DO NATIVE ANTS PLAY A SIGNIFICANT ROLE IN THE REPRODUCTIVE SUCCESS OF THE SAN FERNANDO VALLEY SPINEFLOWER, *CHORIZANTHE PARRYI* VAR. *FERNANDINA* (POLYGONACEAE)?

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Abstract

Previous field studies of the reproductive biology of the San Fernando Valley Spineflower suggested that pollination by ants might be an important feature of this small endangered polygonous taxon. This conclusion was based on observations that native ants were the most abundant floral visitors and were constant to this species. The current controlled study was undertaken to explore more stringently the possibility that native ant vectors were facilitating pollination resulting in viable seed set. Based on our current experimental data, ants can indeed be effective pollinators of this taxon. Although 27% of flowers in the control group (all vectors excluded) set fruit, fruit set in flowers exposed to the native ant *Dorymyrmex insanus* was 57% higher. Further, a 25.7% germination rate was registered for achenes produced in the absence of any vector in contrast to a 61% rate in those produced in the presence of the ant vector. Also touched upon are the association between ant pollination and drier climates, why ant production of inhibitory substances may not be a severe limitation to their function as pollinators, potential effects of invasive Argentine ants, why self pollination may not be a negative attribute for these plants, and conservation and management implications of our results.

Introduction

Recent studies have investigated a variety of factors related to the reproductive success of the San Fernando Valley Spineflower (SFVS), *Chorizanthe parryi* S. Watson var. *fernandina* (S. Watson) Jepson, an endangered California polygonous species formerly thought to be extinct (Jones, et al., 2002; 2004). These studies have demonstrated that the SFVS is at least a facultative selfer with a mixed mating strategy; i.e., has adopted a generalist pollination strategy and is not pollinator limited. It sets abundant fruit and is visited by a substantial variety of potential pollinators including native ants. Indeed, ants were among the most abundant visitors to the flowers of the SFVS. Since at least three different species of native ants were significant visitors to the flowers of the SFVS at the Ahmanson and Newhall Ranch sites, we decided that follow-up studies were warranted to address the following questions:

1. To what degree is the SFVS adapted for ant pollination? How specifically does it meet Hickman's 10 characteristics of "Ant-Pollinated Flowers?"
2. Are ants demonstrably effective pollinators of this species? Are viable seeds produced as a result of ant floral visitations?
3. Are the ants facilitating outcrossing as well as selfing? Is selfing per se an evolutionary constraint in this species?

Materials and Methods

The Plant Species: Formerly distributed in Southern California from Lake Elizabeth in Los Angeles County to near Del Mar in San Diego County (Jones, et al., 2002), the SFVS has been described as being found within coastal sage communities at elevations below 350 m. After being considered extinct for a time (Hickman, 1993), the SFVS was found in two locations where it tends to occur in dense patches of several hundred plants primarily in dry, sandy places within surrounding coastal sage vegetation. It is currently designated as a List 1B.1 plant (Rare, Threatened, or Endangered in California or Elsewhere; seriously endangered in California) by the California Native Plant Society and is State-listed Endangered (CNPS, 2001) and a Federal candidate for similar listing (CNPS, 2005). Stems of the SFVS spread more or less horizontally from the base to form a low, flat-topped, grayish plant 0.2-0.8 dm high and 0.5-4 dm across (see Fig. 1). The sessile flowers are 2.5-3 mm long with a greenish-white tube and 6 white, sparsely hairy lobes, occurring in two series of 3 (Reveal, 1989). Filaments and anthers of the nine stamens are white. The ovary is glabrous and bears three styles. Nectar is present around the base of the ovary and between the filaments. The flowers are protandrous and are produced in late spring, April-June. The fruit is a brown achene, 2.5-3 mm long with a 3-angled beak.

Ants as possible pollination vectors investigation: The specific purpose of this experiment was to determine if there was a statistical difference in fruit-set and seed viability between SFVS plants exposed to potential ant pollinators versus those grown without exposure to a vector (selfs). SFVS achenes were extracted from plants field-collected from the Ahmanson Ranch site 3 (34˚ 25.12' N, 118˚ 35.14' W) during the first week of April 2005 and held in refrigerated storage until they were extracted from the inflorescences.

Figure 1. Photo of the SFVS with pollinator. A. Unopened flower bud; B. Open flower with dehiscing anthers – flowers protandrous; C. Open flower with receptive stigma; D. Post-pollinated flower – perianth retained ; E. Pollen of the SFVS on head of Pyramid Ant, *Dorymyrmex insanus*. Photo by Robert L. Allen.

The extracted achenes were divided into two equal groups. Two separate screened enclosures held forty SFVS plants each evenly spaced on a black plastic tray (43.5 cm square X 60 mm deep) filled with premium grade potting soil and top layered with the commercially produced sterile sand. Approximately 500 sterile female *Dorymyrmex insanus* worker ants were also collected from the Ahmanson Ranch site 3 at the end of the second week of May 2005 and were introduced into a screened enclosure (Enclosure 1, Experimental), whereas no vectors were introduced into the second one (Enclosures 2, control). The flowers from each plant were subsequently counted in each enclosure as was the fruit-set per flower. After all plants had died in each of the two separate enclosures, each individual plant was harvested and placed into a separate numbered clear plastic bag.

Achene Viability Study: In order to determine if the achenes produced with or without ant vectors were viable, we conducted a germination study. The achenes were extracted from the controlled experimental SFVS plants. The one seeded achenes were then subjected to germination tests. Three hundred achenes from Enclosure 1 (with ants) and three hundred from Enclosure 2 (with no potential vectors) were each divided into subgroups of 15 achenes each and placed on moistened seed germination paper and placed in one of four Percival Model E-30B growth chambers set on 11 hours of daylight with 15° C daytime temperature and 10° C nighttime temperature.

Results

Ants as possible pollination vectors. The results, which detail the number of flowers and achenes collected per plant, are shown in Table 1. A comparison of the fruit set from the control plants (from enclosure 2) were compared to the fruit-set from the ant exposed enclosure (1). The result was significant at <0.001 (t=24.612, df=38).

Enclosure 1 with ants present. N=40 plants.				
Character	N=Total produced on all plants	Range	Average	Standard deviation
Number of flowers per plant	4422	51 -144	110.6	22.5
Fruit set in %	3728	55 -100	84.3	13.8
Enclosure 2 without ants present. N=40 plants.				
Character	N=Total produced on all plants	Range	Average	Standard deviation
Number of flowers per plant	4481	44 - 142	112.0	22.3
Fruit set in %	1196	16.8 – 38.5	26.7	5.2

Table 1. Comparison of number of flowers produced per plant and fruit set per plant for the two enclosures. Enclosure 1 (experimental) with ants present. Enclosures 2 (control) lacked ants.

Approximately 27% of the flowers within the control group, which lacked ants set fruit without a vector as compared to 84% fruit-set in flowers exposed to the ant species *Dorymyrmex insanus*. This is clear evidence that this plant can set fruit without a vector, but fruit set with *Dorymyrmex insanus* was higher by approximately 57%. This clearly demonstrates that the ant species *D. insanus* effectively transported pollen when all other pollination vectors were excluded.

Achene Germination: Achenes harvested from the enclosure experiments were tested for germination under controlled growth chamber conditions. Of the 300 achenes produced in the absence of any vector (selfs without a vector), 77 germinated (for 25.7% germination). In contrast, of the 300 achenes produced in the enclosure with ants, 183 germinated (for a 61% germination). Achenes produced via selfing without a vector were less than half as likely to germinate than those produced in association with ant vectors. Achenes produced via selfing showed a lower potential viability than those produced as result of outcrossing ($P<0.01$, $X^2 = 43.22$, df = 1).

Discussion

The pollination biology of this taxon: Individuals of the ant species, *Dorymyrmex insanus*, were effective pollen transporters and facilitated fruit-set in over 50% more flowers than when all pollinators were excluded.

Pollinators – ants and the SFVS: In terms of pollination interactions of the ten characteristics noted by Hickman (1974) in his description of what has been termed the "ant pollination syndrome" nine were documented in the SFVS.

Breeding system – selfing: Germination of achenes produced by selfing without a vector have a lower viability, than achenes produced with the aid of ants. However, our results support the Stebbins (1957) postulate that geographically restricted plants are likely to be self-compatible.

Selfing and ant pollination: In the case of a facultative selfer like the SFVS, ants appear to provide a reliable pollination vector that ensures successful reproduction via both selfing and outcrossing.

Conservation concerns – effects of population size variability and selfing: Small population sizes, resulting in decreased floral display and nectar resources, would be expected to lead to decreases in the number of flying visitors. Our data indicate that during harsh, dry, growing seasons, the SFVS may survive by producing a significant number of progeny via autofertility or by utilizing various native ant species as major pollination vectors. A decrease in the number of floral visitors or the production of a significant number of progeny via selfing with or without a vector would have important genetic implications in terms of interpopulation gene flow.

Argentine ant impacts: The issue of potential Argentine ant impacts on the reproductive biology of plants like the SFVS, which seems to depend on ants for a significant part of their reproductive effort, requires further investigation.

We conclude that the SFVS is neither pollinator limited, nor limited by lack of seed production or seed germination. We suggest that this species may be rare due to the destruction of suitable habitat. Understanding the ecology that promotes the well being of both the SFVS and its insect pollination vectors is of primary concern.

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