

# The plot thickens: does low density affect visitation and reproductive success in a perennial herb, and are these effects altered in the presence of a co-flowering species?

Tracy S. Feldman

Received: 2 November 2007 / Revised: 4 March 2008 / Accepted: 17 March 2008  
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**Abstract** Plants may experience reduced reproductive success at low densities, due to lower numbers of pollinator visits or reduced visit quality. Co-occurring plant species that share pollinators have the potential to facilitate pollination by either increasing numbers of pollinator visits or increasing the quality of visits, but also have the potential to reduce plant reproductive success through competition for pollination. I used a field experiment with a common distylous perennial (*Piriqueta caroliniana*) in the presence and absence of a co-flowering species (*Coreopsis leavenworthii*) in plots with one of four different distances between conspecific plants. I found strong negative effects of increasing interplant distance (related to conspecific density) on several components of *P. caroliniana* reproductive success: pollinator visits to plants per plot visit, visits received by individual plants, conspecific pollen grains on stigmas, outcross pollen grains on stigmas, and probability of fruit production. Although *P. caroliniana* and *C. leavenworthii* share pollinators, the co-flowering species did not affect visitation, pollen receipt or reproductive effort in *P. caroliniana*. Pollinators moved very infrequently between species in this experiment, so floral constancy might explain the lack of effect of the co-flowering species on *P. caroliniana* reproductive success at low

densities. In co-occurring self-incompatible plants with floral rewards, reproductive success at low density may depend more on conspecific densities than on the presence of other species.

**Keywords** Allee effect · Co-flowering · Density · Pollen receipt · Reproductive success

## Introduction

Plants growing at low density may receive fewer pollinator visits (Sih and Baltus 1987; Jennersten 1988; Jennersten and Nilsson 1993; Kunin 1993; Alexandersson and Ågren 1996; Kunin 1997; Steffan-Dewenter and Tschardt 1999) or lower-quality pollinator visits (Groom 1998, Johnson et al. 2003). Pollinators may be less likely to encounter low-density patches of plants than high-density patches (Feldman et al. 2004), may be more attracted to higher-density patches of floral resources (Kunin 1993), and may visit fewer plants within low-density patches (e.g., Sih and Baltus 1987). Reduced visitation may lead to decreased reproductive success (e.g., Sih and Baltus 1987; Kunin 1993; Alexandersson and Ågren 1996; Steffan-Dewenter and Tschardt 1999; Hackney and McGraw 2001; reviewed in Ghazoul 2005). These low-density effects may occur over local (Kunin 1997), population-level (Groom 1998), or community scales (Moeller 2004), that in part depend upon the characteristics of the plants (e.g., mating system) and their pollinators (e.g., mobility and constancy). Low-density populations of plants with decreased reproductive success may experience Allee effects (Allee 1931; Stephens et al. 1999), in which a population is doomed to extinction should it fall below a critical threshold density (Groom 1998; Hackney and McGraw 2001).

Communicated by Florian Schiestl.

T. S. Feldman  
Biology Department, Duke University, Box 90338,  
Durham, NC 27708-0338, USA

T. S. Feldman (✉)  
Plant Biology Division, Samuel Roberts Noble Foundation,  
2510 Sam Noble Parkway, Ardmore, OK 73401-2124, USA  
e-mail: tsfeldman@noble.org

But plant species do not occur in isolation, and many simultaneously blooming plants share pollinator species. These co-flowering species may compete for pollinator visits (e.g., Rathcke 1988). Even if patches with co-flowering species attract more pollinators, such that the number of pollinator visits to plants does not decrease at low densities (e.g., Campbell 1985), heterospecific pollen transfer may increase (e.g., Waser 1978; Feinsinger et al. 1991; Caruso 1999), clogging stigmas (Waser and Fugate 1986), or conspecific pollen may be lost to surrounding heterospecifics (Campbell and Motten 1985). Alternatively, co-flowering species may facilitate pollinator visits (Thomson 1981, 1982; Roy 1994), pollen removal (Johnson et al. 2003), or seed production (e.g., Pellmyr 1986; Lavery 1992; Roy 1996; Moeller 2004; Ghazoul 2006). Increasing numbers of studies have demonstrated greater seed production in the presence of co-flowering species. If pollination facilitation occurs, one plant might mitigate negative effects experienced by another plant at low density by facilitating pollination at low density.

Here, I present an experimental test to determine the relative importance of conspecific and heterospecific densities on pollination and reproductive success of *Piriqueta caroliniana*, a common distylous perennial herb. Distyly in *P. caroliniana* is associated with self-incompatibility (Ornduff and Perry 1964; Tamari et al. 2001). Self-incompatible species may be more sensitive to effects of low density on fecundity than self-compatible species, making *P. caroliniana* a good experimental system. Also, because *P. caroliniana* is distylous, it is possible to manipulate distances to the nearest donors of compatible pollen (plants of the opposite style morph), and to determine relative numbers of compatible versus incompatible pollen grains, as pollen grain sizes differ between style morphs (Li and Johnston 2001). *P. caroliniana* plants co-occur with *Coreopsis leavenworthii* in some populations in Florida, and bloom at the same time (personal observation). Moreover, both of these plant species attract a wide variety of generalist pollinators including bees, butterflies, and flies. In this experiment, I also addressed the possibility of pollination competition or facilitation between *P. caroliniana* and *C. leavenworthii* at low densities. Specifically, I aimed to answer the following questions:

1. Does interplant distance affect visitation patterns, pollen receipt, or reproductive success in *P. caroliniana*?
2. Are low-density effects on visitation patterns, pollen receipt, or reproductive success in *P. caroliniana* altered in the presence of a second plant species with which it shares pollinators?

## Materials and methods

### System

*Piriqueta cistoides* (L.) Griseb. ssp. *caroliniana* (Walt.) Arbo [syn. *Piriqueta caroliniana* (Walt.) Urban] (Turneraceae) is a common perennial plant that grows in sandhills and waste ground from South Carolina (USA) to Cuba and Haiti (Ornduff and Perry 1964). In Florida (USA), it flowers between April and October, but primarily between May and August. In San Felasco Preserve (Florida; 29°44'35"N, 82°26'50"W) where I conducted concurrent field studies of a mapped population, plants produced from zero to two 1-day flowers per day, plants ranged from 0 to 17 m apart (mean 3.7 m), and not all plants bloomed on a given day. Plants may grow several stalks, although usually few stalks on a given plant produce flowers on a given day. *P. caroliniana* is distylous, each plant requiring insects to transfer pollen between plants of the opposite style morph to produce seeds (pollen from plants of the same style morph, including self pollen, is incompatible; Tamari et al. 2001). The pistil in each flower has three main branches, each with two lobes, resulting in a total of six "stigmatic lobes" per flower. *P. caroliniana* flowers attract over 40 species of insects, including Lepidoptera, Hymenoptera, Diptera, and Coleoptera. Lepidopterans, hymenopterans, and dipterans collect nectar, and hymenopterans collect pollen. Dipterans may also feed upon pollen, and coleopterans may feed upon all floral parts. Many of these pollinator species also visit other plant species that bloom at the same time as *P. caroliniana*, such as *Cnidoscolus stimulosus* (Michx.) Engelm and Gray, *Stylisma patens* (Desr.) Myint, *Buchnera americana* L., and *Coreopsis leavenworthii* Torr. and Gray (personal observation). For this study, I focused on *C. leavenworthii* as a co-flowering species, because several of the most common insect visitors to *P. caroliniana* also visit *C. leavenworthii*.

### Field site

The Disney Wilderness Preserve (DWP), Kissimmee, Florida (28°08'19"N, 81°26'52"W), includes many pastures dominated by the invasive forage grass *Paspalum notatum*. Both *P. caroliniana* and *Coreopsis leavenworthii* grow naturally (and bloom) on the outskirts of the pastures and along roadsides on DWP. Several other plant species grow and bloom in the pastures. All of the fields were mowed in early and middle summer, in an attempt to minimize effects of other co-flowering species. Mowing removed blooms of most other species during the experiment, although a few other species still produced small numbers of blooms in the experimental plots. Focal plant

species (in pots) were removed from the field sites prior to mowing.

### Field experiment

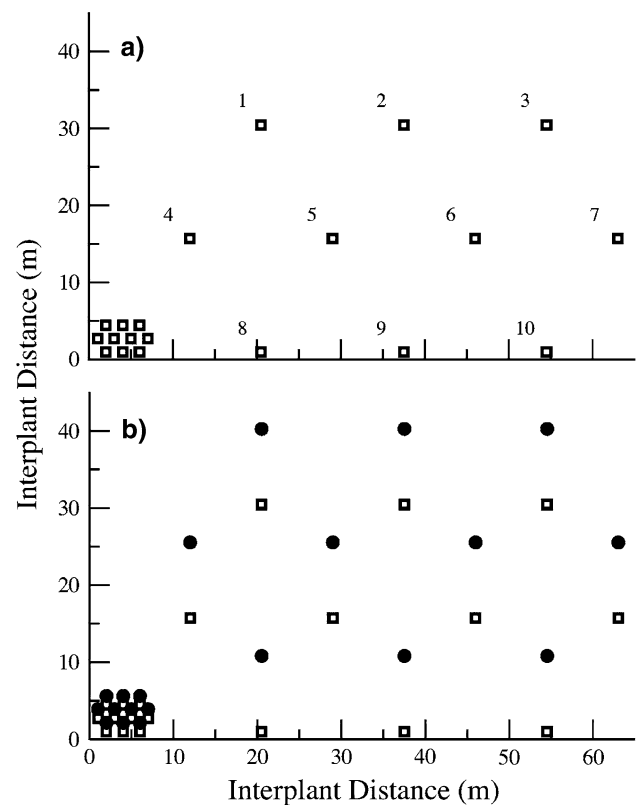
*P. caroliniana* plants used in the field experiment were grown from seed in the Duke University Greenhouse, North Carolina, USA. Seeds germinated in seed trays over heat mats. Plants were watered twice daily, and illuminated for 12 h/day. When plants were about 10 cm tall, I transplanted individual plants into 4" pots. Four weeks later, I transplanted plants into the 6" pots I used in the experiment. *C. leavenworthii* plants were transplanted into pots from natural populations growing less than 1 km from plots. Plants were stored in a screened enclosure at the DWP, and all opened flowers were removed prior to (and between) experimental replicates.

I set up eight experimental plots in three large pastures at the DWP. At least 100 m separated edges of adjacent plots, to increase the chance that pollinators would view adjacent plots as distinct. I planted ten *P. caroliniana* plants in each plot (arranged as in Fig. 1), planting pots in the ground. I randomly assigned each plant to one of four interplant distance treatments (2, 7, 12, or 17 m apart) and one of two co-flowering species treatments, using *C. leavenworthii* (co-flowering species absent, or present and evenly interspersed with *P. caroliniana* plants). Each plant was covered with wire mesh (5 cm diameter holes) to discourage deer herbivory while allowing pollinators access to plants.

I conducted five temporal replicates of each treatment combination between late May and mid August, each lasting 5–6 days. For each temporal replicate, I randomly assigned new assemblages of plants to plots, and plots to density and co-flowering treatments, such that in each of five temporal replicates, neither plots nor plants were used for the same treatment combination twice.

On each experimental day within each replicate, I removed all but one flower from each plant prior to opening (before 0830 hours). This mimicked natural conditions at San Felasco Preserve (mentioned above), as well as in other natural populations (personal observation). To keep the morph ratio at 1:1, if a plant did not flower on a given day, I allowed another plant in the plot with the same style morph to have two flowers. In this way, I allowed ten *P. caroliniana* flowers per plot, and ten *C. leavenworthii* inflorescences (one per plant, with variable numbers of open flowers per inflorescence) in each plot with the co-flowering-present treatment. I marked each chosen *P. caroliniana* flower with colored thread to indicate its treatment combination.

To measure pollinator visitation, I observed plots in a randomly determined order each day, recording pollinator



**Fig. 1** Arrangement of plants within experimental plots. Distances between adjacent conspecific plants in the four interplant distance treatments were 2, 7, 12, and 17 m, respectively, and each treatment occurred with and without the co-flowering species (for a total of eight treatments): **a**, **b**, 2- and 17-m treatments are shown, *Piriqueta caroliniana* plants depicted by open squares, **a** without and **b** with *Coreopsis leavenworthii*, the co-flowering species, depicted by closed circles. **a** Odd-numbered *P. caroliniana* plants were long-styled plants, while even-numbered plants were short-styled. Also, in the experiments, each treatment occurred in plots >100 m from other plots

visits to plants during 15-min periods. During each visit, I recorded the identity of each pollinator (to species, when possible—sometimes identification to species was difficult in the field). Plants were numbered in each plot, and pollinators were followed once they entered the plot and visited at least one plant, to determine the trajectory of their visit to the plot and to measure visitation rate to each plant individually. I stopped recording visits when I observed pollinators leaving the plot.

To measure effects of the treatments on visit quality, I collected one to six stigmatic lobes from two plants of each style morph in each plot, to measure conspecific and heterospecific pollen loads, on 2 days within each temporal replicate, during the afternoons when flowers had closed. Stigmas were mounted on microscope slides with basic fuchsin jelly (Kearns and Inouye 1993), so I could count the number of pollen grains and measure pollen diameter to estimate the number of outcross pollen grains on stigmas.

After each experimental replicate, I tallied fruits on each marked floral node (marked with different colors to denote different treatment combinations). I collected these fruits when they matured to measure both the number of seeds produced per fruit and mean seed mass (of up to five mature seeds).

### Data analysis

For all statistical analyses, I used mixed effects models (Pinheiro and Bates 2000) with appropriate error distributions to describe the data, using the Laplace method for non-normally distributed data and restricted maximum likelihood for normally distributed data. From these models I conducted likelihood ratio tests between full models and models reduced by one variable (testing each effect separately), using the lmer function in R (version 2.4.1; R Development Core Team 2006). In each case, I tested for fixed effects of interplant distance (as an ordered factor), co-flowering treatment (as an unordered factor), and for effects of interactions between interplant distance and co-flowering treatment. I report only significant interaction effects below. I also tested for random effects of temporal replicate and plot. *df* in tests for random effects are equal to 1, regardless of the number of levels in the random effect. This is because likelihood ratio tests for random effects estimate only one parameter: the variance of the random effect.

### Visit quantity

I tested for effects of interplant distance and co-flowering treatment on three measures of visitation (described above): (1) the number of visits to plants per plot visit, measured as the number of plants each pollinator visited from the time it entered the plot until it left (a measure of residency within a plot); (2) visitation rate to individual plants per hour, calculating the number of visits to each plant during each temporal replicate per observation hour, from pollinator trajectories recorded during observation periods; and (3) taxa richness, the number of species per observation hour. When the species identities of pollinators were unknown, I lumped these taxa into broader categories (e.g., other skippers), and treated these as one taxon. I assumed that all measures of visit quantity were Poisson distributed. Because pollinator visits were observed in low-density treatments in which plants were distant from one another, observers may have underestimated the number of pollinator visits to plants in low-density plots. Thus, I conducted a post hoc test on the number of visits received by plants per hour [(2) above], using only the four central plants (nearest to observers and potentially most reliable) in the two low-density treatments. In addition, because

some plants were allowed to have more than one flower per day, this may influence the density of flowers in a plot, and thus could change pollinator visitation patterns. Thus, I conducted a second post hoc test on this data set, using only data from plants that produced one flower. Since the patterns and significance of main effects from both post hoc tests were the same as those of tests using the full data set, I report only the results of tests using the full data set. Further, an analysis of the number of visits to plots per hour (a redundant measure of visit quantity) yielded results that were similar to those for other measures of visit quantity (data not shown), so I did not include it here.

To determine if pollinator taxa differed in the number of visits to plants per plot visit, I conducted separate analyses of deviance for the ten taxa most frequently observed during the experiment (Table 1).

### Visit quality

To measure visit quality, I counted conspecific and hetero-specific pollen grains on mounted stigmas, and determined: (1) the number of conspecific pollen grains per stigma lobe

**Table 1** Insect types observed visiting *Piriqueta caroliniana* during experimental replicates, and their percent abundances. *Numbers in parentheses* indicate the number of observed visits to *Coreopsis leavenworthii* plants during observation periods in treatments with co-flowering plants

| Insect type                                    | Percent of visits to plots ( <i>n</i> = 1,269) | Percent of visits to plants ( <i>n</i> = 2,259) |
|--|--|---|
| Lepidoptera                                    | 66.6   | 69.9  |
| <i>Polites vibex</i> <sup>ab</sup> (3)         | 17.2   | 27.2  |
| <i>Copaeodes</i> sp. <sup>b</sup> (0)          | 16.4   | 2.9   |
| <i>Erynnis</i> spp. <sup>ab</sup> (16)         | 3.2  | 3.4   |
| Other skippers <sup>b</sup> (7)                | 5.4  | 19  |
| <i>Eurema दौरा</i> <sup>ab</sup> (2)           | 19   | 13.8  |
| <i>Strymon melinus</i> <sup>ab</sup> (1)       | 2.3  | 3.5   |
| Other (3)                                      | 3.1  | 0.1   |
| Hymenoptera                                    | 27.6   | 25.9  |
| <i>Agapostemon splendens</i> <sup>ab</sup> (1) | 5.8  | 6.1   |
| <i>Megachile</i> spp. <sup>ab</sup> (4)        | 8.4  | 8.8   |
| Other solitary bees (1)                        | 1.5  | 0.9   |
| <i>Bombus</i> spp. <sup>ab</sup> (2)           | 7.3  | 7.8   |
| Other (8)                                      | 4.6  | 3.7   |
| Diptera  | 4.6  | 3.5   |
| <i>Bombyliidae</i> <sup>ab</sup> (0)           | 4.5  | 3.4   |
| Other (0)                                      | 0.1  | 0.1   |
| <i>Coleoptera</i> (Cantharidae) (0)            | 1.2  | 0.7   |

<sup>a</sup> Taxa observed in all treatments

<sup>b</sup> Taxa used in analyses of visits per plot visit by single taxon

(if pollen grains on more than one lobe were counted, I averaged these values); (2) the number of putative outcross pollen grains per stigma lobe, based upon differences in the sizes of pollen grains produced by each morph (methods described in the [Appendix](#)); (3) the number of pollen grains of *C. leavenworthii* per stigma lobe; and (4) the number of other heterospecific pollen grains per stigma lobe. I assumed that for the number of conspecific, outcross, and heterospecific pollen grains per stigma, data were best described by normal distributions. Because the mean numbers of *C. leavenworthii* pollen grains per stigma were generally low (<5), I assumed these data were best described by a Poisson distribution. Because short-styled plants may be more likely to receive geitonogamous pollen than long-styled plants, I tested for effects of density and co-flowering treatment on putative outcross pollen receipt by each style morph separately. I conducted separate tests on the number of putative incompatible pollen grains received (pollen from the same plant or from other plants of the same style morph), testing both morphs combined and each separately, to determine if stigmas might be clogged by incompatible pollen at low or high densities. In addition, I tested whether estimated seed production is correlated with outcross pollen receipt in *P. caroliniana*, calculating the mean number of seeds produced by each plant (in each replicate) from which stigmas had been collected.

### Reproductive success

Reproductive success of plants was measured in three ways: (1) the probability that a given flower will become a fruit, which hereafter I refer to as “fruit set”; (2) mature seeds produced per fruit; and (3) seed mass of mature seeds. I calculated fruit set by dividing the number of fruits produced by each plant in each replicate by the number of flowers on that plant during the experimental replicate. To calculate seed mass, I weighed up to five seeds per fruit (when available) and averaged these. I transformed fruit set data (which are binomially distributed) using the arcsine square-root transformation, and assumed that fruit set, the number of seeds produced per fruit and seed mass were all normally distributed.

## Results

### Effects of interplant distance

#### Visit quantity and visitor diversity

Insect visitors to *P. caroliniana* plants in the experimental plots included several species of Lepidoptera (66.6% of

visits), Hymenoptera (27.6% of visits), Diptera (4.6% of visits), and Coleoptera (1.2% of visits; Table 1). Beetle visitors were all Cantharidae, which may have been florivores. In addition, at least nine taxa were observed in all treatment combinations (Table 2). At least 14 taxa visited *C. leavenworthii* during the observation periods, moving between flowers of *C. leavenworthii* and *P. caroliniana*. These taxa included the most common visitors to *P. caroliniana*: *Erynnis* spp., *Polites vibex*, other Hesperidae, sphecids wasps, megachilid bees, *Eurema daira*, *Bombus* spp., other Lepidoptera, and other Hymenoptera (Table 1 shows numbers of visits by each taxon to *C. leavenworthii*).

The number of plants that pollinators visited per plot visit decreased 58% with increasing interplant distance (here, percentages indicate the approximate change from the smallest to the greatest interplant distance; Fig. 2a; Table 2). For all of the taxa tested separately, the number of visits to plants per plot visit decreased as interplant distance increased. Two taxa, *P. vibex* and “other Hesperidae”, showed significant negative effects of interplant distance ( $\chi^2 = 24.66$  and  $24.23$ , respectively;  $n = 38$  and  $40$ , respectively;  $df = 3$ ;  $P < 0.001$  in both cases). All other taxa except Bombyliidae showed non-significant negative effects of increasing interplant distance. Individual plants received 82% fewer visits per hour as distances between plants increased (Fig. 2b). Further, taxa richness of pollinators (the number of taxa per observation hour) also decreased 38% as a function of interplant distance (Fig. 2c; Table 2).

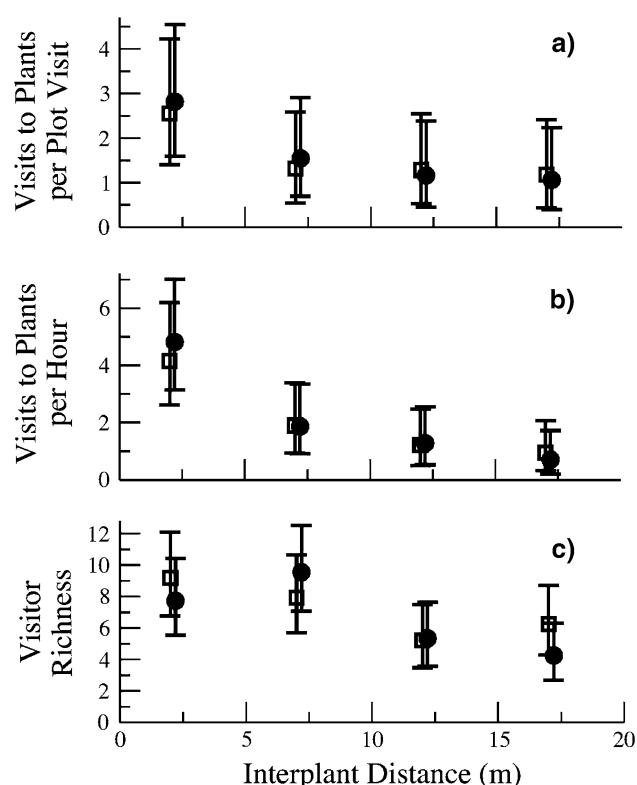
**Table 2** Summary of statistical results for visitation rates to *P. caroliniana* plants in experimental plots. Fixed effects are in **bold**. In each case,  $n = 40$  total plots, or eight plots used in each of five replicates

| Response variable                       | Covariates                 | $df^a$ | $\chi^2$ statistic |
|---|----------------------------|--------|--------------------|
| Number of plants visited per plot visit | <b>Interplant distance</b> | 3      | 8.80*              |
|   | <b>Co-flowering sp.</b>    | 1      | 0.03               |
|   | Plot                       | 1      | 0                  |
|   | Temporal replicate         | 1      | 0                  |
| Visits to plants per hour               | <b>Interplant distance</b> | 3      | 34.65***           |
|   | <b>Co-flowering sp.</b>    | 1      | 0.07               |
|   | Plot                       | 1      | 0                  |
|   | Temporal replicate         | 1      | 0                  |
| Taxa richness of visitors per hour      | <b>Interplant distance</b> | 3      | 16.24**            |
|   | <b>Co-flowering sp.</b>    | 1      | 0.26               |
|   | Plot                       | 1      | 0                  |
|   | Temporal replicate         | 1      | 0                  |

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

<sup>a</sup> Test  $df$  ( $df$  of the full model –  $df$  of models reduced by each variable)



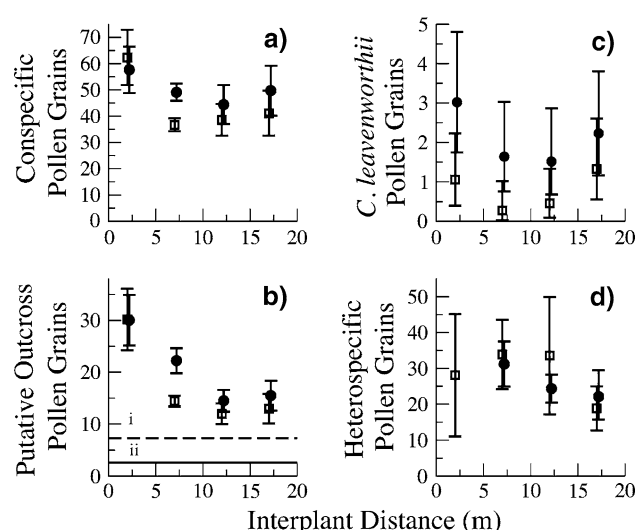


**Fig. 2** Effects of interplant distance and co-flowering treatment on pollinator visitation to *P. caroliniana*. **a** the number of plants visited by pollinators per plot visit, **b** visits received by individual *P. caroliniana* plants per hour decrease with increasing interplant distance, and **c** visitor taxa richness observed in experimental plots per hour decreases with increasing interplant distance. Open squares denote treatments with *P. caroliniana* plants only, and closed circles denote treatments with *C. leavenworthii* plants (mean  $\pm$  95% Poisson confidence intervals)

#### Visit quality (pollen loads)

Conspecific pollen loads on stigmas of *P. caroliniana* decreased 24% with increasing interplant distance (Fig. 3a; Tables 3, 4). The estimated number of putative outcross pollen grains per stigma lobe decreased 53% as a function of interplant distance (Fig. 3b; Tables 3, 4). When I analyzed data separately by style morph, there was still a significant negative effect of interplant distance on outcross pollen receipt (Tables 3, 4). Further, the estimated number of putative incompatible pollen grains per stigma lobe was unaffected by distance (Tables 3, 4).

*C. leavenworthii* pollen loads on stigmas of *P. caroliniana* were unaffected by increasing interplant distance. Stigma lobes received fewer than four *C. leavenworthii* pollen grains on average in all treatments (Fig. 3c; Tables 3, 4). Heterospecific pollen loads (pollen from species not including *C. leavenworthii*) on stigmas of *P. caroliniana* were unaffected by interplant distance (Fig. 3d; Tables 3, 4). Many of the heterospecific pollen grains appeared to be



**Fig. 3** Effects of interplant distance and co-flowering treatment on pollen receipt in *P. caroliniana*. **a** Conspecific pollen load per *P. caroliniana* stigma lobe (mean  $\pm$  SE). **b** The number of putative outcross pollen grains per *P. caroliniana* stigma lobe (mean  $\pm$  SE). The upper dotted line (i) denotes the largest number of seeds observed in a fruit, which is divided by the number of stigmatic lobes per plant to make this value comparable to the other values in the figure (assuming equal seed production per stigmatic lobe). The lower solid line (ii) denotes the mean number of seeds produced per fruit divided by the number of stigmatic lobes per plant. **c** *C. leavenworthii* pollen load per *P. caroliniana* stigma lobe (mean  $\pm$  95% Poisson confidence intervals). **d** Heterospecific pollen load (not counting *C. leavenworthii* pollen) per *P. caroliniana* stigma lobe (mean  $\pm$  SE). Open squares denote treatments with *P. caroliniana* plants only, and closed circles denote treatments with *C. leavenworthii* plants

from grasses, perhaps from the *Paspalum notatum* plants that were dominant in the pastures.

#### Reproductive success

Fruit set decreased 47% with increasing interplant distance (Fig. 4a; Table 5). In addition, both the number of seeds per fruit and seed mass were unaffected by interplant distance (Table 5; Fig. 4a–c).

The mean number of seeds produced per fruit was marginally positively correlated with the number of putative outcross pollen grains per stigma lobe when covariates and random effects were included ( $n = 40$ ;  $df = 1$ ;  $\chi^2 = 3.59$ ;  $P = 0.06$ ). However, mean fruit production was uncorrelated with pollen receipt. Further, the number of putative outcross pollen grains received was much larger than the average number of seeds produced per fruit ( $15.5 \pm 0.3$  seeds; mean  $\pm$  SE) and even than the maximum number of seeds observed in one fruit (44; Figs. 3b, 4b). Although conspecific pollen load and putative outcross pollen load were positively correlated with both visits to plants per plot visit and visits to plants per hour, these effects were not significant in models that included effects

**Table 3** Results from analyses on visit quality (pollen receipt by *P. caroliniana* stigmas): conspecific and putative outcross pollen receipt by *P. caroliniana* stigmas. Fixed effects are in **bold**. In each case,  $n = 40$  total plots, or eight plots used in each of five replicates

| Response variable   | Covariates  | $df^a$ | $\chi^2$ statistic |
|---|---|--------|--------------------|
| Conspecific pollen load per stigma branch                   | <b>Interplant distance</b>                          | 3      | 10.33*             |
|   | <b>Co-flowering sp.</b>                             | 1      | 1.50               |
|   | Plot  | 1      | 0.91               |
|   | Temporal replicate                                  | 1      | 4.84*              |
| Number of putative outcross pollen grains per stigma branch | <b>Interplant distance</b>                          | 3      | 28.43***           |
|   | <b>Co-flowering sp.</b>                             | 1      | 2.30               |
|   | Plot  | 1      | 0.01               |
|   | Temporal replicate                                  | 1      | 1.74               |
| Long style morph  | <b>Interplant distance</b>                          | 3      | 32.95***           |
|   | <b>Co-flowering sp.</b>                             | 1      | 0.92               |
|   | Plot  | 1      | 0                  |
|   | Temporal replicate                                  | 1      | 5.66*              |
| Short style morph   | Interplant distance $\times$ co-flowering treatment | 3      | 7.44†              |
|   | <b>Interplant distance</b>                          | 3      | 16.44***           |
|   | <b>Co-flowering sp.</b>                             | 1      | 2.22               |
|   | Plot  | 1      | 0                  |
| Number of putative self pollen grains per stigma branch     | Temporal replicate                                  | 1      | 0.18               |
|   | <b>Interplant distance</b>                          | 3      | 2.36               |
|   | <b>Co-flowering sp.</b>                             | 1      | 0.57               |
|   | Plot  | 1      | 0.49               |
|   | Temporal replicate                                  | 1      | 2.03               |

†  $P < 0.1$ , \*  $P < 0.05$ ,\*\*\*  $P < 0.001$ <sup>a</sup> Test  $df$  ( $df$  of the full model –  $df$  of models reduced by each variable)

of interplant distance ( $n = 40$ ;  $df = 1$ ;  $\chi^2 = 0.34$  for conspecific pollen load, and  $\chi^2 = 1.69$  for putative outcross pollen load).

#### Effects of co-flowering treatment

There were 65% more *C. leavenworthii* pollen grains on stigmas of plants in the treatments with *C. leavenworthii* plants (here, percentage indicates the percent difference between co-flowering treatments, averaged across

interplant distance treatments; Tables 3, 4; Fig. 3c). Otherwise, there were no significant effects of co-flowering treatment, and no significant interactions between interplant distance and co-flowering treatment, in any measure of visit quantity, visit quality, or reproductive success (Tables 2, 3, 4; Figs. 2, 3, 4). There was a marginally significant interaction effect between interplant distance and co-flowering treatment on the number of putative outcross pollen grains on long stigmas (Tables 3, 4), such that the negative effect of interplant distance was stronger in treatments without the co-flowering species.

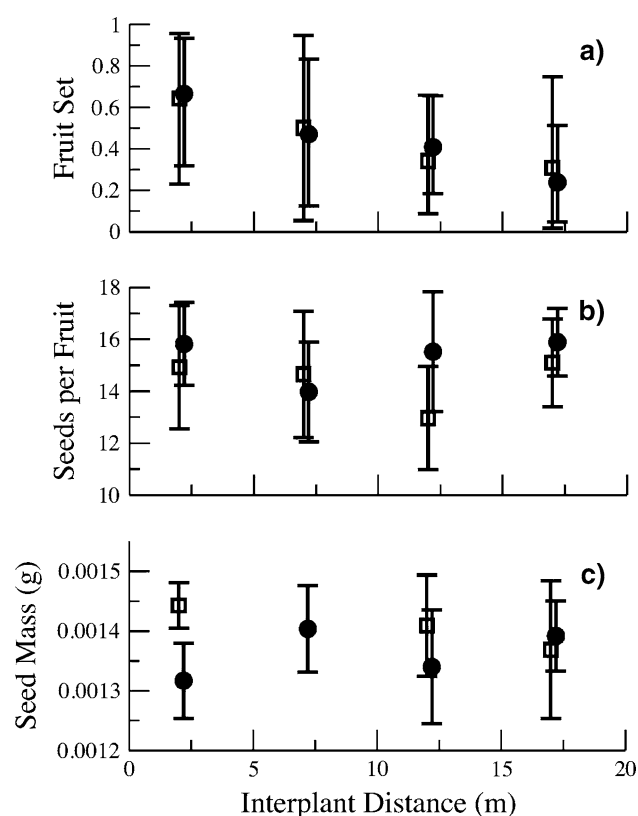
**Table 4** Summary of statistical results for *C. leavenworthii* and other heterospecific pollen receipt by *P. caroliniana* stigmas. Fixed effects are in **bold**. In each case,  $n = 40$  total plots, or eight plots used in each of five replicates

| Response variable                                     | Covariates                 | $df^a$ | $\chi^2$ statistic |
|---|----------------------------|--------|--------------------|
| <i>C. leavenworthii</i> pollen load per stigma branch | <b>Interplant distance</b> | 3      | 4.12               |
|   | <b>Co-flowering sp.</b>    | 1      | 12.95***           |
|   | Plot                       | 1      | 9.17**             |
|   | Temporal replicate         | 1      | 4.25*              |
| Heterospecific pollen load per stigma                 | <b>Interplant distance</b> | 3      | 3.65               |
|   | Co-flowering sp.           | 1      | 0.32               |
|   | Plot                       | 1      | 1.38               |
|   | Temporal replicate         | 1      | 9.50**             |

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ <sup>a</sup> Test  $df$  ( $df$  of the full model –  $df$  of models reduced by each variable)

#### Discussion

*P. caroliniana* plants experienced higher pollinator visitation rates, higher conspecific (and outcross, but not self) pollen loads, and higher fruit set at smaller interplant distances. Several mechanisms might explain these results, supporting two predictions of pollinator behavior in low-density patches of flowers. First, pollinators visited fewer plants per plot visit, which may be because flight costs are greater between widely spaced plants, or because neighboring plants were more difficult to detect in low-density patches. Second, plants received fewer visits per hour in low-density plots, indicating that pollinators are less able to find, or are less attracted to, low-density patches. Further, the negative effect of increasing interplant distance on



**Fig. 4** Effects of interplant distance and co-flowering treatment on reproductive success of *P. caroliniana*. **a** Probability that a *P. caroliniana* flower produces a fruit decreases with increasing interplant distance (mean  $\pm$  95% confidence intervals; these values were back-transformed into proportions by taking the sine of the mean and confidence intervals squared). **b** Seed production per fruit decrease as a function of interplant distance (mean  $\pm$  SE). **c** Mean weight of mature seeds produced by *P. caroliniana* plants are unaffected by interplant distance or co-flowering treatment (mean  $\pm$  SE). *Open squares* denote treatments with *P. caroliniana* plants only, and *closed circles* denote treatments with *C. leavenworthii* plants

visits from one species that visited *P. caroliniana* flowers most frequently (*Polites vibex*; Table 1), may in part drive the decreases in pollen load and reproductive success at low plant densities.

Because the density treatments used in this experiment were within the range of plant densities observed in natural populations, *P. caroliniana* plants growing at lower densities in natural populations may have lower fitness due to reduced visitation and reduced pollen receipt. **Researchers have commonly reported strong effects of conspecific plant density on reproductive success (e.g., Kunin 1997).** These low-density effects have the potential to cause Allee effects, leading to decline (Hackney and McGraw 2001) and even extinction of populations (Groom 1998). In this case, whether the negative effects of low density on individual fitness in this study could lead to population decline in *P. caroliniana* remain untested.

**Table 5** Summary of statistical results for reproductive effort of *P. caroliniana* plants: fruit set, seeds produced per fruit, and seed weight. Fixed effects are in **bold**. In each case,  $n = 40$  total plots, or eight plots used in each of five replicates

| Response variable        | Covariates                 | $df^a$ | $\chi^2$ statistic |
|--------------------------|----------------------------|--------|--------------------|
| Fruit set                | <b>Interplant distance</b> | 3      | 28.32***           |
|                          | <b>Co-flowering sp.</b>    | 1      | 0.36               |
|                          | Plot                       | 1      | 9.81**             |
|                          | Temporal replicate         | 1      | 35.84***           |
| Seeds produced per fruit | <b>Interplant distance</b> | 3      | 1.27               |
|                          | <b>Co-flowering sp.</b>    | 1      | 0.76               |
|                          | Plot                       | 1      | 0                  |
|                          | Temporal replicate         | 1      | 9.20**             |
| Mean seed mass           | <b>Interplant distance</b> | 3      | 3.26               |
|                          | <b>Co-flowering sp.</b>    | 1      | 1.52               |
|                          | Plot                       | 1      | 0.0978             |
|                          | Temporal replicate         | 1      | 0                  |

\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

<sup>a</sup> Test  $df$  ( $df$  of the full model –  $df$  of models reduced by each variable)

In this study, I necessarily confounded patch size and interplant distance. However, larger patches of flowers may attract more pollinators, and plants growing in larger patches may experience greater reproductive success (e.g., Sih and Baltus 1987)—these predictions contradict those for decreasing plant density. Thus, larger patch size per se did not likely influence the results of this study, in which negative effects of low density are demonstrated. In addition, in this study, “interplant distance” is an adequate surrogate for “plant density”. Analyses using plant density (the number of plants in a plot divided by the area occupied by the plot) rather than interplant distance gave the same results (analyses not shown).

**The co-flowering species, *C. leavenworthii*, had no effect (facilitative or competitive) on visitation, conspecific pollen receipt, or reproductive success of *P. caroliniana* plants.** In this study, pollinators made few transitions between *P. caroliniana* and *C. leavenworthii* flowers. Although *P. caroliniana* stigmas received more pollen of *C. leavenworthii* in the presence of the co-flowering species, they received very few (<4 on average) co-flowering pollen grains per stigma branch. Also, pollen grains of *C. leavenworthii* are very small in comparison to *P. caroliniana* pollen grains, covering very small areas of the stigmatic surface. Thus, larger numbers of *C. leavenworthii* pollen grains may be necessary to negatively impact conspecific pollen receipt or germination in *P. caroliniana*.

Recent studies outline several mechanisms by which pollination facilitation may occur (e.g., Feldman et al. 2004; Moeller 2004; Ghazoul 2006). Mechanisms include accelerating pollinator aggregative response to patches of



flowers (Lavery and Plowright 1988; Feldman et al. 2004), accelerating functional response to floral density (possible mechanism in Rathcke 1983; Lavery 1992; Johnson et al. 2003) or to diversity of floral resources (Ghazoul 2006), and increased pollinator numerical response across years in diverse populations (Moeller 2004). All of these studies involve plants that flower at the same time and share pollinators, which was also the case for *P. caroliniana* and *C. leavenworthii*. Perhaps in this system the floral diversity and abundance outside the experimental plots diluted any potential effect of *C. leavenworthii* on visitation. Alternatively, perhaps floral constancy in the plot was so strong that most individual pollinators behaved as if only one plant species was in a given plot (as indicated by the small numbers of transitions observed between the two experimental species). This effect of constancy has recently been invoked to explain reproductive isolation between sympatric species sharing pollinators (Ollerton et al. 2007; Yang et al. 2007). Also, because all replicates of this experiment were conducted over a few weeks, it is impossible to evaluate the potential for pollination facilitation through increased numerical response of pollinators to increased plant density in this system.

Pollinator visitation was not correlated with receipt of conspecific or outcross pollen grains. Pollinator visits varied temporally and spatially as well as with treatment. Variation in pollinator visits, combined with variation in the amount and type of pollen carried (heterospecific or conspecific, self or outcross pollen from long- or short-styled plants), may explain variation in pollen receipt.

Similarly, seed production was not correlated with the estimated number of outcross pollen grains. Plants in all treatments likely received more outcross pollen grains on average than the maximum observed number of fertilized ovules. Several studies have documented similar discrepancies between mean pollen receipt and seed production (Campbell 1991; Caruso 2002). Perhaps either pollen from plants of the same style morph (including self pollen) blocked stigmas, preventing compatible pollen from germinating, or other factors such as below-ground resources limited seed production (Schemske and Horvitz 1988). Also, in my test for a correlation between outcross pollen receipt and seed production, I could not link pollen receipt by individual flowers to seed production in the fruits those flowers produce. Instead, I used mean seed production per plant, which likely ignores important variation in pollen receipt and seed production. In addition, variation in both pollinator visits and pollen receipt may result in the large variation in seed production.

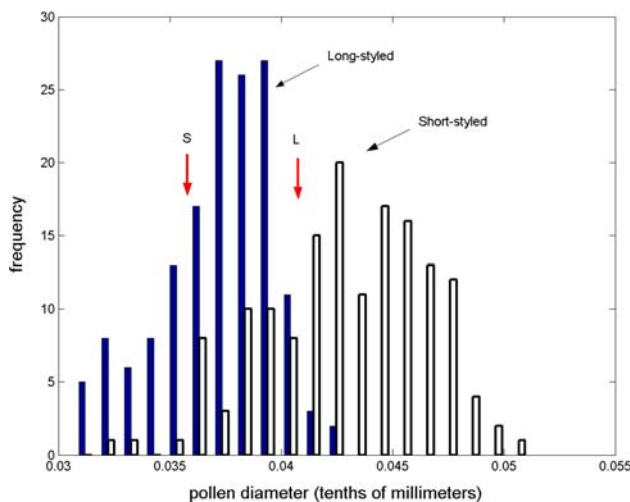
This study is one of very few experimental tests for effects of density of both conspecifics and co-flowering species on individual fitness, defined as fruit and seed production (see also Campbell 1985; Caruso 1999; Lavery

1992; Moeller 2004; Ghazoul 2006; Internicola et al. 2006). The negative individual-level impacts of low conspecific density were strong within the range of densities observed in natural populations. Effects of conspecific density may be more important than those of co-flowering species in many systems. However, co-flowering species may be more important for outcrossing species that produce no floral rewards (e.g., Lavery 1992; Johnson et al. 2003). In addition, factors that affect individual fitness may not alter population growth rates. In long-lived perennial plants, increases in fecundity tend to have little effect on population growth rate (Silvertown et al. 1993), but may increase the contributions of these individual plants to the population growth rate relative to other individuals (Miriti et al. 2001). In these long-lived species, facilitative or competitive effects on growth rates or seedling recruitment may have larger effects on the growth rate of the population than any effects of co-flowering species on pollination. It is important to further characterize the conditions under which co-flowering species affect each other's reproductive success, to aid predictions of facilitative or competitive effects on persistence of species in fragmented or small populations (Spira 2001), delimitation of effective range limits, or establishment and maintenance of introduced species (Brown et al. 2002).

**Acknowledgements** This work was funded by the National Science Foundation (Predoctoral Fellowship and Doctoral Dissertation Improvement Grant) and the Duke University Biology Department (Grant-in-Aid of Research). Thanks to W. F. Morris, for very helpful comments on experimental design and on all aspects of the manuscript. Thanks also to J. S. Clark, M. Rausher, J. Willis, W. G. Wilson, and the members of their labs for useful discussion, comments on drafts of the manuscript, and statistical advice (especially B. M. Bolker, D. M. Bates, I. Fiske, S. Graves, K. Gross, W. A. Ray, and M. Wolosin). Thanks to L. C. Ray and to several reviewers for comments on the manuscript. I am very grateful to Jessi Patti for help with fieldwork, and to Jessica Figueroa and Jena Jamison for help with data entry. M. Byrne, M. Folk, D. Gordon, P. Royston, and S. Woiak (the Nature Conservancy's Disney Wilderness Preserve) provided research support, and M. Cruzan and S. Cole provided additional field advice. All work conducted in this study was in compliance with current United States, North Carolina and Florida laws.

## Appendix

I tested whether distance and co-flowering treatment affect the number of outcross pollen grains on stigmas. To determine the ranges of pollen grain sizes produced by anthers of *P. caroliniana* plants of the two floral morphs, I measured diameters of pollen grains from anthers of five long- and five short-styled *P. caroliniana* plants. I used digital photographs of pollen from anthers to calculate pollen diameters [in pixels;  $75.40 \pm 0.46$  pixels (mean  $\pm$  SE)] using Adobe Photoshop® (Adobe 1999–



**Fig. 5** A frequency histogram of pollen grain diameters (in tenths of millimeters) measured from pollen grains from *P. caroliniana* anthers. Dark bars indicate pollen from long-styled plants, and open bars indicate pollen from short-styled plants. Downward-pointing arrows indicate the lower (for short-styled plants) or upper (for long-styled plants) 95% confidence limits of the distribution

2002), converting these numbers to actual pollen diameters using the number of pixels per micrometer from digital photographs of a stage micrometer taken at the beginning and end of each photographic session.

Pollen grains of short-styled plants have larger diameters on average than pollen grains of long-styled plants [ $4.22 \pm 0.30$  vs.  $3.74 \pm 0.09$   $\mu\text{m}$ , respectively (mean  $\pm$  SE); seven short-styled anthers; nine long-styled anthers;  $df = 10$ ;  $t = 4.57$ ;  $P < 0.01$ ; 10–30 pollen grains were measured per anther]. However, pollen grain sizes of the two style morphs overlap (Fig. 5). Knowing both the style morph of each plant and the size range of pollen grains produced by each morph, I was able to estimate the probability that a given pollen grain was from a plant of the opposite style morph, using the equations:

$$P_L(X) = \frac{S_X}{S_X + \alpha L_X} \quad (1)$$

$$P_S(X) = \frac{\alpha L_X}{S_X + \alpha L_X} \quad (2)$$

where  $X$  indexes the diameter class of a pollen grain,  $S_X$  and  $L_X$  are the fractions of pollen grains produced by short-styled and long-styled flowers, respectively, that fall into size class  $X$ ,  $P_L(X)$  and  $P_S(X)$  are the probabilities that a pollen grain in size class  $X$  found on the stigma of a long- or short-styled flower, respectively, is a compatible outcross pollen grain, and  $\alpha$  is the estimated ratio of the number of pollen grains produced in anthers of long-styled plants versus short-styled plants. In some distylous plants, one style morph produces more pollen than the other

(Li and Johnston 2001). If anthers of long-styled flowers produce more pollen than those of short-styled plants ( $\alpha > 1$ ), then  $P_L(X)$  is smaller and  $P_S(X)$  is larger than if anthers of both style morphs produce equal amounts of pollen.

I measured diameters of pollen grains in digital photographs of mounted stigmas collected during the field experiment (using methods described above). I used maximum likelihood methods to estimate  $\alpha$  by fitting the fraction of pollen grains produced by long-styled anthers ( $f$ ) [ $f/(1-f) = \alpha$  in Eqs. 1 and 2], using data on diameters of pollen grains found on stigmas as the representation of the overall pollen pool. Specifically, I used the equation:

$$P(X, f) = fL_X + (1-f)S_X \quad (3)$$

where  $P(X, f)$  is the probability that a pollen grain randomly chosen from the pollen pool is in size class  $X$ . I assumed that the fractions of pollen grains among size classes followed a multinomial distribution. The log likelihood function of  $f$  is:

$$\log L = \sum_{i=1}^n [X(i) \log(P(X, f))] \quad (4)$$

Using these methods, the estimated fraction of pollen produced by long-styled morphs ( $f$ ) = 0.5893 and  $\alpha$  [ $=f/(1-f)$ ] = 1.4346.

I then determined, for each pollen grain on a given stigma, the probability that it was produced by a plant of the opposite style morph (using the estimate of  $\alpha$  from Eqs. 3 and 4 in Eqs. 1 and 2), and summed these probabilities for the subset of pollen grains measured on that stigma (I measured diameters only for pollen grains in the focal plane of the photograph). I estimated the number of outcross pollen grains per stigma, by dividing the total proportion of outcross pollen by the number of pollen grains measured, and multiplying by the number of pollen grains per stigma lobe.

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