

Accompanying material for a paper published as a Noteworthy Data Set in the *Journal of Pollination Ecology*. Reviewers questioned these analyses and interpretations as being unclear or possibly inappropriate. Because circumstances prevented the authors from making corrections, these interpretations should not be considered to have the endorsement of peer review.

APPENDIX: SPATIOTEMPORAL VARIATION IN POLLINATOR TAXA ON THE SANTA ANA RIVER WOOLY STAR *ERIASTRUM DENSIFOLIUM* SSP. SANCTORUM (MILLIKEN) MASON (POLEMONIACEAE)

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STATISTICAL ANALYSIS

In order to determine if shifts in pollinator taxa occurred across observed years, seasons, and sites, the following analyses were performed with a significance level of $P < 0.05$ and a coefficient of determination of $r^2 > 0.5$. We further compared the results of total abundance recorded in 2008 to the results of abundance of 1995; a Student's T-test was used to compare the means of visitor abundance. We compared abundance when possible, across other years. A one-way ANOVA was performed on abundance (as counts per observation hour) to calculate the variance among the means of years to the average variance within each year. This analysis was repeated for abundance by site and by season. In order to compare shifts in taxa of each of the primary pollinators for 1995 and 2008 a one-way ANOVA was performed to compare, first, the shifts in taxa between years; second, the shifts in taxa among sites; and, third, to compare shifts among seasons. If significant differences were demonstrated by the ANOVA, a Tukey-Kramer HSD Test was performed to test for difference in principal pairs of taxa groupings. 5) To examine the similarity of primary pollinators of each site by season by year between 1995 and 2008 a Bray-Curtis similarity index was performed. 6) To determine the relationship between mean annual rainfall for the years of observation and for the years preceding observation, and abundance of each of the primary pollinator taxon, linear regression analyses were performed. Analyses were performed on Microsoft Excel™ Version 2007 and Kaleidagraph™ Version 4.0.

RESULTS

Pollinators. Pooling across seasons and sites, the primary pollinators in 2008 were hummingbirds, primarily the black-chinned hummingbird (31.7% of visits); Acton giant flower-loving fly (22.9%); western honey bee (21.3%); long-tongued digger bee (7.5%); longhorned digger bees (6.3%); and sweat bees, primarily *Halictus tripartitus* (4.1%); whereas, in 1995 the primary pollinators were longhorn digger bees (24%); hummingbirds (20%); sweat bees, primarily *tripartitus* (19%); bumble bees (13%); and the Acton giant flower-

loving fly (4.4%). There were significant differences between the two years for abundance by pollinator taxa: hummingbirds in comparison to butterflies and moths ($P < 0.001$) and western honey bees in comparison to butterflies and moths ($P < 0.01$).

A comparison of the data taken from the more comprehensive pollination studies of the flowering seasons of 2008 and 1995 and other less comprehensive years including 2000 (Atallah 2001), 1993 and 1994 (Stone 1995), 1991 and 1992 (Erickson 1993), 1989 and 1990 (Muñoz 1991) indicates some variation in abundance and, conversely, some consistency in primary pollinators taxa (Tab. 1).

During the 2000 season, observations were taken during mid and late season but were not taken for complete dawn to dusk periods at each site. Primary pollinators were sweat bees, primarily *Halictus tripartitus*, longhorned digger bees, bumble bees, hummingbirds, and the Acton giant flower-loving fly (Tab. 1). The taxa observed coincide with those present in 1995, although percent totals vary. During the 1993 and 1994 seasons, only one site (Site 5) was observed, from which the principal pollinators included bumble bees, hummingbirds, and the Acton giant flower-loving fly (Tab. 1). Sweat bees were secondary pollinators in both years. Observations in the 1991 and 1992 seasons were derived from two sites (Sites 1 and 5) from which the primary pollinators observed varied. During 1991 and 1992 there was only one primary pollinator observed at Site 1, the long-tongued digger bee. In 1991, the Acton giant flower-loving fly was a secondary pollinator. In contrast, at Site 5, two pollinators were consistent as primary pollinators, in both 1991 and 1992, hummingbirds and the Acton giant flower-loving fly, but a third primary pollinator changed from the white-lined sphinx moth in 1991 to the California bumble bee in 1992 (Tab. 1). During 1989 and 1990 (Site 1 and Site 3), the long-tongued digger bee was the primary pollinator at Site 1, whereas hummingbirds (primarily *Arhilocheus alexandri*) and the Acton giant flower-loving fly were the primary pollinators at Site 3 (Tab. 1).

2) Annual pollinator abundance. A comparison of the relative abundance of pollinator total visits in the two most comprehensive years 1995 and 2008 revealed a greater overall abundance of visits across sites and seasons for 2008 (Tab. 2). In 1995 there were 833 total recorded visits (Fig. 2) by a total of ten different taxa, in comparison to a total of 14,414 recorded visits (Fig. 2) by 17 taxa in 2008. During 1995, three taxa: sweat bees (19%), hummingbirds (24%), and digger bees (20%) contributed 63% of the total visits (Tab. 2). During 2008 western honey bees (21%), hummingbirds (32%), and the Acton giant flower-loving fly (23%) accounted for over 75% of all visits (Tab. 2). All three primary pollinators in 2008 exhibited a high number of visits per foraging bout. A one-way ANOVA comparing the abundance of pollinators for 1995, 2000, and 2008 indicated no significant differences ($P > 0.5$).

3) Pollinator abundance across blooming seasons. A one-way ANOVA of abundance by season (1995 and 2008) indicates no significant difference in pollinator abundance among early, mid, and late season ($P = 0.9$; see supplementary material).

4) Primary pollinator contribution across seasons. A one-way ANOVA of pollinator taxa abundance by season indicates a significant difference between 1995 and 2008 ($P = 0.03$), no significant difference within 2008 ($P = 0.7$), and no significant difference within 1995 ($P > 0.5$).

5) Comparison of site seasonal diversity between and within 1995 and 2008. Employing a Bray-Curtis similarity analysis of primary pollinator raw data for each site by season and by year, the array of primary pollinators of the sites in 2008 were not significantly similar to the array of primary pollinators in 1995. Exceptions are the site 5 mid-season 1995 and site 5 mid-season 2008 (45.53) and site 5 mid-season 2008 and early season 1995 (41.01). However, within 2008 and within 1995, there were several similarities, especially in mid- and late season data for 2008. In 2008, significant similarities occurred for site 1 during mid and late periods (75.12); site 2 during mid and late periods (53.56); and site 3 during mid and late season data (54.29). Site 5 did not show as close a similarity as the other sites for mid and late season data (37.47). In 1995, significant similarities occurred for site 1 during mid and late periods (65.64) and early and late (58.49) and early and mid-season (56.93). Thus, site 1 was the most consistent in the pollinator array throughout the seasons. In 1995, site 2 was only similar during mid and early season (41.18), whereas site 3 was similar in mid and late (53.33) and early and late (46.15), and finally site 5 was most similar in early and mid (83.14), slightly less similar between mid and late (49.14) and early and late season (46.07).

Across sites within the same year, the greatest similarity occurred between site 5, 2008 and site 2, 2008 (69.12). These sites represent a relatively younger seral stage site, site 2, and a relatively older seral stage site, site 5. Additionally, site 5, 2008 shared 51.01 similarity with site 3, 2008. Both sites represent older seral stage sites. Site 5 shared the greatest similarity with all other sites in 2008. In 1995, site 3 and site 2 were most similar (55.51 similarity). When using only raw

data numbers, the relatively small abundance of recorded pollinators in 1995 and the relative high abundance of pollinators made comparison difficult across the years.

Site abundance comparison. A one-way ANOVA of the abundance by sites for 1995, 2000 and 2008 indicates no significant difference in abundance by year and sites ($P = 0.2$).

Primary pollinator patterns at sites. A one-way ANOVA of the abundance of each of the primary pollinator contribution by site and by year resulted in no significant difference within 1995 ($P = 0.5$), within 2000 ($P = 0.45$), but a marginal significant difference was shown within 2008 ($P = 0.05$). No significant difference was shown amongst sites and years ($P = 0.6$).

Additionally, the ratio of visits of select taxa and all recorded visits for the observed years of 1995, 2000, and 2008, was compared across Sites 1, 2, 3, 5 (Tab. 2). From these data, the principal contributing pollinators for each site could be determined. In 1995, the largest contributions to overall visits for Site 1 were bumble bees (10%), butterflies and moths (5.3%) and hummingbirds (3.6%); for 2000, longhorned digger bees (2.6%); and for 2008, long-tongued digger bees (5.7%) and western honey bees (4.8%). In 1995, the largest contributions to overall visits for Site 2 were sweat bees (12.5%); for 2000, the Acton giant flower-loving fly (9.7%), sweat bees (9.7%), and hummingbirds (6.3%); for 2008, hummingbirds (3.6%). In 1995, the largest contributions to overall visits for Site 3 were sweat bees (2.9%); for 2000 hummingbirds (12.8%), the Acton giant flower-loving fly (12.2%), sweat bees (12.2%), and digger bees (4.4%); for 2008 hummingbirds (19.7%), the Acton giant flower-loving fly (15.4%) and western honey bees (9.1%). In 1995, the largest contributions to overall visits for Site 5 were hummingbirds (10.1%) and digger bees (18.4%); in 2000 hummingbirds (27%), the Acton giant flower-loving fly (5%), and sweat bees (4.9%); in 2008 hummingbirds (7.3%), the Acton giant flower-loving fly (4.4%), and western honey bees (Tab. 2).

Environmental Influence: mean Annual Rainfall and Primary Pollinator Abundance. 1995 was considered a "wet" year, whereas 2008 was considered a "dry" year (Tab. 3). Rainfall during other years of less comprehensive pollination studies includes "dry" years, 1989, 1990, 1994 and 2000 (19.96 cm), and "wet" years, 1991, 1992, 1993, and 1995 (Tab 3). Rainfall for 1993 and 1995 years was the highest, whereas rainfall during 1989, 1990, and 2000 years was the lowest. During January, February, and March, in 1995 total rainfall was 81% of total annual rainfall, and in 2008, 51.5% of total. Abundance of primary pollinators observed for years when data were available was correlated to mean annual precipitation for the year of observation and correlated to mean annual precipitation for the year before observations to elicit trends in pollinator abundance (Tab. 4). Although the abundance of several taxa demonstrated a strong relationship to precipitation, only two taxa demonstrated a significant negative relationship, the longhorned digger bees of the genus *Melissodes*, with rainfall of year of observation ($P = 0.01$), and the collective grouping of butterflies and moths with rainfall of the year prior to observations ($P = 0.05$), positive relationship (Tab. 4).

DISCUSSION

Overall, there was no statistical indication that pollinator abundance (number of visits) varied across sites, seasons or years. However, there were shifts in taxa acting as primary pollinator across seasons, suggesting that *Eriastrum* is a pollen and nectar resource for a diversity of taxa. Furthermore, this together with its blooming phenology may enable *Eriastrum* to take advantage of the natural seasonal and yearly cycling of the available visitors within their temperature and weather tolerances. A review of many years of past observations is limited to an examination by site as many observations were only taken at one or two of the populations. Of the nine years of complete and partial observations, hummingbirds and the Acton giant-flower loving fly were primary pollinators for eight and nine of those years respectively, the long-tongued digger bee and sweat bees for five years, bumble bees for four years, digger bees for three years, whereas the white-lined sphinx moth was the primary visitor for only one year (1991) and lepidopterans in general for two (Tab. 5).

Although *Eriastrum* is a generalist, there are two taxa that seem pivotal to its reproductive success, which are hummingbirds and the Acton giant-flower loving fly. Both the Acton giant flower-loving fly and hummingbirds are well-suited to pollinate the relatively large and long floral tubes of *Eriastrum* although they do have different habitat requirements. The Acton giant flower-loving fly has hairs on its body for pollen adhesion and they actively collect both pollen and nectar. Their long proboscis enables them to reach nectar in the long corolla of *Eriastrum*, which has an average length of 16-25 mm (Stone 1995). Pollination efficiency experiments by Stone (1995) and Dorsett et al. (2001) determined that they were effective pollinators, requiring only one stigmatic contact (Stone 1995). Furthermore, together with hummingbirds and white-lined sphinx moths, the Acton giant flower-loving fly visits many flowers and, unlike butterflies, and the long-tongued digger bee, flies long distances, between isolated populations of *Eriastrum*, promoting interpopulation gene exchange (Erickson 1993).

Hummingbirds are not only a frequent, but an effective pollinator (Anderson et al. 2001; Boyd 2004). Engel & Erwin (2003) noted that fertilization success is both a function of visits and the receipt of pollen to the stigma, and hummingbirds exhibit both high number of visits and effective pollen transfer. Furthermore, hummingbirds have been noted to exhibit high constancy (Aldridge & Campbell 2007). Similar to the behaviour of the Acton giant flower-loving fly, hummingbirds were noted by Muñoz (1991), Erickson (1993), Stone (1995), and Dorsett et al. (2001), to visit many flowers on one plant and also many plants. Muñoz (1991) noted specifically that they were monolectic. Erickson (1993) noted the constancy of hummingbirds and the behaviour of methodically working up and down one side of an *Eriastrum* plant before moving to next plant. Stone (1995) also noticed that birds usually had patches of pollen on their bodies and beaks, as was also observed in 2008. These behavioural observations suggest the conclusion that hummingbirds exhibit high constancy and effectiveness as pollinators of *Eriastrum*. Further, Dorsett et al. (2001) noted relatively high

experimental pollinator efficiency, although bumble bees and Acton giant flower-loving flies were more efficient. Stone (1995) supported the effectiveness of hummingbirds in pollinator efficiency experiments with the resultant finding that two stigmatic contacts of hummingbirds produced equivalent seed set to pollination by Acton giant flower-loving flies.

Native bees, especially sweat bees of the family Halictidae, particularly the species *H. tripartitus* and *H. farinosus*, the longhorned digger bees (*Melissodes* spp.), and the long-tongued digger bee (*Micranthora flavocincta*), have been primary or secondary pollinators for many years of observation. Of the primary and secondary taxa recorded, sweat bees appear to exhibit a decline in either overall abundance and or in percent contribution from 1994 to 2008, although not statistically significant. Sweat bees tend to be observed in years where there were multiple primary pollinators. Interestingly, in the past when long-tongued digger bees were observed, sweat bees were not.

Over the years long-tongued digger bees have been primary pollinators at Site 1 (in 1989-1992 and 2008) and secondary pollinators at Site 3 (in 2008). Long-tongued digger bees and bumble bees are unique among native bees in that they showed repeated high abundance at one particular site, although, as noted before, consistent site data are not available for all sites. Observations have taken place at Site 1 during seven of the nine years and, of those seven, long-tongued digger bees have been primary pollinators for five, and in some cases the only primary pollinators. They also seem to be very abundant at one site in the same year when they are uncommon or missing at other sites. This is true in 1990, when they were common at Site 1 and rare at Site 3 and in 1991 and in 1992 when they were common at Site 1 and rare at Site 5. Interestingly, long-tongued digger bees have been consistently rare at Sites 2 and 5 for all years of observation.

During observations in 2008, Hofmann (2010) observed long-tongued digger bees to be monolectic, a behaviour that was also noted by Muñoz (1991), who also indicated that long-tongued digger bees did not visit as many flowers on a per foraging bout as other pollinators, and by Erickson (1993), who also observed that long-tongued digger bees would finish visiting the flowers on one plant and then, unlike other visitors, move to another plant 10 m or more away instead of the adjacent plant. This habit could account for a lower number of recorded visits in some studies if the insect moved out of the sub-population. Also, the small size of long-tongued digger bees may have caused it to be overlooked by observers.

Bumble bees were the primary pollinators in four of the nine years of study, 1992, 1993, 1994, and 1995 at both a younger site (Site 1) and an older seral stage site (Site 5), and although not as primary visitors, they were also observed in 1989, 1990, 1991, 2000, and 2008. During observations in 1995, bumble bees were principal pollinators, contributing over 10% of the visitor abundance, especially at Site 1, with 110 visits, representing 35% of the total visits for this site. However, they were uncommon at Sites 2 and 3. Data before 1995 did not examine Site 2, so there are no trends to observe,

whereas during the only earlier year of observations at Site 3, in 1990, bumble bees were present in low abundance.

Previous pollination efficiency studies by Erickson (1993), Stone (1995) and Dorsett et al. (2001) studied the effectiveness of bumble bees as pollinators and found them to be the most effective of the primary pollinators. Further pollen load analysis determined that bumble bees are effective pollinators, exhibiting 40% constancy, slightly lower than hummingbirds and the Acton giant flower-loving fly (Dorsett et al. 2001). The hairy bodies of bumble bees would enable the incidental collection and transfer of pollen. Stone (1995) observed pollen on many portions of the thorax and abdomen and noted that the bees were repeatedly grooming and packing; this was also noted in studies by Thompson et al. (1982), and Mayfield et al. (2001). No observers mentioned nectar robbery by the bees. However, Stone (1995) noted that the queen bumble bees would bend the flowers in such a way that there was no direct stigmatic contact.

Differing interplant distances and ranges of pollinators are important to gene flow within a population and across populations as gene flow depression can be a serious threat with imperilled plants (Aizen and Feinsinger 1994; Heschel and Paige 1995; Fischer and Matthies 1997; Torres et al. 2002). Bumble bees are known to have long flight distances between plants, up to three km, depending on wind speed (Greenleaf et al. 2007; Pasquet et al. 2008), which would enable them to affect interpopulation gene flow.

In 2008, western honey bees were among the primary pollinators for the first time in all the years of observation. They can be very effective pollinators of native plants (Butz-Huryn 1997; Chamberlain & Schlising 2008), especially in the face of declining native bee populations. They were a frequent visitor to *Eriastrum* and exhibited high constancy. The behaviour of the bees was noted during observations in 2008, when most western honey bees visited multiple flowers on the same plant and multiple plants, and often several bees would be seen foraging together. Stone (1995) noted multiple pollen contacts on the bodies of bees and Dorsett et al. (2001) also observed high constancy. During the early season, many bees were observed collecting either the blue colour pollen or the cream colour pollen of *Eriastrum*, especially in morning and evening, and although pollen samples were not obtained, close examination of the bees revealed consistency of pollen colours. During 2008, surrounding the sub-populations of *Eriastrum*, several bee-pollinated flowers were in bloom, although with the exception of non-native *Brassica* spp. and *Eriogonum fasciculatum*, bees were not noted in abundance on any of these plants. Bees seen visiting *Brassica* spp. and *Eriogonum fasciculatum* seemed to be monolectic in their foraging and did not visit *Eriastrum* or vice-versa; thus, pollen would not have been comingled.

The white-lined sphinx moth has not been observed in great abundance in the years of observation since 1991. This species is most active at dusk, although activity will continue throughout the day. Like many lepidopteran taxa, this species may have natural population cycling that span several years. This may have been the cause of their abundance in 1991. In 1991 this species represented one of the primary pollinators, but the actual number of visits was not high in comparison to

total numbers of visitors in 2008; therefore, a few individuals could account for the recorded visits. Erickson (1993) observed that white-lined sphinx moths exhibited high rates of visitation to the same and different plants in each foraging bout, suggesting it is effective as a vector of pollen. Herrera (1987) concluded that it was effective in gene exchange from plant to plant via pollination although, as concluded by Boyd (2004), when compared to hummingbirds, these moths, were not as effective. White-lined sphinx moths were also noted by Erickson (1993), to seek shelter in scale broom, a sub-shrub found more commonly at Sites 1 and 2 than Site 5, although many other shrubs could offer protective resting areas at all sites.

Considering all pollinators, in mid-season, when *Eriastrum* displays the greatest floral display (75% in bloom), the greatest abundance of visitors would be expected, however this was not the case in 2008 or 1995, therefore, the natural history of the pollinators or the weather conditions could be more important determining factors. As phenology is contingent upon rainfall and temperatures and does not necessarily occur annually at the same time, pollinator abundance and also diversity could depend on the time of year during which the bloom occurs. However, overall abundance of pollinators was not correlated to rainfall (Tab. 4), as years considered wet, such as 1995, exhibited low overall abundance; whereas years considered “dry” such as 2008, exhibited high overall abundance and other years varied. However due to the variances in observation methodology, overall abundance is difficult to compare across years and definitive conclusions cannot be drawn.

A strong negative inverse relationship of the abundance of longhorned digger bees to mean rainfall in year of observation may be due to abundant rainfall having a negative impact on larvae by flooding ground nests or increasing fungal or microbial growth (4). Thus, dry years may be beneficial to the survival of the larvae. Although not significant, sweat bees also exhibited a strong negative relationship with rainfall, which may also be due to their ground nesting behaviour. This speculation is further supported with strong correlations for sweat bees and longhorned digger bees with the previous years' mean rainfall. Interestingly there was no similar correlation for long-tongued digger bees and rainfall in either year. Long-tongued digger bees are also ground nesting bees, but perhaps there is a difference in their response to microbial or fungal threats caused by the increased rainfall. Hummingbirds also exhibited a strong negative correlation to abundance of rainfall in year of observation, which is the opposite of expected results based on other research that positively correlates rainfall in arid regions with hummingbird abundance (Wethington, et al. 2005). Further, hummingbirds are unique as one of the principal pollinators, in that they also consume insects or potentially other pollinators for protein. Therefore, it would be reasonable to assume they would be more abundant in wet years, when more insects should be available. The only taxa to exhibit a strong positive correlation of abundance to mean annual rainfall were the collective grouping of moths and butterflies. This group demonstrated as strong positive correlation to rainfall from the previous year and a strong positive correlation to rainfall of the year of

observation, which could indicate overall abundance of the insects or their visitation.

Eriastrum has been able to attract a variable array of pollinators across the years, throughout the seasons and throughout the day. The morphology of the flowers and pollen and nectar rewards of this plant have broad appeal, enabling *Eriastrum* to attract visitors even when phenology shifts. However, as one of the subpopulations of *Eriastrum* has already been reduced to a negligible size (Site 4) and remaining subpopulations persist in ever decreasing and isolated patches, that may not be large enough to attract pollinators and the persistence and survival of *Eriastrum* may be doubtful. Both small and large pollinators seem important to *Eriastrum*, with small native bees generally visiting within the patches and larger pollinators and non-native bees travelling between patches. Shrinking abundance of *Eriastrum* could make it energetically unfeasible for pollinators to remain monolectic or oligolectic, or to remain in the area at all, which will certainly impact seed set. According to research by Dorsett et al. (2001), the high variance of nectar reward also supports the generalist attraction of *Eriastrum* and promotes multiple flower visits. Ultimately pollinator presence for *Eriastrum* remains vulnerable, especially considering anthropogenic impacts.

In general, threats to native pollinators including habitat loss and alteration, pesticide use, introduction of disease, introduction of invasive species, global climate change, and threats to native plants are all leading to decline of many pollinators including several native bee species, some butterflies, bats and hummingbirds (Buchmann and Nabhan 1996; Kearns et al. 1998; Winter et al. 2006; and National Research Council 2007). However, there is limited knowledge

of pollinator life histories and their ecology and limited data from long-term population studies, which makes it difficult to accurately assess the extent of loss of pollinators, when limited evidence of the decline, extirpation, or extinction only exists for a few species and for others there is no baseline data at all and their status is completely unknown (National Research Council 2007).

Conclusions

Based upon this research, the status and decline of *Eriastrum* seems to be due to habitat alterations and not, at this time, the limitation of available pollinators for reproductive success. It could be assumed, then, that this species could persist if cyclic flooding of the Santa Ana River were to reoccur, which would result in restoration of suitable habitat for *Eriastrum* and its pollinators and additionally, if the restored habitat were then protected from human impacts and disturbance.

Successful management leading to the survival of *Eriastrum* must be inclusive of the preservation of pollinators, including their various habitat requirements, which are sometimes contradictory to the requirements of *Eriastrum*. Of the nine years of study both hummingbirds and the Acton giant flower-loving fly have been primary pollinators for eight years; thus, these two taxa seem to be especially critical and their persistence could be intimately tied to the reproductive success of *Eriastrum*. Further, a trend in decline of several species of native bees, including sweat bees, at least two genera of digger bees, and two or more species of bumble bees may be representative of a larger trend and may be cause for concern for the reproductive success of *Eriastrum*.