86: 905-913.
- Baker, H.G. 1955. Self-compatibility and establishment after long distance dispersal. *Evolution* 9(3): 347-349.
- BANKS, J.A. 1980. The reproductive biology of *Erythronium propullans* Gray and sympatric populations of *E. albidum* Nutt. (Liliaceae). *Bulletin of Torrey Botanical Club* 107(2): 181-188.
- BURD, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* 60(1): 83-139.
- CANE, J.H. 2001. Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology* 5: 3.
- CAVIERES, L.A., A.P.G. PEÑALOZA & M.T.K. ARROYO. 1998. EFECTOS DEL TAMAÑO Y DENSIDAD DE FLORES EN LAS TASAS DE VISITAS DE INSECTOS POLINIZADORES EN *Alstroemeria pallida*. *Gayana Botánica* 55(1): 1-10.
- Clampitt, C.A. 1987. Reproductive biology of *Aster curtus* (Asteraceae), a Pacific Northwest endemic. *American Journal of Botany* 74(6): 941-946.
- CLARK, J.A., J.M. HOESKSTRA, P.D. BOERSMA & P. KAREIVA. 2002. Improving U.S. Endangered Species Act recovery plans:

- key findings and recommendations of the SCB recovery plan project. *Conservation Biology* 16(6): 1510-1519.
- COLWELL, R.K. 2009. ESTIMATE S: Statistical estimation of species richness and shared species from samples, version 8.2.0. <http://vicero.y.eeb.uconn.edu/EstimateS>.
- DI CASTRI, F. & E.R. HAJEK. 1976. *Bioclimatología de Chile*. Ediciones de la Universidad Católica de Chile, Santiago. 128 pp.
- ELLSTRAND, N.C. & D.R. ELAM. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242.
- FAEGRI, K. & L. VAN DER PIJL. 1980. *The principles of pollination ecology*. Pergamon Press, Oxford. 244 pp.
- FIEDLER, P.L. & S.J. AHOUSE. 1992. Hierarchies of cause: towards an understanding of rarity in vascular plant species. In: P.L. Fiedler & S.K. Jain (eds.), *Conservation Biology: the Theory and Practice of Nature Conservation, Preservation and Management*, pp. 23-47. Chapman & Hall, New York.
- FINER, M.S. & M.T. MORGAN. 2003. Effects of natural rates of geitonogamy on fruit set in *Asclepias speciosa* (Apocynaceae): evidence favoring the plant's dilemma. *American Journal of Botany* 90(12): 1746-1750.
- FISCHER, M. & D. MATTHIES. 1998. Effects of population size on performance of a rare plant *Gentianella germanica*. *Journal of Ecology* 86(2): 195-204.
- GARGANO, D., T. GULLO & L. BERNARDO. 2009. Do inefficient selfing and inbreeding depression challenge the persistence of the rare *Dianthus guliae* Janka (Caryophyllaceae)? Influence of reproductive traits on a plant's proneness to extinction. *Plant Species Biology* 24(2): 69-76.
- GASTON, K.J. & W.E. KUNIN. 1997. Rare-common differences: an overview. In: W.E. Kunin & K.J. Gaston (eds.), *The biology of rarity: causes and consequences of rare-common differences*, pp. 12-29. Chapman & Hall, London, England.
- GÓMEZ, J.M. & R. ZAMORA. 2006. Ecological factors that promote the evolution of generalization in pollination systems. In: N.M. Waser & J. Ollerton (eds.), *Plant-pollinator interactions, from specialization to generalization*, pp. 145-165. University of Chicago Press, Chicago, USA.
- GÓMEZ, P., S. HAHN & J. SAN MARTÍN. 2009. Estructura y composición florística de un matorral bajo plantaciones de *Pinus radiata* D. Don en Chile Central. *Gayana Botánica* 66(2): 252-264.
- GONZÁLEZ, A.V. & M.F. PÉREZ. 2010. Pollen limitation and reproductive assurance in the flora of the Coastal Atacama Desert. *International Journal of Plant Sciences* 171(6): 607-614.
- GOULSON, D. 2003. *Bumblebees: their behaviour and ecology*. Oxford University Press, Oxford. 234 pp.
- HAHN, S. & P. GÓMEZ. 2008. Hallazgo de *Adesmia bijuga* Phil. (Fabaceae) en la zona costera, Región del Maule, Chile central. *Gayana Botánica* 65(1): 119-121.
- HAMRICK, J.L. & M.J.W. GODT. 1989. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions: Biological Sciences* 351: 1291-1298.
- HAMRICK, J.L., M.J.W. GODT, D.A. MURAWSKI & M.D. LOVELESS. 1991. Correlations between species traits and allozymes diversity; implications for conservation biology. In: D.A. Falk & K.E. Holsinger (eds.), *Genetics and Conservation of Rare Plants*, pp. 75-86. Oxford University Press, New York, USA.
- HARDER, L.D. & S.C.H. BARRETT. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512-515.
- HEINRICH, B. 1983. Insect foraging energetics. In: C.E. Jones & R.J. Little (eds.), *Handbook of experimental pollination biology*, pp. 187-214. Van Nostrand Reinhold, New York, USA.
- HERLIHY, C.R. & C.G. ECKERT. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416: 320-323.
- HILL, L.M., A.K. BRODYA & C.L. TEDESCO. 2008. Mating strategies and pollen limitation in a globally threatened perennial *Polemonium vanbruntiae*. *Acta Oecologica* 33: 314-323.
- HOLSINGER, K.E. & J.D. THOMSON. 1994. Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *American Naturalist* 144(5): 799-812.
- JENNERSTEN, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology* 2(4): 359-366.
- KAYE, T.N. 1999. From flowering to dispersal: reproductive ecology of an endemic plant, *Astragalus australis* var. *olympicus* (Fabaceae). *American Journal of Botany* 86(9): 1248-1256.
- KIRK, W.D.J. 1987. HOW MUCH POLLEN CAN THIRPS DESTROY? *ECOLOGICAL ENTOMOLOGY* 12(1): 31-40.
- KNIGHT, T.M., J.A. STEETS, J.C. VAMOSI, S.J. MAZER, M. BURD, D.F. CAMPBELL, M.R. DUDASH, M.O. JOHNSTON, R.J. MITCHELL & T-L. ASHMAN. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36: 467-497.
- LAMONT, B.B., P.G. KLINKHAMER & E.T.F. WITOWSKI. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* – a demonstration of the Allee effect. *Oecologia* 94(3): 446-450.
- LAVERGNE, S.M., M. DEBUSSCHE & J.D. THOMPSON. 2005. Limitations on reproductive success in endemic *Aquilegia viscosa* (Ranunculaceae) relative to its widespread congener *Aquilegia vulgaris*: the interplay of herbivory and pollination. *Oecologia* 142(2): 212-220.
- LLOYD, D.G. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Science* 153(3): 370-380.
- LLOYD, D.G. & D.J. SCHOEN. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Science* 153(3): 358-369.
- MARON, J.L., J.K. COMBS & S.M. LOUDA. 2002. CONVERGENT DEMOGRAPHIC EFFECTS OF INSECT ATTACK ON RELATED THISTLES IN COASTAL VS. CONTINENTAL DUNES. *ECOLOGY* 83(12): 3382-3392.
- MEHRHOFF, L.A.III. 1983. Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70(10): 1444-1453.
- MORALES, C., A. TRAVESET & N. RAMÍREZ. 2009. Especies invasoras y mutualismos planta-animal. En: R. Medel, M. Aizen & R. Zamora (eds.), *Ecología y Evolución de interacciones planta-animal*, pp. 247-261. Editorial Universitaria, Santiago, Chile.

- MÜNZBERGOVÁ, Z. 2005. Determinants of species rarity: Population growth rates of species sharing the same habitat. *American Journal of Botany* 92(12): 1987-1994.
- OOSTERMEIJER, J.G.B. 2000. Population viability analysis of the rare *Gentiana pneumonanthe*: the importance of genetics, demography, and reproductive biology. In: A.G. Young & G.M. Clarke (eds.), *Genetics, Demography, and Viability of Fragmented Populations*, pp. 313-334. Cambridge University Press, Cambridge, England.
- PATE, J.S. & S.D., HOPPER. 1993. Rare and common plants in ecosystems, with special reference to the south-west Australian flora. In: E.D. Schulze & H.A. Mooney (eds.) *Biodiversity and Ecosystem Function*, pp. 293-325. Springer, Berlin.
- PHILIPPI, R.A. 1884. Descripción de algunas plantas nuevas chilenas. *Anales de la Universidad de Chile* 65: 57-65.
- POWELL, K.I., K.N. KRAKOS & T.M. KNIGHT. 2011. Comparing the reproductive success and pollination biology of an invasive plant to its rare and common native congeners: a case study in the genus *Cirsium* (Asteraceae). *Biological Invasions* 13(4): 905-917.
- RATHCKE, B.J. & E.S. JULES. 1993. Habitat fragmentation and plant-pollinator interactions. *Current Science* 65: 273-277.
- RICHARDSON, D.M., N. ALLSOPP, C.M. D'ANTONIO, S.J. MILTON & M. REJMANEK. 2000. Plant invasions-the role of mutualisms. *Biological Reviews* 75(1): 65-93.
- ROSSETTO, M., G. JEZIERSKI, S.D. HOPPER & K.W. DIXON. 1999. Conservation genetics and clonality in two critically endangered eucalypts from the highly endemic southwestern Australian flora. *Biological Conservation* 88: 321-333.
- RUIZ, T. & M.T.K. ARROYO. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10(3): 221-230.
- RYMER, P.D., R.J. WHELAN, D.J. AYRE, P.H. WESTON & K.G. RUSSEL. 2005. Reproductive success and pollinator effectiveness differ in common and rare *Persea* species (Proteaceae). *Biological Conservation* 123(4): 521-532.
- SAUNDERS, N.E. & D.S. SEDONIA. 2006. Reproductive biology and pollination ecology of the rare Yellowstone Park endemic *Abronia ammophila* (Nyctaginaceae). *Plant Species Biology* 21(2): 75-84.
- SCHEMSKE, D.W., B.C. HUSBAND, M.H. RUCKELSHAUS, C. GOODWILLIE, I.M. PARKER & J.G. BISHOP. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75(3): 584-606.
- SHI, X., J. WANG, Y.C. ZHANG, J.F. GASKIN & B.R. PAN. 2010. Pollination ecology of the rare desert species *Eremosparton songoricum* (Fabaceae). *Australian Journal of Botany* 58(1): 35-41.
- SOKAL, R.R. & F.J. ROHLF. 1995. *Biometry. The principles and practice of statistics in biological research*, 3rd edn. W.H. Freeman and Co., New York. 887 pp.
- STEFFAN-DEWENTER, I. & T. TSCHARNTKE. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121: 432-440.
- TEAR, T.H., J.M. SCOTT, P.H. HAYWARD & B. GRIFFITH. 1995. Recovery plans and the endangered species act: are criticisms supported by data? *Conservation Biology* 9(1): 182-195.
- TEDESCO, S.B., M. DALL'AGNOL, M.T. SCHIFINO-WITTMANN & J.F.M. VALLS. 2000. Mode of reproduction of Brazilian species of *Adesmia* (Leguminosae). *Genetics and Molecular Biology* 23(2): 475-478.
- TSCHARNTKE, T. & R. BRANDL. 2004. Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology* 49: 405-430.
- VAN KLEUNEN, M. & S.D. JOHNSON. 2007. Effects of self-compatibility on the distribution range of invasive European plant in North America. *Conservation Biology* 21(6): 1537-1544.
- WISE, M.J. & J.J. CUMMINS. 2002. Nonfruiting hermaphroditic flowers as reserve ovaries in *Solanum carolinense*. *The American Midland Naturalist* 148(2): 236-245.
- WYATT, R. & S.B. BROYLES. 1994. Ecology and evolution of reproduction in milkweeds. *Annual Review of Ecology and Systematics* 25: 423-441.
- YOUNG, A.S., S-M. CHANG & R.R. SHARITZ. 2007. Reproductive ecology of a federally endangered legume, *Baptisia arachnifera*, and its more widespread congener, *B. lanceolata* (Fabaceae). *American Journal of Botany* 94(2): 228-236.

Recibido: 11.11.11  
Aceptado: 20.04.12