

## RESEARCH PAPER

# Patterns and sources of variation in pollen deposition and pollen tube formation in flowers of the endemic monoecious shrub *Cnidoscolus souzai* (Euphorbiaceae)

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## Keywords

*Cnidoscolus souzai*; monoecious, pollen grain; pollen limitation; pollen tube; Yucatan Peninsula.

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## ABSTRACT

Pollen deposition and pollen tube formation are key components of angiosperm reproduction but intraspecific variation in these has rarely been quantified. Documenting and partitioning (populations, plants and flowers) natural variation in these two aspects of plant reproduction can help uncover spatial mosaics of reproductive success and underlying causes. In this study, we assess variation in pollen deposition and pollen tube formation for the endemic monoecious shrub *Cnidoscolus souzai* throughout its distribution range in Mexico, and determine how this variation is structured among populations, plants and flowers. We also infer the relative importance of pollen quantity and quality in determining pollination success in this species. While we found no evidence suggesting that pollen receipt limits *C. souzai* reproduction across 19 populations, we did find extensive variation in pollen load size and pollen tube number per flower. Total variation in pollen receipt and pollen tube number was mostly explained by intra-individual and among-population variance. Furthermore, pollen load size had a stronger effect on the number of pollen tubes at the base of the style than pollen germination rate, suggesting that pollen quantity may be more important than quality for pollen tube success in *C. souzai*. Our results suggest that both small within-plant flower differences and broad-scale differences in community attributes can play an important role in determining pollination success. We emphasise the need to evaluate patterns and sources of variation in pollen deposition and pollen tube formation as a first step in understanding the causes of variation in pollination success over broad spatial scales.

## INTRODUCTION

Successful sexual reproduction of angiosperms in natural populations is highly dependent on the transfer of male gametes (*i.e.* pollen) to and from flowers of potential mates. Therefore, documenting patterns of natural variation in pollen deposition in flowers and uncovering the potential causes of such variation are of key importance for understanding the mechanisms that determine successful plant reproduction and to identify potential opportunities for selection in nature (Burd 1995; Herrera 2002). However, to date, only a handful of studies have documented natural variation in pollen receipt by flowers (*e.g.* Niesenbaum 1994; Plitmann & Levin 1996; Quezada *et al.* 2001; Herrera 2004; Bernasconi *et al.* 2007) and even fewer have attempted to identify its underlying causes (Niesenbaum 1994; Herrera 2004; Alonso 2005).

Evaluating patterns of natural variation in pollen receipt across wide spatial scales can also help to uncover potential spatial mosaics in the frequency and magnitude of pollen limitation (when ratio of pollen to ovule number  $<1$  = pollination deficit; Herrera 2002) across a species distribution range (Herrera 2002; Bernasconi *et al.* 2007). Evaluating pollination success at such broad scales can be a daunting task using

traditional (*i.e.* manipulative) techniques for assessing pollen limitation (Parra-Tabla *et al.* 1998; Ashman *et al.* 2004; Knight *et al.* 2005; Alonso *et al.* 2012). Thus, methods that allow for broad sampling of the pollination environment are an important first step in evaluating the potential for pollen limitation over a regional and even across a species entire distribution range. Furthermore, studies that evaluate the incidence of pollen shortage for a single species over broad spatial scales are crucial if we aim to fully assess the effects of environmental conditions and community attributes on pollination success (Herrera 2002; Vamosi *et al.* 2006; Alonso *et al.* 2010; Sargent *et al.* 2011; Arceo-Gómez & Ashman 2014a). This is particularly important given the accelerated rate of human-mediated disturbances that are altering the biotic and abiotic conditions that influence plant reproductive success in natural populations (*e.g.* Traveset & Richardson 2006; Bjerknes *et al.* 2007; Memmott *et al.* 2007; Hegland *et al.* 2009; Forrest *et al.* 2010).

Evaluating how intraspecific variation in pollen receipt is structured spatially at different hierarchical levels of organisation (*i.e.* populations, plants within populations and flowers within plants) can provide important insights into the factors underlying variation in plant reproductive success in nature (Herrera 2002). For instance, greater among-population

variance would suggest that environmental conditions and community attributes, such as plant density (e.g. Knight 2003; Spigler & Chang 2008), co-flowering diversity (e.g. Schuett & Vamosi 2010; Sargent *et al.* 2011; Arceo-Gómez & Ashman 2014b) and the abundance and composition of the pollinator community (e.g. Moeller 2006; Gómez *et al.* 2010), are the major drivers of pollination success. In contrast, greater among-plant variance would indicate that intrinsic plant traits that affect pollinator foraging behaviour, such as flower (e.g. Totland 2001; Fishman & Willis 2008) and floral display size (e.g. Sandring & Ågren 2009), may play a more important role. Finally, if greater variance in pollen deposition is observed among flowers within individual plants, a frequently dismissed level, then fine-scale differences among flowers in traits such as size, colour and shape and/or stochastic events would be the main factors underlying intraspecific variation in pollination success (Herrera 2002, 2004). Knowledge of the main source(s) of variation in pollen receipt is also important for determining the potential for natural selection to act on floral and plant traits that influence pollen deposition and fertilisation success (Herrera 2002, 2009), as well as for the evolution of plant reproductive strategies (Burd 1995).

The amount of conspecific pollen deposited on stigmas can determine not only the quantity but also the quality of the progeny produced (Winsor *et al.* 2000; Herrera 2002; Bernasconi *et al.* 2007) by affecting the intensity of competition among male gametophytes (Snow 1986; Winsor *et al.* 2000; Herrera 2004; Alonso *et al.* 2012). Therefore, pollen load size has been typically viewed as a strong indicator of pollination success. However, large pollen loads do not always lead to higher ovule fertilisation, as pollen germination and pollen tube growth can also be influenced by other factors, such as environmental conditions and pollen–stigma interactions (Herrero & Johnson 1980; Stephenson *et al.* 1992). Thus, in addition to documenting natural pollen loads on stigmas, evaluating the number of successful pollen tubes that enter the ovary (hereafter, pollen tube success) is also necessary in order to have a more complete understanding of the reproductive environment experienced by plants in natural populations (Herrera 2002; Alonso *et al.* 2012). Although factors such as late-acting self incompatibility can disrupt the link between pollen tube formation and seed production, pollen tube success has often been recognised as a reliable estimator of reproductive success (e.g. Winsor *et al.* 1987; Waser & Price 1991; Arceo-Gómez & Ashman 2014b), but this interpretation should be taken with caution. Nevertheless, evaluating how pollen tube success relates to pollen load size and pollen germination rate can help to elucidate the potential relative importance of factors affecting the quantity (*i.e.* pollen load size) and/or the quality (*i.e.* pollen germination rate) of the pollen received for plant reproductive success (Alonso *et al.* 2012).

In this study, we sampled 19 populations of the endemic *Cnidocolus souzae* (Euphorbiaceae), a monoecious, self-compatible shrub distributed in the Yucatan Peninsula (southern Mexico,) to address the following goals: (i) assess the magnitude of variation in pollen load size and pollen tube success across this species' distribution range in Mexico, and in doing so assess the potential for geographic mosaics of pollen limitation; (ii) determine how variation in pollen load size and tube

success are partitioned at different hierarchical levels of organisation, namely populations, plants and flowers, to better understand the underlying causes of variation in pollination success in this species; and (iii) infer the relative importance of the quantity *versus* quality of pollination in determining *C. souzae* pollen tube success by examining how the number of pollen tubes at the base of the style relates to pollen load size and pollen germination rate, respectively. In addressing the above, this study will be the first to describe the patterns and potential causes of variation in pollination success in a monoecious species across a broad spatial scale.

## MATERIAL AND METHODS

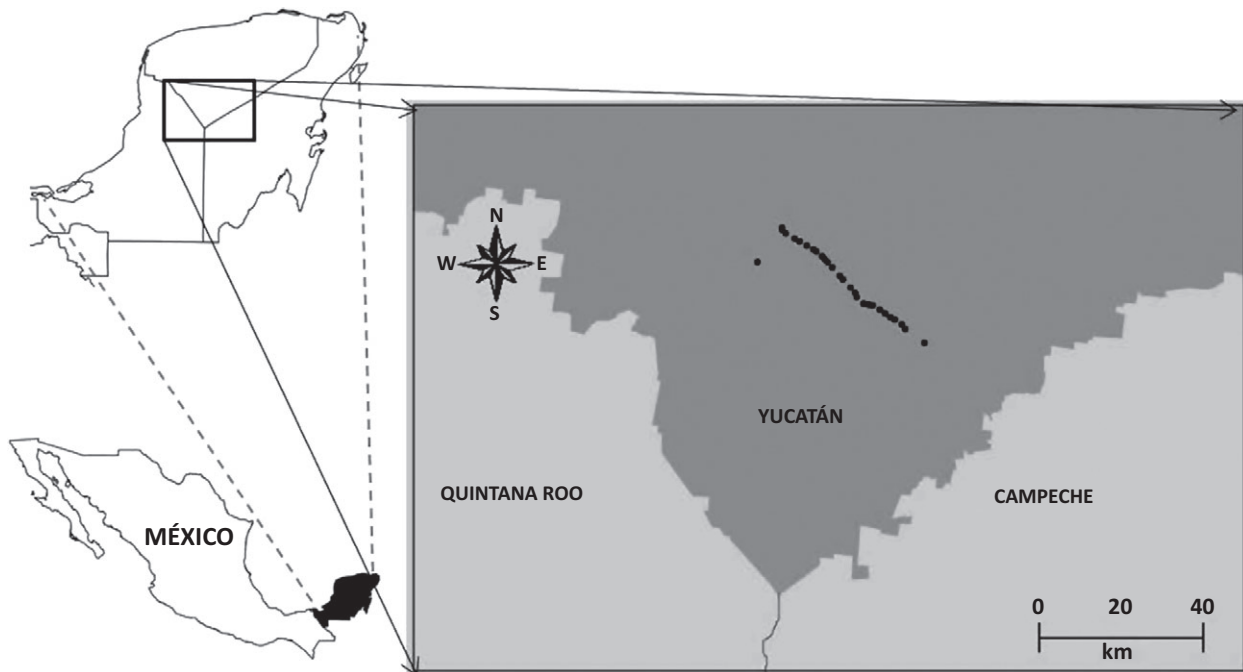
### Study species

*Cnidocolus souzae* (Euphorbiaceae) is a self-compatible, monoecious (male and female flowers on the same individual) tropical shrub with a very narrow distribution in the Yucatan Peninsula in Mexico and in small areas of Belize and Guatemala (Standley & Steyermark 1949). *C. souzae* produces small (*ca.* 2-cm long) white actinomorphic flowers that bloom during the rainy season (July–October), remain open for 1 day and are visited by at least 15 species of butterfly and ten species of bee (Parra-Tabla unpublished research). Although self-compatible, *C. souzae* is entirely dependent on pollen vectors for successful pollen transfer from male to female flowers, which typically have four ovules (Parra-Tabla & Herrera 2010). As with other *Cnidocolus* species flowers are arranged in inflorescences with a dichotomous ramification pattern with female flowers at the base and male flowers above (Standley & Steyermark 1949).

### Study populations and sampling design

To evaluate spatial variation in conspecific pollen load size and pollen tube success in flowers of *C. souzae* we sampled 19 populations distributed along a northwest to southeast transect within the Yucatan Peninsula (Fig. 1), thus covering most of this species' distribution range in Mexico. Distance between populations ranged from 0.5 to 5.0 km. (mean: 2.9 km). Populations of *C. souzae* are small (<70 plants), occur in open patches of tropical dry forest, and experience similar climatic conditions (Parra-Tabla, personal observations).

Due to the aggregate distribution of plants within populations, we established three to five blocks per site and marked three to five plants within each block, for a total of 12–23 plants sampled per population (Table 1), depending on the abundance of *C. souzae* at each site. All sites were visited during peak flowering, and three wilted female flowers were collected per plant (36–69 total flowers per population) and stored in individual microcentrifuge tubes with a formalin-acetic-alcohol (FAA) solution. Since flowering phenology does not vary among populations (Parra-Tabla unpublished research), all sites were visited during a 3-day window to reduce temporal variation in pollination success across populations. Styles from each flower were softened and stained with decolourised aniline blue (Arceo-Gómez & Ashman 2011). We recorded the number of conspecific pollen grains on the stigma, the number of pollen grains that germinated and produced tubes in the first 0.5 cm of the style (hereafter, germinated pollen), and the



**Fig. 1.** Map showing the location of the 19 *Cnidocolus suozae* populations evaluated in this study. All populations are located in the south of the Yucatan Peninsula, along *C. suozae* narrow distribution range in Mexico.

**Table 1.** Mean ( $\pm$ SE) pollen load size, pollen tube success (number of pollen tubes at the base of the style), pollen germination rate and their respective coefficients of variation (CV) for each one of the 19 populations studied. The percentage of styles with more pollen tubes than ovules per flower and the total number of plants sampled per population are also given.

population no.	number of plants	mean ( $\pm$ SE) conspecific pollen load size	CV conspecific pollen load size	mean ( $\pm$ SE) pollen tube success	CV pollen tube success	mean ( $\pm$ SE) conspecific pollen germination rate	CV conspecific pollen germination rate	% styles with > four tubes
1	18	246.5 $\pm$ 26.2	77.4	17.3 $\pm$ 1.5	63	0.12 $\pm$ 0.01	115.1	90
2	19	105.3 $\pm$ 17.7	120.1	22.9 $\pm$ 2.8	88.2	0.36 $\pm$ 0.03	66.3	82
3	18	108 $\pm$ 12.2	85.6	44 $\pm$ 5	86.9	0.46 $\pm$ 0.02	46.6	93
4	13	134.8 $\pm$ 18.1	102.3	31.3 $\pm$ 3.7	91.6	0.33 $\pm$ 0.02	57.8	83
5	14	59 $\pm$ 8.5	76.9	13.8 $\pm$ 1.9	75.6	0.3 $\pm$ 0.03	67.5	89
6	19	248.3 $\pm$ 35.2	72.3	64.8 $\pm$ 10.2	80.2	0.25 $\pm$ 0.01	35.7	96
7	15	101.8 $\pm$ 19.1	132.9	14.2 $\pm$ 2.5	124.8	0.27 $\pm$ 0.03	102.4	72
8	16	283.8 $\pm$ 39.3	60.4	27 $\pm$ 5.2	84.4	0.12 $\pm$ 0.02	89.3	100
9	17	168.1 $\pm$ 23.8	73.6	26.7 $\pm$ 4.1	80.3	0.18 $\pm$ 0.01	49.3	100
10	18	227.5 $\pm$ 42.8	92.1	55.2 $\pm$ 12.3	109.2	0.29 $\pm$ 0.03	56	87
11	23	110 $\pm$ 24	102.3	14.5 $\pm$ 2.8	91.7	0.16 $\pm$ 0.02	62.1	77
12	14	316.7 $\pm$ 37.7	75.4	30.4 $\pm$ 4.3	90.1	0.11 $\pm$ 0.01	93.9	97
13	12	232.9 $\pm$ 45.1	79.8	38.2 $\pm$ 10.2	110.2	0.26 $\pm$ 0.06	101.8	88
14	12	109.4 $\pm$ 16	101.5	11 $\pm$ 2	130.4	0.24 $\pm$ 0.03	107.3	73
15	12	100.9 $\pm$ 22.2	103.3	10.5 $\pm$ 1.8	83.3	0.15 $\pm$ 0.02	87.3	81
16	13	151.4 $\pm$ 28.6	102	17.2 $\pm$ 3.2	100.5	0.21 $\pm$ 0.03	98.8	82
17	12	52.4 $\pm$ 9.9	128.5	9.4 $\pm$ 1.4	106.7	0.28 $\pm$ 0.03	83.1	61
18	14	92.3 $\pm$ 16.7	116.2	20.1 $\pm$ 3.8	122.2	0.28 $\pm$ 0.02	62.3	73
19	12	106.7 $\pm$ 14.1	92.5	11.9 $\pm$ 1.4	82.5	0.22 $\pm$ 0.03	97.9	87

number of conspecific pollen tubes reaching the base of the style (*i.e.* pollen tube success) using a fluorescence microscope (Nikon e200; Nikon, Tokyo, Japan). We estimated conspecific pollen germination rate per flower by dividing the number of pollen grains that germinated by the total number of pollen grains on the stigma.

#### Data analyses

##### *Spatial variation in pollen load size and pollen tube success*

We performed a nested ANOVA (proc glm; SAS Institute, Cary, NC, USA) to evaluate the statistical significance of differences in conspecific pollen load size and pollen tube success among

populations, blocks nested within populations and plants nested within blocks. All factors were considered random effects in the model. Residuals from both models exhibited a normal distribution (K-S test,  $P > 0.05$ ). Means  $\pm$  SE are reported throughout the paper.

We estimated the contribution of population, block nested within population, plant nested within block and flower nested within plant (*i.e.* residual variation) to the total amount of variation observed in conspecific pollen load size and pollen tube success in each model by partitioning the variance into each of the different components (proc varcomp; SAS). To further evaluate the variability in conspecific pollen load size and pollen tube success we also calculated the coefficient of variation (CV) for each response variable and used ANOVA to test for differences in CV among populations, blocks nested within population and plants nested within blocks (proc glm; SAS). These latter analyses allowed us to further evaluate if the degree of variation in conspecific pollen load size and pollen tube success differed significantly among populations, blocks and plants within a population (Herrera 2009). It is worth pointing out that we were unable to detect any large-scale spatial structure in pollen receipt, since population mean conspecific pollen load size is not related to a population location on the sampled transect (Fig. 1;  $r = -0.02$ ,  $P = 0.9$ , data not shown). Furthermore, Moran's I (proc variogram; SAS) showed no evidence of spatial autocorrelation in conspecific pollen receipt across all sites ( $Z = -0.46$ ,  $P = 0.6$ ). These results suggest a lack of spatial autocorrelation in the dataset and further suggest that any variation in pollination success among populations would be due to inherent differences among them or to temporally fluctuating patterns of pollinator abundance.

#### Contribution of pollen quantity and quality to variation in pollen tube success

To evaluate whether the quantity and quality components of pollination contribute to pollen tube success we performed a multiple regression (proc reg; SAS) using population means where conspecific pollen load size (*i.e.* quantity component) and pollen germination rate (*i.e.* quality component) were predictors of pollen tube success. We then estimated standardised regression coefficients (stb; Landis 2005) to infer the relative importance of quantity and quality of pollination for pollen tube success. A variance inflation factor was estimated to test for potential multicollinearity influencing our results. Residuals from this model exhibited a normal distribution (K-S test,  $P > 0.05$ ).

## RESULTS

### Spatial variation in pollen load size and pollen tube success

*Cnidocolus souzae* flowers received on average  $146.7 \pm 5.9$  conspecific pollen grains and produced  $24.1 \pm 1$  pollen tubes that reach the ovary across all populations. However, there was extensive spatial variation in both response variables across the 19 studied populations, with populations receiving, on average, 52.4–283.8 conspecific pollen grains and forming between 9.4 and 64.8 successful pollen tubes per flower (Table 1). Accordingly, we found significant variation in conspecific pollen load size and pollen tube success among populations as well as among blocks and plants (plant individual range for pollen

load size:  $2.5 \pm 0.5$ – $672.6 \pm 143.0$  and tube success:  $1.5 \pm 0.5$ – $184.0 \pm 9.7$ ) within populations (Table 2).

The estimation of variance components for conspecific pollen load size revealed within-plant variation (*i.e.* residual) as the most important source of variation (70%), followed by variation among populations (17%), blocks within a population (10%) and plants within blocks (3%). Likewise, variation among flowers within a plant explained most of the variation in successful pollen tubes (70%), followed by variation among populations (18%), blocks within a population (7%) and plants within blocks (5%). Analysis of the CV for conspecific pollen load size and pollen tube success in *C. souzae* flowers revealed significant differences among populations, but not among blocks or plants within a population, for both response variables (Table 2).

### Contribution of quantity and quality of pollination to variation in pollen tube success

Population mean conspecific pollen load size and germination rate significantly affected the number of successful pollen tubes that reached the ovary across the 19 populations ( $r^2 = 0.75$ ,  $P < 0.0001$ ,  $N = 19$ ; Fig. 2). Furthermore, standardised regression coefficients indicated that conspecific pollen load size had a stronger effect on pollen tube success (stb = 0.95) compared to pollen germination rate (st = 0.76; Fig 2). The variance inflation factor showed no effect of multicollinearity influencing our results (VIF < 2).

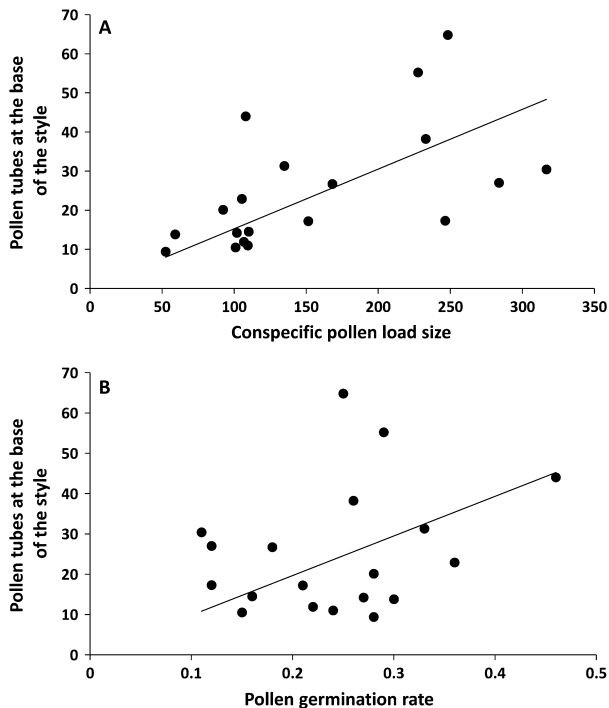
## DISCUSSION

We found substantial variation in conspecific pollen load size and pollen tube success in female flowers of *C. souzae* throughout its distribution range in Mexico, with population differences of up to five and seven orders of magnitude in the number of pollen grains and tubes, respectively (Table 1). However, we found little evidence suggesting pollen limitation of reproductive success across the 19 populations studied, as indicated by three major lines of evidence. First, the least successful population received on average 13 times more conspecific pollen and produced > than two times more pollen tubes than ovules per flower (pop 17 in Table 1). Second, <5% of all flowers sampled received fewer conspecific pollen grains than ovules per flower (*i.e.* <4). Third, the percentage of styles with a larger number of successful pollen tubes than number of

**Table 2.** ANOVA results for the effects of population, block nested within population and plant nested within block on pollen load size, pollen tube success (number of pollen tubes at the base of the style) and their respective coefficients of variation (CV).

source	pollen load size		pollen tube success		CV pollen load size		CV pollen tube success	
	df	F	df	F	df	F	df	F
population	18	<b>9.9**</b>	18	<b>10.1**</b>	18	<b>2.11**</b>	18	<b>1.92*</b>
block (population)	70	<b>2.8**</b>	70	<b>2.6**</b>	70	0.84	70	0.89
plant (block)	27	<b>2.1**</b>	27	<b>2.1*</b>	27	0.73	27	0.51

Values in bold are statistically significant, \* $P < 0.01$ , \*\* $P < 0.001$ .



**Fig. 2.** Effect of (A) population mean pollen load size ( $stb = 0.95$ ,  $P < 0.001$ ,  $N = 19$ ) and (B) pollen germination rate ( $stb = 0.76$ ,  $P < 0.001$ ,  $N = 19$ ) on average pollen tube success (number of pollen tubes at the base of the style) for each population.

ovules per flower was consistently high across all populations (mean: 84%, range: 61–100%; Table 1), particularly when compared to what has been reported for other species (e.g. *Ballota hirsuta* 25%, *Rosamarinus officinalis* 30% and *Teucrium rotundifolium* 32%; Herrera 2004; also see Alonso *et al.* 2013). It is worth nothing, however, that in many cases more than one pollen grain per ovule is needed for successful fertilisation. Nonetheless, these results suggest that the potential for pollen quantity (amount of pollen) and quality (number of successful pollen tubes) limitation of reproductive success in *C. souzae* is low throughout its distribution range in Mexico. Such large pollen loads and high pollen tube success could be attributed, at least partly, to this species' generalist pollination system (>25 different flower visitors) and to high visitation rates observed at all sites (Parra-Tabla personal observation). In addition, *Apis mellifera*, one of the main pollinators of *Cnidocolus* species in Yucatan (Rodríguez 2004; Arceo-Gómez *et al.* 2009; Parra-Tabla & Herrera 2010), is known to be a highly efficient pollinator, able to carry and deposit large amounts of pollen (e.g. Tepedino 1981; Freitas & Paxton 1998), likely contributing to the large conspecific pollen loads in *C. souzae*. Another important factor likely contributing to potential low pollen limitation in this species is the small number of ovules available for fertilisation (~4), which limits the number of conspecific pollen grains required for successful reproduction. It is also important to point out that despite the fact that pollen germination rate in *C. souzae* is very low (<50% in all populations; Table 1), flowers still had, on average, six successful pollen tubes per ovule, suggesting that flowers may need to receive large conspecific pollen loads in order to compensate for low-quality pollen. Because this species is

self-compatible and floral displays tend to be large, low-quality pollen deposition could result from a high degree of geitonogamous pollination. Furthermore, the large conspecific pollen loads and the high number of pollen tubes found in *C. souzae* flowers also suggest that microgametophyte competition can be intense. Especially because pollen arrival and germination likely take place synchronously (Snow 1986; Spira *et al.* 1992) as flowers of *C. souzae* remain open for <1 day (Parra-Tabla personal observation; also see Parra-Tabla & Herrera 2010). Having said this, a detailed study on the intensity of microgametophyte competition across populations of *C. souzae* is needed in order to assess the importance of this phenomenon as an agent of selection on traits that influence pollen performance in this species.

Although there was little evidence suggesting pollen limitation in *C. souzae*, flowers of this species varied extensively in conspecific pollen load size and in the number of successful pollen tubes (Table 1), indicating large differences in the magnitude of pollination success. Variance partitioning showed that variation in conspecific pollen load size and pollen tube success was mainly determined by within-plant differences, which accounted for 70% of the total variance observed for these variables. It is important to acknowledge this residual variation may include biologically relevant differences among the smallest sampling units (*i.e.* flowers within a plant), as well as other sources of variation (e.g. count errors). However, a high degree of variation among flowers within plants (e.g. 60–92%; Herrera 2004) and even among styles within flowers has been shown in at least eight other plant taxa (e.g. Levin 1990; Niesenbaum 1994; Herrera 2002, 2004). Therefore, it is possible that a substantial portion of residual variation in conspecific pollen load size and tube success was due to flower differences and stochastic events that affect fine-scale patterns of pollen deposition and pollen performance within plants, which have been shown to be important factors determining differential pollination success in natural plant populations (Niesenbaum 1994; Herrera 2002, 2004). For instance, pollinator foraging patterns could be a strong driving factor leading to small-scale (within-plant) variability as different pollinators (individuals and/or species) may carry different pollen loads of different quantity and quality (e.g. proportion of heterospecific and conspecific pollen; Arceo-Gómez *et al.* 2016). Furthermore, all flowers within plants may not receive equal numbers of visits, depending on their position in the inflorescence. Thus, these two factors may contribute to the large within-plant variability in the stigmatic pollen loads observed. It is also possible that small differences in light (Feng *et al.* 2000) and water availability (Lush *et al.* 1998) among flowers can affect the rate of pollen germination and the directionality of pollen tube growth, respectively, and this could contribute to high amounts of within-plant variation in pollen tube success, but this has not been evaluated. Detailed studies on the mechanisms underlying intra-individual variation in patterns of conspecific pollen deposition and pollen tube success are needed if we aim to fully comprehend the factors that affect plant reproductive success in natural populations.

Among-population differences were the second most important factor determining variation (17–18%) in *C. souzae* pollination success. Accordingly, analyses of the degree of variability (CV) in conspecific pollen load size and pollen tube success also revealed significant among-population differences.

Studies with other congeneric species of *Cnidocolus* (e.g. *C. aconitifolius*) sharing a similar pollinator community and floral characteristics, and conducted in the same study area, have also shown a high level of variation in pollination success (pollen tube formation) among populations (Parra-Tabla & Herrera 2010). These results suggest that large-scale differences in plant and pollinator community attributes and/or environmental conditions, which have been shown to influence the pollination environment (e.g. Herrera 2002; Knight 2003; Gómez *et al.* 2010; Schuett & Vamosi 2010; Arceo-Gómez & Ashman 2014a), may also play an important role in determining differential pollination success in *C. souzae* and other species of this genus. It is also worth noting that we found a small contribution of among-block variance to total variation in *C. souzae* pollination success, suggesting that the location of a plant within a population or the characteristics of the surrounding plant neighbourhood (e.g. density or diversity) have the potential to influence pollination success (e.g. Spigler & Chang 2008; Jakobsson *et al.* 2009) in this species.

A closer look at the underlying factors potentially driving population-level variation in pollination success for *C. souzae* suggests that factors that affect the amount rather than the quality of conspecific pollen reaching the stigmas are more important drivers of differential pollination success in this species. Such factors may include differences in the co-flowering context (density and diversity), which have been shown to alter pollinator foraging behaviour through facilitation or competition processes (e.g. Knight 2003; Moeller 2004; Bell *et al.* 2005; Schuett & Vamosi 2010; Sargent *et al.* 2011; Arceo-Gómez & Ashman 2014a), and/or differences in the abundance and identity of the pollinator community, which can directly influence the amount of pollen deposited on stigmas (e.g. Moeller 2006; Gómez *et al.* 2007, 2010). However, detailed studies that assess spatial variation in plant and pollinator community composition are needed in order to have a complete understanding of

the community attributes that may influence pollination success in *C. souzae*. Finally, factors affecting conspecific pollen quality also appear to have an impact on pollen tube success in *C. souzae*, but to a much lesser extent (~20%) than factors affecting pollen load size, as suggested by the relationship between conspecific pollen germination rate and pollen tube success, a result that is surprising given the low pollen germination rate in this species.

Overall, this study lends support to studies that have proposed within-plant variance as the most important source of variation in pollination success in natural plant populations (Levin 1990; Niesenbaum 1994; Herrera 2002, 2004), and thus we emphasise the need to understand the causes and consequences of such variation if we are to fully comprehend the factors affecting plant reproductive success. Our results also suggest that among-populations differences in community attributes, in this case affecting pollen quantity to a larger degree than pollen quality, can play an important role in determining pollination success at broad spatial scales. Therefore, we highlight the importance of conducting studies that evaluate sources of variation in pollen receipt and pollen tube formation as an important first step towards understanding patterns and causes of differential pollination success in plant species over broad spatial scales.

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