

Pollen limitation, fruit abortion, and autonomous selfing in three populations of the perennial herb *Ruellia nudiflora*

LUIS ABDALA-ROBERTS,* DENIS MARRUFO-ZAPATA,† GERARDO ARCEO-GÓMEZ‡ and VICTOR PARRA-TABLA†

*Department of Ecology and Evolutionary Biology, University of California, Irvine, California, ‡Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; and †Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, México

Abstract

Multiple factors determine plant reproductive success and their influence may vary spatially. This study addresses several factors influencing female reproductive success in three populations of *Ruellia nudiflora*, specifically we: (i) determine if fruit set is pollen-limited and if pollinator visitation rates are related to this condition; (ii) estimate fruit set via autonomous self-pollination (AS) and relate it to the magnitude of herkogamy; and (iii) evaluate if fruit abortion is a post-pollination mechanism that determines the magnitude of pollen limitation. At each site we marked 35 plants, grouped as: unmanipulated control (C) plants subjected to open pollination, plants manually cross-pollinated (MP), and plants excluded from pollinators and only able to self-pollinate autonomously (AS). Fruit set was greater for MP relative to C plants providing evidence for pollen limitation, while a tendency was observed for lower fruit abortion of MP relative to C plants suggesting that fruit set is influenced not only by pollen delivery *per se*, but also by subsequent abortion. In addition, although pollinator visits varied significantly among populations, the magnitude of pollen limitation did not, suggesting that pollinator activity was not relevant in determining pollen limitation. Finally, fruit set tended to decrease with the degree of herkogamy for AS plants, but this result was inconclusive. These findings have contributed to identify which factors influence reproductive success in populations of *R. nudiflora*, with potentially relevant implications for population genetic structure and mating system evolution of this species.

Keywords: herkogamy, pollen limitation, pollinators, reproductive assurance, *Ruellia nudiflora*.

Received 31 January 2012; revision received 16 April 2012; accepted 18 May 2012

Introduction

Plant reproductive success is determined by intrinsic characteristics of the reproductive system of a species, as well as by extrinsic biotic and abiotic factors. On the one hand, it is widely recognized that abiotic factors such as light or nutrient availability can have strong effects on plant reproductive success (e.g. Herrera 1995; Kilkenny & Galloway 2008), and the same holds true for biotic factors such as plant antagonists and mutualists (Strauss & Zangerl 2002). On the other hand, characteristics of a plant's reproductive system such as sexual expression, self-incompatibility, mating strategies, the occurrence of

autonomous self-pollination and flower traits are all key features that influence plant reproductive success (Wiens 1984; Barrett & Harder 1996; Eckert *et al.* 2006; Harder & Routley 2006).

Pollen limitation, the degree to which pollen supplementation increases fruit or seed set relative to natural pollination (Ashman *et al.* 2004; Knight *et al.* 2005), is a common factor influencing plant reproductive success in natural populations and may be modulated by extrinsic factors as well as plant reproductive traits. For instance, many studies have shown that nutrient availability and pollinator abundance can have an impact on pollen limitation (e.g. Campbell & Halama 1993; Brookes *et al.* 2008). Moreover, the quality and quantity of pollen received may result in selective fruit abortion in order to reallocate

Correspondence: Victor Parra-Tabla
Email: ptabla@uady.mx

resources to other flowers and fruits (Stephenson 1981; Burd 1988; Parra-Tabla *et al.* 1998; Banuelos & Obeso 2005), thus influencing the degree of pollen limitation. Independently of extrinsic factors, however, several traits of the plant's reproductive system are thought to have evolved to reduce the negative effects of pollen limitation. For example, autonomous self-pollination (hereafter AS), a fairly common condition in angiosperms (Schoen *et al.* 1996), can occur when anthers come into contact with the stigma of the same flower through some mechanical process (see studies in Wang *et al.* 2005), and is assumed to provide reproductive assurance when pollinators are scarce or unpredictable (Eckert *et al.* 2006). In addition, floral traits such as herkogamy (i.e. distance between anthers and stigma), may reduce reproductive success via AS by decreasing the efficiency with which self-pollen is delivered to the stigma (e.g. amount of pollen deposited; Vaughton *et al.* 2008) and thus influence the degree to which AS contributes to reproductive assurance, as well as pollen limitation.

Extrinsic factors and plant reproductive traits may vary spatially, affecting plant reproductive success differently across sites, with this having relevant ecological and evolutionary consequences across a species' distribution range (Barrett *et al.* 2001). For example, it has been shown that geographic variation in the degree of herkogamy can affect outcrossing rates and the genetic structure of populations (e.g. Herlihy & Eckert 2004; Medrano *et al.* 2005; Takebayashi *et al.* 2006). On the other hand, spatial variation in pollinator abundance and diversity (Gomez *et al.* 2007) as well as in resource availability can lead to spatial differences in the magnitude of pollen limitation (Knight *et al.* 2005). Hence, a multipopulation approach can provide a more robust understanding of how reproductive traits and extrinsic factors interact to affect plant fitness differentially across sites, as well as shed light on ecological factors responsible for spatial variation in the outcomes of selection (Moeller & Geber 2005; Vanhoenacker *et al.* 2006; Parra-Tabla & Herrera 2010).

Ruellia nudiflora (Acanthaceae) is a self-compatible perennial herb with a broad distribution throughout the state of Yucatan, Mexico. It has a mixed reproductive system as it produces chasmogamous (hereafter CH) and cleistogamous (hereafter CL) flowers, the latter of which obligately self-pollinate. In addition, CH flowers frequently exhibit delayed AS (in the absence of pollinators, ca 70% of flowers set fruit via this mechanism; Abdala-Roberts *et al.* 2009) and exhibit approach herkogamy (i.e. stigma above the level of anthers). Although AS is likely a relevant mechanism for reproductive assurance and could reduce pollen limitation in this species, it is also possible that the quantity and/or quality of pollen delivered to the stigma through this mechanism are not sufficient for successful fruit formation (Pflugshaupt *et al.* 2002; Vaughton

et al. 2008). Moreover, the degree of herkogamy may influence the efficiency of pollen deposition and thus fruit set via AS, with this effect potentially varying across populations based on the degree of herkogamy. In addition, high rates of fruit abortion have been observed in some populations of *R. nudiflora* (from 20 to 30%; Carbajal-Borges P, 2008, unpubl. data) which may allow plants to reallocate resources to flowers that have received greater pollen loads or pollen of higher quality (e.g. allogamous; Charlesworth & Charlesworth 1987). Accordingly, the contribution of AS to reproductive assurance and the strength of pollen limitation measured as fruit set in this species may be contingent upon fruit abortion.

Our first goal was to determine if *R. nudiflora* is pollen-limited and if this condition varies in magnitude across populations. Although this species is highly dependent on autonomous pollination, we expected that manipulating pollen quality and/or quantity would alter the degree of pollen limitation and female reproductive success. In addition, we expected that *R. nudiflora* populations with greater pollinator visitation rates would be less pollen-limited. Our second goal was to determine if fruit abortion is a mechanism that governs the magnitude of pollen limitation. We predicted that greater loads of allogamous pollen achieved through manual cross-pollination (simulating high end of pollen load sizes received by flowers of this species) would result in a lower abortion rate, while AS would lead to higher abortion given that this mechanism results in the deposition of smaller loads of self-pollen (Wang *et al.* 2004; Vaughton *et al.* 2008). Here we define self-pollen as being of lower quality relative to allogamous pollen because the former may incur costs of inbreeding (although predictions of outcrossing effects on inbreeding may vary; see Takebayashi & Delph 2000). Our third goal was to measure fruit set via AS and test for a relationship between herkogamy and fruit set from AS. We predicted a negative relationship given that greater anther–stigma separation would result in a lower efficiency of pollen deposition from autonomous self-pollination, either at the moment of anther dehiscence or when the corolla falls upon floral senescence (with anthers coming into contact with the stigma). Our study represents a significant contribution to studies on pollination ecology because it provides a simultaneous evaluation of multiple factors influencing plant reproductive success. Furthermore, by using a multipopulation approach it will advance our understanding on how the individual contributions of reproductive traits and extrinsic factors shape plant reproductive success across sites.

Methods

Study species

Ruellia nudiflora Englem. and Gray Urb. (Acanthaceae) is a perennial herb that measures 20–30 cm in height, and is

distributed from Texas to southeast Mexico (Standley *et al.* 1974; Tripp 2007). It is found mostly in disturbed open areas (Tripp 2007) and has a wide distribution throughout the state of Yucatan (Mexico). It produces CH flowers which have an average lifespan of 10 hours and are visited by bee species, such as *Apis mellifera* and *Trigona fulviventris*, as well as butterfly species, of which one of the most common is *Microtia elva*. The CH flowers are self-compatible, herkogamous (Marrufo-Zapata 2011), and exhibit delayed AS during corolla dehiscence because stamens are fused to the corolla and anthers come into contact with the stigma when the corolla falls off the plant (Abdala-Roberts *et al.* 2009). Pollen may also come into contact with the stigma upon anther dehiscence if herkogamy is low. In addition, this species also produces closed CL flowers that obligately self-pollinate. Fruits from both flower types are dry and dehiscent, with CL and CH fruits producing 9.44 ± 0.02 (mean and SE) and 13.27 ± 0.16 seeds, respectively (Abdala-Roberts *L*, unpubl.). A previous study showed reduced phenological overlap between these two reproductive functions, with CL flower production being greater before and after the peak of CH flower production (Munguía-Rosas *et al.* 2012). Although likely varying during ontogeny, fruit production of adult plants is CL-skewed (Munguía-Rosas *et al.* 2012)

Study sites and pollination experiment

We used three populations of *R. nudiflora* found in the state of Yucatan (southeast Mexico): Sinanche in the north ($21^{\circ}13'25.25''\text{N}$, $89^{\circ}11'27.79''\text{W}$), Xmatkuil in the center ($20^{\circ}51'59.07''\text{N}$, $89^{\circ}37'28.43''\text{W}$) and Hobonil in the south of the state ($20^{\circ}1'1.49''\text{N}$, $89^{\circ}1'10.19''\text{W}$). Distance between sites ranged from 60 to 112 km. Previous work with *R. nudiflora* at the study area has grouped populations in Yucatan into three regions with contrasting levels of herbivory and climatic conditions (see Ortegón-Campos *et al.* 2009; Abdala-Roberts *et al.* 2010). Accordingly, preliminary data indicated that these regions exhibited differences in pollinator abundance (mean = 0.346 ± 0.092 [SD] to 0.758 ± 0.089 visits per minute) and herkogamy (0.90 ± 0.68 to 2.53 ± 0.68 mm) (Marrufo-Zapata 2011). Thus, for the present study we a priori selected one population that was representative (closest to mean value) of each region based on climatic, herkogamy, and pollinator abundance levels. Within each population, in June 2009, we selected 35 plants that were similar in size (height and leaf number) and flower number (based on visual counts) as well as separated by a distance of at least 2 m. On day 1 (first week of July 2009), for each plant we marked five CH flower buds using cotton thread and entire plants were randomly assigned to one of three treatment groups: control or open pollination (C = 10 plants), autonomous

self-pollination (AS = 15 plants), and manual pollination (MP = 10 plants) (n per population = 35 plants). Flower buds were randomly chosen within each plant and in most cases plants did not have more than five buds at a given time. The next morning (day 2), between 0700 and 0730 hours, we performed the following manipulations on recently open marked flowers: AS plants were covered with pollinator enclosures (ca 30 cm diameter by 40 cm high cylinder made of nylon tulle mesh and wire) and marked flowers were left untouched. Marked flowers of MP plants were emasculated (prior to anther dehiscence) and subsequently pollinated with a mixture of pollen from five non-experimental plants of the same population; after being pollinated, MP plants were covered with the same type of enclosure cage. Given that MP plants remained with enclosures instead of being subject to subsequent open pollination events (as is commonly reported in pollen limitation studies, i.e. 'supplemental' pollen treatment), we consider that this treatment represented a conservative measure of pollen limitation. Finally, marked flowers of C plants were not manipulated and plants were free of enclosures and exposed to open pollination. Although C plants could also autonomously self-pollinate, we considered that this would be the adequate treatment to use as control, as these plants would reflect natural levels of fruit set, which would necessarily have to take into account AS. The following morning (day 3), enclosures were removed from MP and AS plants. The effect of the enclosure *per se* on reproduction was assumed to be negligible given the short period of time plants were covered (24 hours). In the case of AS plants, we also measured herkogamy (in mm) for three flowers (different from those marked for the pollination experiment) using a digital caliper.

Each population was visited on days 4 and 11 to record the following: (i) number of initiated fruits (on day 4: these represent young fruits with seeds that are not fully elongated) and (ii) number of mature fruits (on day 11: these fruits are fully elongated and dry). Initial fruit set was then calculated as the number of initiated fruits per plant/number of flower buds marked per plant, while final fruit set was the number of mature fruits/number of buds marked. The difference between initiated and mature fruits for each plant was used to obtain the proportion of aborted fruits calculated as: (number of initiated fruits – number of mature fruits)/number of initiated fruits (i.e. percent of initiated fruits that were aborted).

For all statistical analyses we used Proc GLIMMIX in SAS version 9.1 (SAS Institute 2002, Cary, NC) and included population (fixed, three levels), pollination treatment (fixed, three levels), and their interaction as main effects in the models. Data for final fruit set and proportion of aborted fruits met the assumptions of homogeneity of variance and normality. For initial fruit set, however,

we used a generalized linear model that assumed a gamma error distribution with a log link function given that the residuals were not normally distributed even after transformation. When main effects were significant, we used the LSMEANS statement (for estimation of least-square means) to test for mean differences between levels of a given effect. Additionally, using only AS plants we performed an ANCOVA in Proc GLIMMIX to test for a relationship between average herkogamy (covariate) and final fruit set (response variable) including population as a main effect; a preliminary analysis showed that the covariate did not interact significantly with the population effect ($F_{2,37} = 1.14$, $P = 0.33$) based on which this interaction term was removed from the model. We also tested if populations differed in average herkogamy using a one-way ANOVA with population as main effect. Residuals for both the ANCOVA and one-way ANOVA were normally distributed. Except indicated otherwise, for final fruit set and abortion we present least-square means and standard errors as descriptive statistics, while for initial fruit set we present back-transformed least-square means and 95% confidence limits given that this model used the log link function (gamma distribution).

Pollinator visitation

During the second week of July 2009 (i.e. flowering peak), we conducted observations of pollinator visitation rates to individual plants at each population. Observations were carried out from 0900 to 1100 hours when pollinators are most abundant using three to four 15-m transects within each population, along which every *R. nudiflora* plant encountered on the transect was observed for 2 min to record flower visitations ($n = 47$ plants per population); a distance of 2 m from the plant was found to be adequate for pollinator observation based on previous surveys. We recorded only visitors that came into contact with reproductive flower parts. For each sampled plant we also counted the total number of open flowers. Pollinator visitation rate was calculated for each plant as the total number of visitations per minute. Given that ca 60% of the floral visitations recorded corresponded to Hymenoptera, we conducted analysis for total visitation rate and hymenopteran visitation rate separately.

Generalized linear models were used in Proc GLIMMIX (SAS version 9.1), for which the response variables were total visitation rate per plant, per minute and hymenopteran visitation rate per plant, per minute. The main effect in both cases was population (fixed, three levels), while number of open flowers per plant was used as covariate. Previously, residuals showed a significant deviation from normality even after transformation. Thus, for both models we assumed an exponential error distribution which showed an adequate fit to the data, using the

log link function. When the population effect was significant, we tested for mean differences between sites using the LSMEANS statement and report Bonferroni-corrected P -values. Back-transformed least-square means and 95% confidence limits are reported as descriptive statistics.

Results

Initial fruit set

The proportion of flowers initially setting fruit was high (0.87 ± 0.02 ; raw mean \pm SE). Results from the generalized linear model indicated a significant effect of pollination treatment ($F_{2,94} = 4.03$, $P = 0.02$), for which only the MP group (0.89 [0.74 , 1.04]; back-transformed least-square mean and confidence limits) differed significantly from the C group (0.60 [0.50 , 0.74]) ($t = 2.82$, $DF = 94$, $P = 0.01$); the AS group showed an intermediate value (0.70 [0.60 , 0.83]) and did not differ from the first two ($t \leq 1.85$, $DF = 94$, $P \geq 0.07$). In addition, the effect of population was also significant ($F_{2,94} = 12.65$, $P < 0.0001$), with Sinanche having the highest mean value (0.95 [0.79 , 1.12]), differing significantly from Xmatkuil which had the lowest mean (0.51 [0.42 , 0.61]) ($t = 4.97$, $DF = 94$, $P < 0.0001$), but not from Hobonil, which showed an intermediate value (0.77 [0.64 , 0.93]) ($t = 1.76$, $DF = 94$, $P = 0.24$); Xmatkuil and Hobonil also differed significantly ($t = 3.14$, $DF = 94$, $P = 0.006$). The treatment \times population interaction was not significant ($F_{4,94} = 1.57$, $P = 0.19$) as the magnitude of difference among pollination treatment level means was similar across sites.

Proportion of aborted fruits

The overall mean proportion of aborted fruits was 0.37 ± 0.03 (raw mean and SE). The generalized linear model indicated a significant effect of treatment ($F_{2,94} = 20.81$, $P < 0.0001$); the greatest mean value was for the AS treatment (0.55 ± 0.04), differing significantly from the C treatment (0.28 ± 0.05) and the MP treatment (0.17 ± 0.04) ($t \geq 4.34$, $DF = 94$, $P < 0.0001$; Fig. 1); the MP treatment showed the lowest mean value, although no detectable difference was found relative to the open pollination (C) treatment (marginal difference: $t = 1.67$, $DF = 94$, $P = 0.09$; Fig. 1). The population (Xmatkuil: 0.31 ± 0.05 ; Sinanche: 0.41 ± 0.05 ; Hobonil: 0.37 ± 0.04) and treatment \times population effects were not significant ($F_{2,94} = 0.85$, $P = 0.43$ and $F_{4,94} = 0.57$, $P = 0.68$, respectively).

Final fruit set

The overall mean for final fruit set was $0.56 (\pm 0.03$ [SE]), much lower than the overall mean for initial fruit set. The general linear model showed a strong effect of treatment

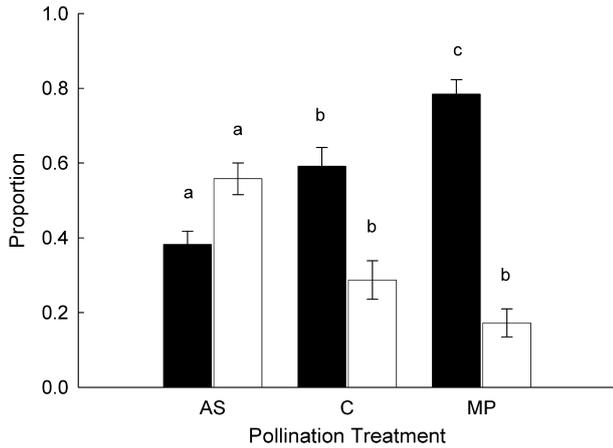


Fig. 1 Least-square mean (\pm SE) values for final fruit set and proportion of aborted fruits of *R. nudiflora* plants subjected to each pollination treatment: AS, autonomous self-pollination; C, control or open pollination; MP, manual cross-pollination. Different letters indicate significant differences ($P < 0.05$) among treatment level means for a given response variable. ■, final fruit set; □, fruits aborted.

($F_{2,94} = 24.36$, $P < 0.0001$), for which all three treatment levels differed significantly ($P \leq 0.002$) describing the opposite trend relative to fruit abortion; the highest mean value was observed for the MP treatment (0.78 ± 0.04), followed by the C treatment (0.59 ± 0.05), and finally the AS treatment (0.38 ± 0.04) which had the lowest fruit set (Fig. 1). On the other hand, the population (Xmatkuil: 0.53 ± 0.05 ; Sinanche: 0.57 ± 0.05 , Hobonil: 0.56 ± 0.05) and treatment \times population effects were not significant ($F_{2,94} = 0.38$, $P = 0.68$ and $F_{4,94} = 0.62$, $P = 0.64$, respectively), the latter indicating that the observed differences in final fruit set among treatments were similar across populations (e.g. the magnitude of increase in fruit set from C to MP plants ranged from 1.3-fold to 1.4-fold among populations; Fig. 2).

Pollinator visitation rates

The generalized linear model revealed a significant population effect on total visitation rates ($F_{2,136} = 7.72$, $P = 0.0007$) for which the greatest average rate of total visitations was observed for Hobonil (2.81 visits [1.99, 3.98], back-transformed least-square mean and confidence limits) followed by Xmatkuil (1.77 [1.25, 2.51]) and Sinanche (1.65 [1.17, 2.34]) ($t \geq 2.78$, $DF = 136$, $P < 0.01$); the latter two did not differ significantly ($t = 0.93$, $DF = 136$, $P = 0.35$). Flower display size had a weak but significant positive effect on total visitation rates (visit rate = $0.012 \times \text{flowers} + 0.40$; $F_{1,136} = 3.86$, $P = 0.05$). In addition, results for hymenopteran visitation rates also showed significant differences between populations

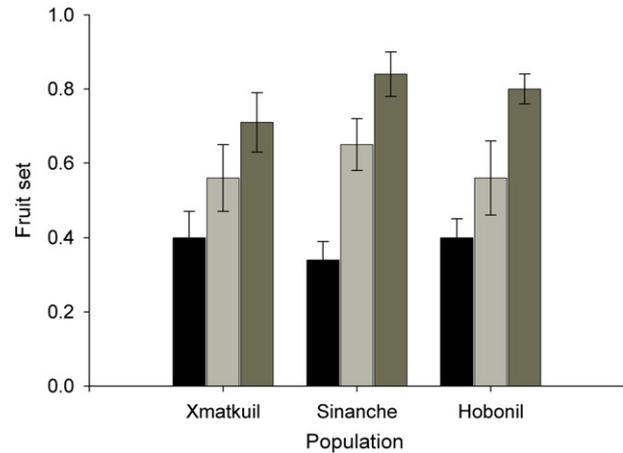


Fig. 2 Least-square means and SE for final fruit set of each pollination treatment (AS, autonomous self-pollination; C, open pollination; MP, manual cross-pollination) presented separately for each *R. nudiflora* population studied. ■, AS; □, C; ■, MP.

($F_{2,136} = 16.29$, $P < 0.0001$), as well as a significant effect of flower display size (visit rate = $0.008 \times \text{flowers} + 0.19$; $F_{1,136} = 8.44$, $P = 0.004$). In this case, however, Hobonil (1.69 [1.31, 2.18]) and Xmatkuil (1.66 [1.28, 2.18]) did not differ from each other ($t = 0.15$, $DF = 136$, $P > 0.9$) and showed a higher average visitation rate compared to Sinanche (1.20 [0.95, 1.58]) ($t \geq 4.96$, $DF = 136$, $P < 0.0001$).

Herkogamy and fruit set from autonomous self-pollination

We found that populations varied up to ninefold in the magnitude of herkogamy ($F_{2,42} = 167.56$, $P < 0.0001$), with all three populations differing significantly from each other in the following order: Hobonil had the highest average value (2.92 ± 0.11 mm), followed by Xmatkuil (1.09 ± 0.11) and finally Sinanche (0.32 ± 0.08). The ANCOVA revealed a negative, albeit non-significant, relationship between herkogamy and final fruit set (fruit set = $-0.16 \times \text{herkogamy} + 0.91$, $R^2 = 0.59$; $F_{1,39} = 3.16$, $P = 0.08$) (Fig. 3). The population effect on final fruit set was not significant (Xmatkuil: 0.40 ± 0.07 ; Sinanche: 0.34 ± 0.05 ; Hobonil: 0.43 ± 0.05 ; $F_{2,39} = 2.13$, $P = 0.13$), showing that the probability of setting fruit via AS did not vary among sites.

Discussion

Numerous studies have reported pollen limitation in plants (see studies in Burd 1994; Knight *et al.* 2005), which suggests that this phenomenon is common in natural plant populations. Accordingly, we detected this condition for the highly selfing *R. nudiflora*, which indicates that

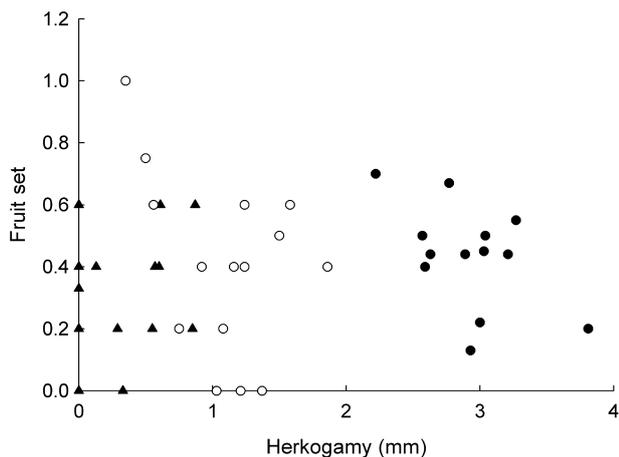


Fig. 3 Scatter plot representing the relationship between mean herkogamy (stigma–anther distance in mm) and final fruit set from autonomous self-pollination for *R. nudiflora* plants sampled in each population. ▲, Sinanche; ○, Xmatkuil; ●, Hobonil.

manual pollination, by increasing pollen quantity (larger load) and potentially also pollen quality (mixture of cross-pollen) may result in a significant increase in fruit set relative to control plants. Although greater fruit set for manually pollinated flowers may have resulted from resource reallocation from unmanipulated flowers and fruits (Zimmerman & Pyke 1988; Parra-Tabla *et al.* 1998; Knight *et al.* 2005), *R. nudiflora* typically produces between five and 10 flowers during the peak of the flowering season, which could have precluded short-term manipulation effects. It is also possible that increased resource allocation from cleistogamous to manually pollinated chasmogamous reproduction also could have influenced estimates of pollen limitation. However, previous data suggest that this factor may not be as relevant given that chasmogamous and cleistogamous reproduction exhibit limited phenological overlap (Munguía-Rosas *et al.* 2012). Regardless of these considerations, estimates of pollen limitation for multiple points during the flowering season as well as across seasons would be desirable in order to estimate the overall magnitude of pollen limitation and its net effect on lifetime fitness for this species (Ashman *et al.* 2004).

Spatial variation in pollinator visitation and pollen limitation

Although pollen limitation has been studied intensively over the past three decades, geographic variation for and the underlying factors influencing this condition have received less attention (Ashman *et al.* 2004; Knight *et al.* 2005; Alonso *et al.* 2010; Schuett & Vamosi 2010). Contrary to predictions, our results indicated that the magnitude of

pollen limitation (measured in terms of final fruit set) was consistent across populations. Although low statistical power may have limited the ability to detect a significant population by treatment interactions (β from 0.2 to 0.5 for initial and final fruit set; not presented in results), the magnitude of differences among pollination treatments was similar among populations (Fig. 2), suggesting that this null result would persist even if power was higher.

Even though the magnitude of pollen limitation did not vary spatially, pollinator visitation rates did differ significantly among sites (twice as high mean value for Hobonil relative to the other two populations), suggesting that pollinator abundance did not limit fruit set during the study season. This result is not surprising given that *R. nudiflora* is self-compatible and produces fruits autonomously. Accordingly, fruit set in this species may be more influenced by abiotic conditions such as resource availability (Ågren *et al.* 2008). However, the effect of pollinator service on fruit abortion rates via changes in pollen quality and quantity cannot be ruled out (see discussion ahead). Furthermore, pollinator visitation rates may vary considerably during the flowering season and among years as shown in previous studies (e.g. Gomez *et al.* 2007), warranting multiple surveys within and between seasons.

Fruit abortion as a modulator of pollen limitation and reproductive success

Ruellia nudiflora plants aborted more than one-third of the fruits they produced and the observed pattern was to some extent the opposite to that for final fruit set. Plants only being able to set fruit autonomously showed the highest mean value of proportion of aborted fruits, whereas manually pollinated plants showed the lowest mean value. Although we were unable to detect significant differences in fruit abortion between control and manually pollinated plants, the latter group did show a tendency for a lower mean value (28% and 17%, respectively). In addition, the difference in final fruit set between manually pollinated and control plants was more pronounced (32% greater for MP) compared to the difference in initial fruit set between these two treatments (17% greater for MP). These findings suggest that fruit abortion influenced the magnitude of pollen limitation in *R. nudiflora*. Indeed, previous studies have shown that if the quality or quantity of pollen delivered to stigmas are not optimal, resources may be reallocated to other fruits resulting in the abortion of fruits of lower quality (Stephenson 1981; Burd 1994). This was likely the case for fruits from autonomous self-pollination, which are more frequently aborted because they result from reduced pollen loads or because self-pollen is of low quality (Pflug-

shaupt *et al.* 2002). Further experiments with *R. nudiflora* will address the relative importance of pollen quantity and quality (Aizen & Harder 2007) by controlling each factor independently. It is important to note that other factors may have influenced fruit abortion rates in this study. In particular, a noctuid seed predator that attacks fruits of *R. nudiflora* (Abdala-Roberts *et al.* 2010) could have influenced abortion independently of our pollination treatments, thus warranting a further examination of seed predator effects within this context (Burkhardt *et al.* 2009).

Ecological and mating system implications of autonomous selfing and herkogamy

Previous field observations have shown high rates of self-pollination via autonomous self-pollination for chasmogamous flowers in *R. nudiflora* (Abdala-Roberts *et al.* 2009). Similarly, in this study initial fruit set for plants only being able to self-pollinate was close to 70%. However, once fruit abortion was taken into account, plants only being able to self-pollinate autonomously exhibited the lowest final fruit set of all three treatments (<40%). Despite such high abortion, autonomous self-pollination may still reduce the magnitude of pollen limitation in this species. Indeed, although ca 40% fruit set via autonomous selfing cannot be extrapolated as a direct contribution to fruit set of control plants (due to differences in resource allocation patterns between autonomous selfing and control plants, as the latter were subjected to both cross- and self-pollination), without autonomous self-pollination, differences in fruit set between control and manually pollinated plants would have been greater if some proportion of control flowers was not visited by pollinators. Having an emasculated open pollination treatment (in addition to an unmanipulated open pollination treatment) to compare with manually pollinated plants would be useful to test for pollen limitation in the absence of autonomous self-pollination and determine the importance of this reproductive mechanism for fitness of *R. nudiflora* (Herlihy & Eckert 2002; Vaughton *et al.* 2008).

Even though selfing is a common condition in *R. nudiflora*, genetic analyses have indicated elevated outcrossing rates ($t_m > 0.90$) and high genetic diversity values for populations of this species (Marrufo-Zapata 2011). Although this seems contradictory based on what we know about the reproductive system of *R. nudiflora*, it is likely that populations undergo two successive ecological filters that remove a large amount of the progeny from self-pollination. One occurs during fruit formation via fruit abortion, and the other may take place subsequently via reduced viability of seedlings from self-pollination, presumably due to inbreeding depression (Herlihy & Eckert 2002). Indeed, a previous study showed that cleistogamous seedlings have

a lower competitive ability relative to potentially outcrossed chasmogamous seedlings (Chalé-Uc 2011). Thus, the effects of pollen quality and quantity determined by the plant's mating system and pollinator service may act in concert with ecological filters to influence *R. nudiflora* population genetic diversity and structure.

Although the degree of herkogamy differed significantly among populations, we did not find differences among populations in final fruit set via autonomous self-pollination, suggesting no relationship between reproductive success through this mechanism and anther-stigma separation across sites. Although the ANCOVA showed a tendency for a negative effect of herkogamy on fruit set via autonomous self-pollination, this finding was inconclusive, thus warranting further research addressing the influence of herkogamy on reproductive success (Vaughton *et al.* 2008) and selfing rates (Barrett 2002) in *R. nudiflora*.

Conclusions

Our findings show spatially consistent pollen limitation across the *R. nudiflora* populations studied. Moreover, results suggest that pollen limitation was underlain by high levels of fruit abortion, which appear to be contingent on pollen quality and quantity. We also found that although autonomous self-pollination resulted in high levels of fruit abortion, it promotes reproductive assurance in this species. Our results emphasize that simultaneously studying reproductive traits and extrinsic factors is a necessary approach to achieve an integral understanding of the factors controlling plant reproductive success and mating patterns.

Acknowledgments

The authors would like to thank L. Loria who kindly provided logistic help and accommodation to conduct research at Sinanche, and D. Campbell and two anonymous reviewers who provided valuable comments on an earlier version of the manuscript. Thanks also to J. Erasles and J. Solorio for authorization to use the study sites at Hobonil and Xmatkuil, respectively. The Consejo Nacional de Ciencia y Tecnología provided the financial support to conduct this research as part of a grant awarded to V. Parra Tabla (SEP 2004-CO1-4658A/A1).

References

- Abdala-Roberts L., Parra-Tabla V., Salinas-Peba L., Diaz-Castelazo C. & Delfin-Gonzalez H. (2010) Spatial variation in the strength of a trophic cascade involving *Ruellia nudiflora* (Acanthaceae), an insect seed predator and associated parasitoid fauna in Mexico. *Biotropica* **42**: 180–187.

- Abdala-Roberts L., Parra-Tabla V., Salinas-Peba L. & Herrera C. M. (2009) Noncorrelated effects of seed predation and pollination on the perennial herb *Ruellia nudiflora* remain spatially consistent. *Biological Journal of the Linnean Society* **96**: 800–807.
- Ågren J., Ehrlén J. & Solbreck C. (2008) Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology* **96**: 334–345.
- Aizen M. A. & Harder L. D. (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* **88**: 271–281.
- Alonso C., Vamosi J. C., Knight T. M., Steets J. A. & Ashman T. L. (2010) Is reproduction of endemic plant species particularly pollen limited in biodiversity hotspots. *Oikos* **119**: 1192–1200.
- Ashman T. L., Knight T. M., Steets J. A., Amarasekare P., Burd M., Campbell D. R., Dudash M. R., Johnston M. R., Mazer S. J., Mitchell R. J., Morgan M. T. & Wilson W. G. (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**: 2408–2421.
- Banuelos M. J. & Obeso J. R. (2005) How is fruit production regulated in the dioecious fleshy-fruited shrub *Rhamnus alpinus*? *Basic and Applied Ecology* **6**: 249–259.
- Barrett S. C. H., Dorken M. E. & Case A. L. (2001) A geographical context for the evolution of plant reproductive systems. In: Silvertown J. & Antonovics J. (eds). *Integrating Ecology and Evolution in a Spatial Context*. Blackwell Science, Oxford, pp. 341–364.
- Barrett S. H. C. (2002) Sexual interference of the floral kind. *Heredity* **88**: 154–159.
- Barrett S. H. C. & Harder L. D. (1996) Ecology and evolution of plant mating. *Trends in Ecology and Evolution* **11**: 73–79.
- Brookes R. H., Jesson L. K. & Burd M. (2008) A test of simultaneous resource and pollen limitation in *Styloidium armeria*. *New Phytologist* **179**: 557–565.
- Burd M. (1988) 'Excess' flower production and selective fruit abortion: a model of potential benefits. *Ecology* **79**: 2123–2132.
- Burd M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* **60**: 84–139.
- Burkhardt A., Delph L. F. & Bernasconi G. (2009) Benefits and costs of pollinating, seed-eating insects: the effect of flower size and fruit abortion on larval performance. *Oecologia* **161**: 8–98.
- Campbell D. R. & Halama K. J. (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* **74**: 1043–1051.
- Chalé-Uc S. (2011) Efecto del sistema de apareamiento en la germinación, crecimiento, reproducción, tasa fotosintética y supervivencia de *Ruellia nudiflora* (Engelm y Gray) Urban (Acanthaceae) bajo condiciones experimentales de densidad de siembra (BSc. dissertation). Universidad Autónoma de Yucatán, Mexico.
- Charlesworth D. & Charlesworth B. (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**: 237–268.
- Eckert C. G., Samis K. E. & Dart S. (2006) Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In: Harder L. D. & Barrett S. H. C. (eds). *Ecology and Evolution of Flowers*. Oxford University Press, New York, pp. 183–203.
- Gomez J. M., Bosch J., Perfectti F., Fernandez J. & Abdelaziz M. (2007) Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* **153**: 597–605.
- Harder L. D. & Routley M. B. (2006) Pollen and ovule fates and reproductive performance by flowering plants. In: Harder L. D. & Barrett S. H. C. (eds). *Ecology and Evolution of Flowers*. Oxford University Press, New York, pp. 61–80.
- Herlihy C. R. & Eckert C. G. (2002) Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* **416**: 320–323.
- Herlihy C. R. & Eckert C. G. (2004) Experimental dissection of inbreeding and its adaptive significance in a flowering plant *Aquilegia canadensis* (Ranunculaceae). *Evolution* **58**: 2693–2703.
- Herrera C. M. (1995) Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* **76**: 1516–1524.
- Kilkenny F. F. & Galloway L. F. (2008) Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* **155**: 247–255.
- Knight T. M., Steets J. A., Vamosi J. C., Mazer S. J., Burd M., Campbell D. R., Dudash M. R., Johnston M. O., Mitchell R. J. & Ashman T. L. (2005) Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution and Systematics* **36**: 467–497.
- Marrufo-Zapata D. (2011) Variación geográfica en la hercogamia y tasas de entrecruzamiento poblacional en *Ruellia nudiflora* (Acanthaceae) en la península de Yucatán (MSc. dissertation). Universidad Autónoma de Yucatán, Mexico.
- Medrano M., Herrera C. M. & Barrett S. C. H. (2005) Herkogamy and mating patterns in the self-compatible daffodil *Narcissus longispathus*. *Annals of Botany* **95**: 1105–1111.
- Moeller D. A. & Geber M. A. (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution* **59**: 786–799.
- Munguía-Rosas M. A., Parra-Tabla V., Ollerton J. & Cervera C. (2012) Environmental control of reproductive phenology and the effect of pollen supplementation on resource allocation in the cleistogamous weed *Ruellia nudiflora* (Acanthaceae). *Annals of Botany* **109**: 343–350. DOI:10.1093/aob/mcr284.
- Ortega-Campos I., Parra-Tabla V., Abdala-Roberts L. & Herrera C. M. (2009) Local adaptation of *Ruellia nudiflora* (Acanthaceae) to biotic counterparts: complex scenarios revealed when two herbivore guilds are considered. *Journal of Evolutionary Biology* **22**: 2288–2297.
- Parra-Tabla V. & Herrera C. M. (2010) Spatially inconstant direct and indirect effects of herbivory on floral traits and pollination success in a tropical shrub. *Oikos* **119**: 1344–1354.
- Parra-Tabla V., Vargas M. F. & Eguiarte F. L. (1998) Is *Echeveria gibbiflora* (Crassulaceae) pollen limited?: an experimental test. *Functional Ecology* **12**: 591–595.
- Pflugshaupt K., Kollman J., Fischer M. & Roy B. (2002) Pollen quantity and quality affect fruit abortion in small populations of a rare fleshy-fruited shrub. *Basic and Applied Ecology* **3**: 319–327.
- SAS (2002) SAS, Version 9.1. SAS Institute Inc., Cary, NC.
- Schoen D. J., Morgan M. T. & Bataillon T. (1996) How does self-pollination evolve? Inferences from floral ecology and molecular evolution. *Philosophical Transactions of the Royal Society B* **351**: 1281–1290.
- Schuetz M. E. & Vamosi J. C. (2010) Phylogenetic community context influences pollen delivery to *Allium cernuum*. *Evolutionary Biology* **37**: 19–28.

- Standley P., Williams L. & Gibson D. (1974) Flora of Guatemala. *Fieldiana Botany* **24**: 328–462.
- Stephenson A. G. (1981) Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* **12**: 253–279.
- Strauss S. Y. & Zangerl A. (2002) Plant–insect interactions in terrestrial ecosystems. In: Herrera C. M. & Pellmyr O. (eds). *Plant–Animal Interactions. An Evolutionary Approach*. Blackwell Publishing, Oxford, pp. 77–106.
- Takebayashi N. & Delph L. F. (2000) An association between a floral trait and inbreeding depression. *Evolution* **54**: 840–846.
- Takebayashi N., Wolf D. E. & Delph L. F. (2006) Effect of variation in herkogamy on outcrossing within a population of *Gilia achilleifolia*. *Heredity* **96**: 159–165.
- Tripp E. (2007) Evolutionary relationships within the species rich genus *Ruellia* (Acanthaceae). *Systematic Botany* **32**: 628–649.
- Vanhoenacker D., Ågren J. & Ehrlen J. (2006) Spatio-temporal variation in pollen limitation and reproductive success of two scape morphs in *Primula farinosa*. *New Phytologist* **169**: 615–621.
- Vaughton G., Ramsey M. & Simpson I. (2008) Does selfing provide reproductive assurance in the perennial herb *Bulbina vagans* (Asphodelaceae). *Oikos* **117**: 390–398.
- Wang Y., Zhang D., Renner S. S. & Chen Z. (2005) Self-pollination by sliding pollen in *Caulokaempferia coenobialis* (Zingiberaceae). *International Journal of Plant Sciences* **166**: 753–759.
- Wang Y.-Q., Zhang D.-X. & Chen Z.-Y. (2004) Pollen histochemistry and pollen: ovule ratios in Zingiberaceae. *Annals of Botany* **94**: 583–591.
- Wiens D. (1984) Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* **64**: 47–53.
- Zimmerman M. & Pyke G. H. (1988) Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* **131**: 723–738.