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# Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae)

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**Abstract** In this paper we evaluate the effects of forest fragmentation on male (pollen removal, pollen load, and pollen tubes) and female reproductive success (fruit- and seed-set) of *Cestrum parqui*, a self-incompatible, pollination-specialist plant species. We also measure focal individual conspecific density to account for possible density-related effects that could influence the response variables. We calculate an index which incorporates male and female fitness and gives an integrated assessment of overall reproductive success. Forest fragmentation strongly affected the amount of pollen grains on stigmas and number of pollen tubes as well as seed-set, decreasing from continuous forest to small forest fragments, whereas focal individual conspecific density failed to explain any of the variability for the studied variables. Declines in overall reproductive success (i.e. male and female) in small forest fragments are ascribed to decreases in both the quality and quantity of pollination. Self-incompatibility coupled with a specialist pollination system may be particularly important traits determining the negative fragmentation effects observed in *C. parqui*. Logarithmic regression models described the behaviour of the variables along the fragmentation size gradient, allowing us to detect a threshold below which the effects of fragmentation begin to negatively affect reproductive success in *C. parqui*. Our results emphasize the importance of evaluating both components of the total plant fitness, as well as including simultaneously several aspects of pollination and reproduction processes when assessing the effects of forest fragmentation on plant reproductive success.

**Keywords** Female fitness · Habitat fragmentation · Male fitness · Patch size · Plant-pollinator interaction

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## Introduction

Most plant populations and communities in the world have been subjected to processes of land clearing and environmental destruction by humans, which have led to the subdivision of originally continuous habitats into smaller, more isolated patches (e.g. Saunders et al. 1991; Jules et al. 1999). Such habitat fragmentation implies the reduction of local abundance of species, and an increase in isolation between populations, along with changes in the surrounding environment, thus affecting many ecological processes at both population and community levels (e.g. Saunders et al. 1991; Rathcke and Jules 1993). The reduction in plant population size is likely to affect the total plant reproductive output, which translates into increased inbreeding, loss of genetic variability, decreased seed production, and eventually increased risk of population extinction (e.g. Lamont et al. 1993; Young et al. 1996; Jules and Rathcke 1999; Cruzan 2001; Jacquemyn et al. 2002). Animal-pollinated plants, in particular, can be influenced by habitat alterations either directly through changes in the reproductive population structure and distribution or indirectly, because of population variations in the local assemblage of pollinators and/or changes in their behaviour and flight patterns (e.g. Aizen and Feinsinger 1994a; Didham 1996). Such alterations in composition, frequency, and behaviour of flower visitors may alter pollination efficiency determining a decrease in the quantity and/or quality of pollen delivered to the stigmas and therefore in seed and fruit production (Rathcke and Jules 1993; Aizen and Feinsinger 1994a; Murcia 1996; Jules and Rathcke 1999). Moreover, small populations have shown increased inter-plant variability of these reproductive variables (Oostermeijer et al. 1998; Jacquemyn et al. 2002), thus the degree of variability of plant reproductive traits denotes the (un)certainty of successful reproduction in reduced plant populations (Oostermeijer et al. 1998).

Plant responses to habitat fragmentation may vary greatly among species according to the degree of dependence on pollinators for successful reproduction (Bond 1994; Murcia 1996). The combination of self-incompatibility and pollination specialization implies a high degree of reproductive dependence on mutualism (Bond 1994). For this reason, self-incompatible plants with specialist pollination systems are expected to be more vulnerable to habitat fragmentation because any decrease in abundance, loss, or exchange of a single pollinator from its reduced pollinator assemblage could lead to complete reproductive failure (Bond 1994; Aizen et al. 2002). Studies oriented to detect effects of habitat fragmentation on plant species with both of these traits combined are scarce in the literature.

Plant reproductive success has been commonly assessed in terms of successful production of fruits and maturation of seeds; that is, estimated solely from the female function of plants (Stanton et al. 1992). However, this is a partial view that only reflects the maternal investment in the plant reproductive output. Natural selection on plant reproductive traits may act differentially through female and male function. For instance, a hermaphrodite plant with a low seed-set may be highly successful as a pollen donor, but this could not be assessed by estimating solely female fitness, thus it is important to evaluate both aspects of the plant reproductive output. Male fitness may be estimated through pollen removal (e.g. Stanton et al. 1992; Queller 1997), pollen dispersal (e.g. Campbell 1989, 1991), pollen deposition on conspecific stigmas and pollen tubes in the style (e.g. Bertin 1988; Waser and Price 1991).

Most of the studies evaluating plant reproduction in fragmented habitats have also followed the female-biased criterion when choosing response variables, thus assessing the potential effects of fragmentation on fruit and/or seed production. In this regard, the patterns found are inconsistent, showing a variety of responses depending on the species studied. Some authors have shown that habitat fragmentation clearly diminishes fruit-set (Aizen and Feinsinger 1994a; Cunningham 2000a, 2000b; Parra-Tabla et al. 2000; Rocha and Aguilar 2001; Wolf and Harrison 2001; Murren 2002; Fuchs et al. 2003; Quesada et al. 2003) and seed-set (Ågren 1996; Groom 1998; Morgan 1999; Rocha and Aguilar 2001; Jacquemyn et al. 2002), whereas some others have detected neutral (Fritz and Nilsson 1994; Murcia 1996; Costin et al. 2001; Cascante et al. 2002; Murren 2002) or positive responses of female reproductive success to habitat fragmentation (Aizen and Feinsinger 1994a; Fritz and Nilsson 1994; Cunningham 2000a).

In contrast, the effects of habitat fragmentation on male fitness have been largely ignored until recently. Only a small number of studies have included and evaluated some male fitness variables, revealing also disparity in their response to habitat fragmentation. Decreases were observed in pollen dispersal distance (Spears 1987), pollinia removal (Parra-Tabla et al. 2000), number of pollen grains deposited on the stigmas (Ghazoul et al. 1998; Rocha and Aguilar 2001; Quesada et al. 2003), and in pollen tube number at the base of the style (Aizen and Feinsinger 1994a; Ghazoul et al. 1998; Cascante et al. 2002). No effects were detected in the rates of pollinia removal (Murren 2002), nor in pollen loads (Cascante et al. 2002), and an increase in pollen tubes was found in one hemi-parasite vine species (Aizen and Feinsinger 1994a).

In conclusion, actual evidence from studies on the effects of habitat fragmentation on plant-pollinator interactions and plant fitness (reviewed by Jules and Rathcke 1999; Jacquemyn et al. 2002; Aizen et al. 2002), suggests that no generalizations can be made and that is difficult to find predictable patterns (Jacquemyn et al. 2002; Aizen et al. 2002).

Moreover, the density of conspecifics characterized by the spacing between conspecific individuals (Kunin 1997) may have also strong effects on ecological interactions (e.g. Feinsinger et al. 1991; Kunin 1993, 1997). Conspecific plant density can either have positive or negative effects on plant reproduction (Rathcke 1983). For instances, high plant densities may be associated with higher inter-plant pollinator visits (Feinsinger et al. 1991; Kunin 1993), whereas low densities may increase within-plant pollen transfer with different consequences on the reproductive success of the plant species depending on its breeding system (e.g. Rathcke 1983). Consequently, high focal individual conspecific density may counteract the effects of small population size due to habitat fragmentation, particularly in self-incompatible species.

In this study we evaluate the effects of habitat fragmentation on male and female fitness of naturally occurring populations of the self-incompatible, pollination-specialist, *Cestrum parqui* L'Herit., a common forest shrub species of the Chaco Serrano from central Argentina. Furthermore, we measure conspecific density at the focal individual level to control for possible density-related effects that could influence the response variables. Specifically, we address the following questions:

1. Does forest fragmentation affect male (pollen removal, deposition, and pollen tubes in the style) and female fitness (fruit- and seed-set) equally?
2. Are the effects of fragmentation strengthened, counterbalanced, or diminished by focal individual conspecific density?
3. How is the overall reproductive success (male and female) of the plant shaped by habitat fragmentation?
4. Is it possible to detect a fragmentation size threshold below which the effects on plant reproduction become discernible?

## Materials and methods

### The species

*Cestrum parqui* L'Herit. (Solanaceae) is a self-incompatible shrub (Morales and Galetto 2003) ca. 1.5 m high, widely distributed in many biogeographic regions of Argentina. Its flowers are hermaphroditic and last for up to 7 days. Flowers are grouped in terminal and axilar inflorescences, each containing six to 25 yellow tubular flowers 20–30 mm long (Romanutti and Hunziker 1998). The species presents a specialized pollination system, with nocturnal moths (family Sphingidae) as main effective pollinators (R. Aguilar, unpublished data). Flowering begins in September and lasts until February.

### Study sites

Based on Landsat ETM satellite images (1 January 1999), six forest fragments of different size were selected forming a fragmentation size gradient (Table 1). The selected study sites are located along 50 km near the road E-53, which connects the city of Córdoba with Río Ceballos. All fragments are located at altitudes ranging from 500 to 600 m a.s.l., have comparable south-east orientation, vegetation stratification, and are included within the same biogeographic region (Chaco Serrano Forest), thus share similar climatic conditions.

### Selection of plant individuals

Sampling was carried out during the flowering season of 1999–2000. Five to eight individuals were selected per study site. In order to account for possible limitations in resource availability due to impoverishment of habitat characteristics in small forest fragments, which may have detrimental effects, particularly on female reproductive success (Stephenson and Bertin 1983), we carefully selected focal individual plants of comparable size, each presenting a similar number of flowers at the moment of sampling (c.a. 150–200) in all forest fragments studied. Each focal individual was separated by at least 10 m from one another within each study site. The appropriate scale at which to assess density is not always clear and it depends on the scale of the mechanisms limiting reproduction. However, pollen travel mediated by animal pollinators is in general strongly leptokurtic, with most of the pollen transport occurring between near-neighbours (e.g. Olensen and Warncke 1989). Thus, the number of conspecifics in a radius of 3 m around each focal individual was assessed to account for focal individual conspecific density. In all, 41 focal plants were selected.

### Pollen production and ovule number

To calculate pollen removal and seed-set, it was necessary to measure pollen production and ovule number. Mature buds from each of several inflorescences per individual were cut and fixed in formalin and alcohol at each study site (*n*=10–15 per individual; a total of ~400 buds). Total pollen content of one anther per flower was cautiously placed on an England Finder counting chamber slide with a drop of aniline blue and counted under a Zeiss Axiolab light microscope. Total pollen production per flower was calculated by multiplying the observed number by the total number of anthers per flower. Total ovule number was counted in the same flowers under a magnifying glass. Although both of these variables are expected not to be affected by forest fragmentation nor focal individual conspecific density they were also subjected to statistical analysis.

### Pollen removal, pollen loads, and pollen tubes in the style

Senescent flowers, which had been exposed to natural pollination, were selected from each of several inflorescences per individual and carefully mounted on a slide with double adhesive tape (*n*=10–12 per individual). Slides were cautiously placed in slide cases to avoid damage of the stigma and losses of pollen content from the anthers. At the laboratory, total remnant pollen grains from each previously hydrated open anther was quantified following the same procedure explained for pollen production. Pollen removal was calculated as the difference between mean pollen production per flower of each individual focal plant and remnant pollen in anthers from senescent flowers. Pollen load on the stigma was counted in the same flowers. Stigmas were cut, placed on a graduated slide and softened with a drop of 8 N sodium hydroxide for 3 h. They were rinsed with water, dyed with a drop of aniline blue and gently squashed under a cover slip. Total pollen content on the stigma surface was counted under Zeiss Axiolab light microscope. Similarly, other sets of senescent flowers (*n*=10–15 per individual) were collected as previously described, to quantify the number of pollen tubes at the base of the style. Pollen tubes were observed using fluorescent microscopy following Martin (1959). Overall, a total of ~700 flowers were used to measure these variables.

### Natural production of fruits and seeds

Mature buds were marked and left exposed to open pollination to assess for natural fruit- and seed-set (*n*=15–20 per individual). After 6–7 weeks fruits were collected and quantified. Fruit-set was calculated as the proportion of fruits produced from total marked flowers per individual. Total number of seeds produced per fruit was counted at the laboratory under a magnifying glass. Seed-set was expressed as the proportion of seeds produced from the mean number of ovules per flower.

We propose an integrated measure to evaluate overall plant reproductive success by including male and female reproductive variables at the individual level. We calculate the overall reproductive success index, which may be taken as a multiplicative fitness function that assumes values from 0 to 1 and is expressed as: [(pollen removal/pollen production)×(pollen tubes/pollen load)]×[(fruits/marked flowers)×(seeds/ovules)].

### Statistical analyses

As a first approach to conservatively test for the effects of habitat fragmentation on the reproductive variables studied we carried out a multivariate ANOVA (MANOVA). Two factors were considered: fragment size was taken as a fixed effect whereas focal individual conspecific density was included in the model as a random effect nested within fragment size. Fragments were classified in three size categories, small, medium, and large (fragments A–B, C–D, and E–F respectively; Table 1), thus transforming the fragmentation gradient in one factor with three levels. We verified the homogeneity of variance-covariance matrices and that residuals from the MANOVA were normally distributed. The MANOVA was followed by univariate ANOVAs to explore which variables contributed to significant MANOVA effects.

Linear regression analyses were carried out as a subsequent analysis to detect for possible relationships between the forest

**Table 1** Gradient of fragment sizes of a Chaco Serrano forest in central Argentina

| Forest fragment | Area (ha) | Distance to nearest forest fragment (km) | Matrix characteristic |
|---|---|---|---|
| A | 0.40 | 0.5 | Soybean crop and highway verge |
| B | 1.20 | 0.7 | Soybean crop |
| C | 5.30 | 0.5 | Soybean crop and road verge |
| D | 13.60 | 0.6 | Corn and soybean crops |
| E | 92.10 | 0.5 | Corn crop and highway verge |
| F | >300 | – | Natural reserve |

fragmentation size gradient and male and female reproductive traits measured. Dependent variables were log- and arcsin-transformed prior to analyses in order to meet normality and homoscedasticity.

The coefficient of variation (CV) was calculated for the overall (i.e. male and female) reproductive success index for each population in order to evaluate the (un)certainty of pollination and reproductive success within and among the fragmented populations studied (Oostermeijer et al. 1998). Subsequently, Pearson moment correlations were used to check if forest fragment area had an effect on population CVs of overall reproductive success. The statistical program SPSS (1999) was used for the analyses.

## Results

Conspecific densities of focal plant individuals ranged from 0 to 3 individuals in a 3-m radius. This naturally occurring range of densities of focal plants was found at most of the forest fragments studied.

Plant individuals produced similar amounts of pollen grains per flower regardless of the size of the fragment they grew in and their particular conspecific density (univariate ANOVA results $F$ =5.23, $P$ =0.24; $F$ =2.01; $P$ =0.47, respectively). The same occurred for the numbers of ovules per flower, which were neither significantly different at any of the forest fragments nor at any focal individual conspecific density ($F$ =1.12, $P$ =0.78; $F$ =3.44; $P$ =0.55, respectively). On average, each flower produces 20,454±386 pollen grains and 12.5±1.14 ovules.

Results of the MANOVA showed that reproductive variables in *C. parqui* are significantly affected by forest fragmentation but not by focal individual conspecific density (Table 2). Results from each univariate ANOVA indicate that forest fragmentation has particular significant negative effects on pollen loads on the stigma, pollen tubes in the style, seed-set, and on the overall reproductive success of the studied populations of *C. parqui* (Table 3). In contrast, pollen removal and fruit-set were not significantly different among the three forest fragment categories established for this analysis (Table 3). Univariate ANOVAs confirmed that focal individual conspecific density does not affect any of the reproductive variables measured. Thus, this source of variation was excluded from the subsequent analyses.

Linear regression analyses with replicates were subsequently carried out, taking the mean value of each individual as a replicate at each forest fragment. Therefore, we were able to partition total sums of square and test for deviations from linearity (Zar 1984). In all cases, deviations from linearity were not significant thus we pooled these with the error mean square. Linear regression analyses showed that forest fragment area had significant effects on pollen loads, pollen tubes, seed-set, and on the overall (i.e. male and female) reproductive success (Table 4). Pollen removal and fruit-set were not significantly affected by forest fragmentation (Table 4).

Logarithmic regression models were used to plot the relationship between each back-transformed male and female reproductive variable, which were previously found to be significantly affected by the fragmentation size gradient (Fig. 1A–D). This model allows one to detect the critical threshold at which the reduction in size of forest fragments begins to affect the performance of each reproductive variable. It can be observed that the amount of pollen grains on stigmas seems to strongly begin to decrease after the 92-ha forest fragment (Fig. 1A). On the other hand, the number of pollen tubes in the style and seed-set present comparable values all along the three larger fragments. After the ~14-ha fragment, however, these values begin to drop strikingly (Fig. 1B, C). The overall plant reproductive success index clearly reveals that *C. parqui* is susceptible to drastic decreases in sexual reproduction if growing in forest patches of 5 ha or less (Fig. 1D). The lower limit of fragment size from which overall reproduction for this species is highly affected is somewhere between fragment areas of ~14 and 5 ha (Fig. 1D).

Coefficient of variation of overall reproductive success index for each population proved to be negatively correlated with fragment size (Fig. 2). That is, intra-population (plant-to-plant) variation of male and female reproductive variables is higher in small forest fragments and it decreases as forest fragments increase in size.

## Discussion

The results obtained clearly indicate that forest fragmentation (namely the reduction in size and isolation of formerly continuous forest) has a strong negative effect on both male and female reproductive success of *C. parqui*. There are several possible reasons for these results.

The significantly lower amount of pollen grains on stigmas of *C. parqui* in small fragments may be reflecting a decreased number of visits by sphingid moths compared to large fragments, which results in reduced pollination quantity. Animal-pollinated plants located in small, isolated populations are more likely to be pollination- or pollen-limited than plants in larger populations, as has been observed by several authors (e.g. Aizen and Feinsinger 1994a; Ågren 1996; Ghazoul et al. 1998; Morgan 1999; Jacquemyn et al. 2002; Wilcock and Neiland 2002; Fuchs et al. 2003; Quesada et al. 2003). This is mostly because when forest fragments are too small

**Table 2** Results of a nested multiple ANOVA evaluating the effects of forest fragmentation on male (pollen removal, pollen loads, pollen tubes), female (fruit- and seed-set), and overall reproductive success (*ORS*). Focal individual conspecific density is the nested factor

| Effect | *df* | Wilks' λ | *F* | *P* -level |
|---|---|---|---|---|
| Forest fragment size | 12, 48 | 0.117 | 10.06 | 0.0002 |
| Conspecific density (Forest fragment size) | 54, 126 | 0.192 | 0.586 | 0.716 |

**Table 3** Results of univariate ANOVAs ($F$ -ratios) of forest fragments and conspecific density effects

| Effect | *df* | Pollen removal | Pollen load | Pollen tubes | Fruit-set | Seed-set | ORS |
|---|---|---|---|---|---|---|---|
| Forest fragment size | 2 | 0.514 | 14.941** | 29.356** | 0.058 | 8.907** | 3.371** |
| Conspecific density (Forest fragment size) | 9 | 0.645 | 0.675 | 0.394 | 0.445 | 0.275 | 0.497 |
| Error | 29 | | | | | | |

** $P$ <0.01

and isolated from other conspecific populations, pollinators may find it difficult to locate them or may not be sufficiently attracted to even make a visit (e.g. Ågren 1996; Groom 1998). The potential ability of a particular pollinator to move among fragments will in turn depend on its natural capability to fly long distances and on its behavioural response to habitat disruption (reviewed by White et al. 2002). In this regard, some studies, including the species-specific wasp pollinator system (Nason et al. 1998), have shown extensive pollen flow into fragmented populations due to the ability of certain animal vectors to move between isolated fragments, thus counteracting the presumed effects of physical isolation (Nason and Hamrick 1997; White et al. 2002). Nocturnal sphingid moths are known to fly relatively large distances and could therefore connect distant populations (e.g. Cascante et al. 2002). Nevertheless, the flying capability of a pollinator itself does not guarantee the successful arrival at a small, isolated forest fragment. The foraging patterns and flight behaviour of the pollinators can also be affected by habitat fragmentation with consequences for the quantity and quality of pollination (e.g. Aizen and Feinsinger 1994a; Didham 1996; Wilcock and Neiland 2002). Several works have demonstrated that forest fragmentation may change the behaviour of long-distance flying pollinators by inhibiting or restricting their travel potential (e.g. Powell and Powell 1987; Didham 1996). An extreme case was observed in isolated populations of *Banksia goodii*, where complete pollination failure was proved to occur because the bird pollinators, which were able to connect populations, avoided the smallest isolated forest patches that were less rewarding to visit (Lamont et al. 1993).

Moreover, habitat fragmentation usually alters the local assemblage of pollinators through reduction in the richness and abundance and/or replacement of pollinator species (e.g. Aizen and Feinsinger 1994a, 1994b; Murcia 1996). As a consequence, other non-specific generalist pollinators may be more abundant in smaller fragments and therefore affect pollination quality (Murcia 1996). These pollinators are typically more ineffective in transferring conspecific pollen, and would mostly transfer either heterospecific or self-pollen loads to stigmas. The quality of pollen transferred is crucial for self-incompatible plants. Thus, such inefficient pollination is likely to have particularly strong effects in *C. parqui* due to its self-incompatible system, as denoted by the lower number of pollen tubes and seeds found in flowers and fruits from smaller fragments. High diurnal frequency of flower visits of the introduced honey bees (*Apis mellifera*) in the studied forest fragments (R. Aguilar, personal observation) may

**Table 4** Linear regression analyses with replication of male and female reproductive variables in relation to forest fragment size (FFS). Fitted parameter values and ANOVA on regression are given. In all cases tests of deviations from linearity were not significant, thus residual MS are shown

| Variable | Parameter | *t* | *P* | ANOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Source | *df* | MS | *F* | *P* | $R^2$ |
| Log pollen removal | | | | | | | | | |
| Intercept | 9.78 | 173.2 | <0.001 | Regression | 1 | 0.02 | 1.23 | 0.15 | 0.08 |
| FFS | 0.27 | 1.47 | 0.15 | Residual | 39 | 0.01 | | | |
| Log pollen load on the stigma | | | | | | | | | |
| Intercept | 2.53 | 30.27 | <0.001 | Regression | 1 | 0.52 | 14.13 | 0.001 | 0.54 |
| FFS | 0.73 | 3.76 | <0.001 | Residual | 39 | 0.14 | | | |
| Log pollen tubes in the style | | | | | | | | | |
| Intercept | 2.36 | 25.47 | <0.001 | Regression | 1 | 1.35 | 17.78 | 0.001 | 0.68 |
| FFS | 0.85 | 4.22 | <0.001 | Residual | 39 | 0.08 | | | |
| Arcsin fruit set | | | | | | | | | |
| Intercept | 0.75 | 45.04 | 0.001 | Regression | 1 | 0.12 | 3.06 | 0.475 | 0.05 |
| FFS | 0.43 | 2.47 | 0.475 | Residual | 39 | 0.08 | | | |
| Arcsin seed set | | | | | | | | | |
| Intercept | 2.08 | 34.3 | 0.001 | Regression | 1 | 0.19 | 11.98 | 0.001 | 0.31 |
| FFS | 0.67 | 4.83 | 0.001 | Residual | 39 | 0.06 | | | |
| Arcsin overall reproductive success | | | | | | | | | |
| Intercept | 1.74 | 21.57 | 0.001 | Regression | 1 | 0.14 | 5.94 | 0.001 | 0.49 |
| FFS | 0.54 | 3.38 | <0.001 | Residual | 39 | 0.02 | | | |

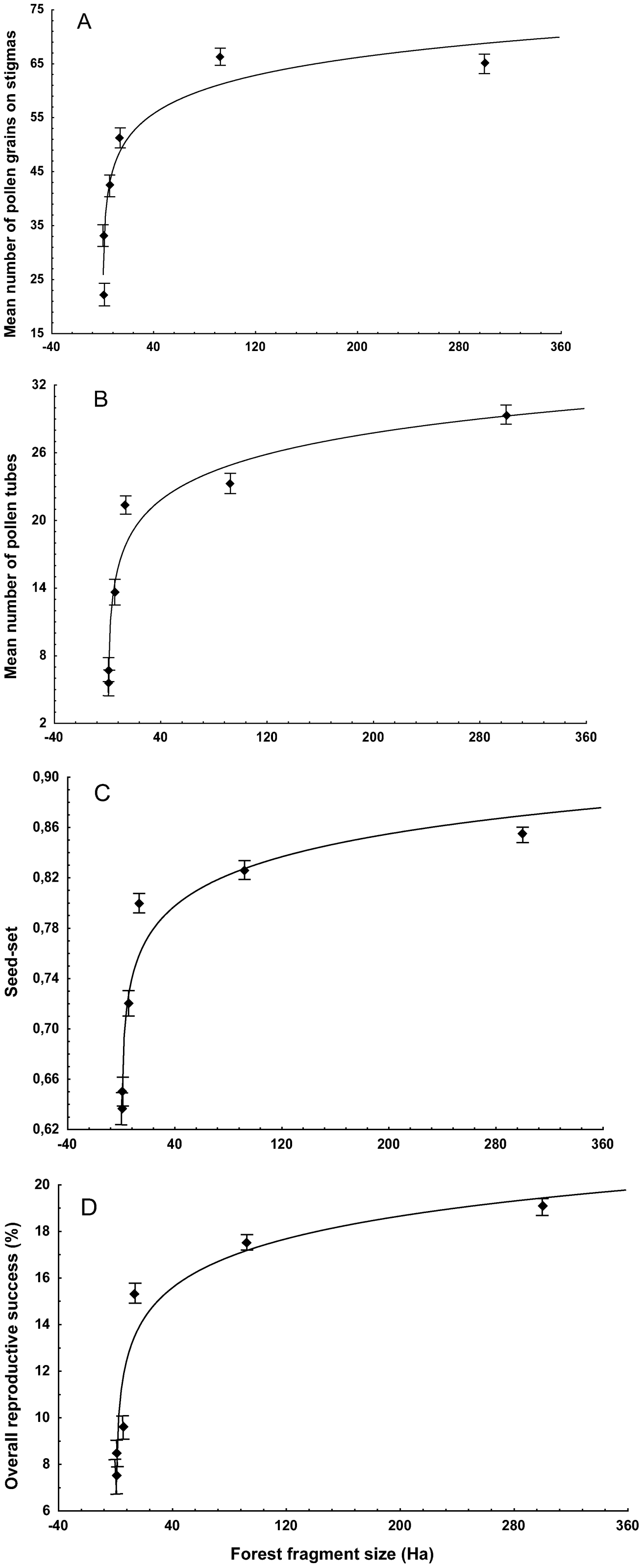

**Fig. 1** Relationships between forest fragment size and mean (±1 SE) pollen loads (**A**), pollen tubes (**B**), seed-set (**C**), and overall reproductive success (**D**). Logarithmic fit significant at $P < 0.05$

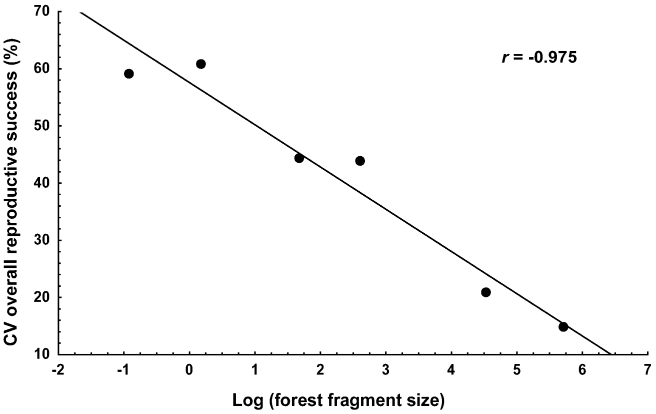

**Fig. 2** Coefficient of variation (*CV*) of overall reproductive success index and log-transformed forest fragment area. The Pearson correlation coefficient ($r$) significant at $P < 0.05$ is shown

account for the low-quality pollination observed, as has been previously reported in north-western regions of the Chaco Serrano forest, where honey bees' visits to flowers tended to increase and those of native insects to decrease in small forest fragments (Aizen and Feinsinger 1994b). Accordingly, flowers of *C. parqui* presented similar amounts of pollen removed from the anthers in all the studied fragments but different pollen loads on stigmas. This indicates that a higher proportion of pollen loss occurred in small fragments due either to inefficient pollen transfer to stigmas mediated by generalist pollinators or to a higher presence of pollen robbers which could have masked effective lower pollen removal in small fragments.

In addition to the lower mean values of most reproductive variables found in smaller forest fragments, the observed higher plant-to-plant variability in the overall reproductive success index in smaller fragments (Fig. 2), implies that there is higher uncertainty of successful reproduction in these populations, which as previously pointed out, may be due to reduced pollination efficiency. Therefore, it seems plausible to suggest that the reduction in seed production in *C. parqui* was mainly due to decreases in both the quality and quantity of pollination in small forest fragments.

On the other hand, focal individual conspecific density had no significant effects on any of the reproductive variables measured, i.e. within the same forest fragment, focal individuals with low conspecific densities presented similar reproductive output as individuals with high conspecific densities. This implies that for any given plant individual of *C. parqui* growing in the studied populations, the effects of forest fragmentation are not ameliorated, compensated, nor strengthened in any way by its particular conspecific density within the 3-m-radius covered in this study. Yet, we can not discard the possibility that density effects at this radius may not represent the actual intra-population pollen flow and, consequently, conspecific densities taken at larger distances may show different results.

In hermaphroditic plants, fitness is obtained via female and male functions, each of them supplying, on average, half of the plant's total fitness (Bertin 1988). Both functions contribute equally to the next generation; that is, there is a capability for genetic transmission through seeds as well as through pollen (Bertin 1988; Stanton et al. 1992; Queller 1997). Moreover, natural selection on plant reproductive traits may act differently through female and male function, and depending on the direction of selection it will tend to maximize either female or male mating success (Campbell 1989). This highlights the important roles of both female and male fitness components for the evolution of floral traits and other reproductive characters in hermaphroditic plants (e.g. Stephenson and Bertin 1983; Campbell 1989). In this paper, we observed that female and male function of flowers of *C. parqui* were equally affected and showed a similar negative trend toward habitat fragmentation. Pollen removal was the only male trait that did not present changes throughout the fragmentation gradient. This was probably due to the higher presence of pollen robbers and/or generalist pollinators in small forest fragments (see above). Similarly, fruit-set, a reproductive trait widely used to assess plant reproductive success, did not significantly decrease with increasing fragmentation. Although lower pollen loads in small fragments yielded reduced seed number per fruit, such small pollen loads were yet sufficient to trigger comparable proportions of fruit production throughout the fragments. In this regard, if we had only taken fruit-set and/or pollen removal as response variables, we might have arrived at the misleading conclusion that sexual reproduction in *C. parqui* is not affected by forest fragmentation. Our results emphasize the importance of evaluating both components of the total plant fitness, as well including simultaneously several aspects of pollination and reproduction.

Disturbance thresholds are relevant at different scales, from the maintenance of genetic diversity (Ouborg et al. 1991) to population and metapopulation viability (Gilpin and Soulé 1986). In this context, habitat fragmentation research could make major contributions to forest management. As Boutin and Hebert (2002) pointed out, in order to make such a contribution, the focus of the research should be the study of threshold effects in habitat fragmentation on species persistence. Once the threshold ranges have been identified, these could then be used in landscape protection models to produce future landscapes that maintain compositional, structural, and functional biodiversity typical of the region. The effects of fragmentation on male and female reproductive variables of *C. parqui* became evident beyond a particular fragment size, indicating the existence of a fragmentation threshold below which the effects are clearly revealed. In the fragmentation gradient studied here, this threshold is somewhere between fragments of 5 and ~14 ha. Thus, plant populations of *C. parqui* growing in such forest fragments may be exposed to a higher risk of extinction.

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