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# A MIXED POLLINATION SYSTEM IN
## *PENSTEMON PSEUDOSPECTABILIS* M. E. JONES (SCROPHULARIACEAE)

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Abstract.—A population of *Penstemon pseudospectabilis* M. E. Jones (Scrophulariaceae) on Cave Creek in Cochise County, Arizona, was used in an experimental test of reproductive fitness with three caging treatments: all flying pollinators excluded, hummingbirds excluded, and no exclusion. Twenty plants were chosen and three shoots on each used in the experiment. The flowers were 25.6 (s.d. = 1.5) mm long, the end diameter was 8.5 (s.d. = 1.0) mm, the tube opening was 6.5 (s.d. = 1.0) mm, and the greatest diameter, 75% distal from the receptacle, was 10.1 (s.d. = 0.8) mm (N = 59). Floral nectar contained 11.7% (s.d. = 2.9%) fructose, 13.8% (s.d. = 2.7%) glucose, and 74.5% (s.d. = 5.4%) sucrose (N = 74). There was some evidence, significant only for fructose, that nectar-sugar composition varies between morning and evening. Larger floral dimensions were correlated with lower sucrose and higher hexoses. Casual observation showed *Xylocopa* sp., small bees, flies, and hummingbirds to be visitors. There was no sign of nectar robbing. Five percent of flowers set seed with all pollen vectors excluded, 44% with hummingbirds excluded, and 63% with no exclusion. Seed set per fruit was 2 with all excluded, 23 with hummingbirds excluded, and 46 with no exclusion. Mean seed set on pollinated flowers was 60, with a range of 2 to 192. Multiple linear regression showed the fraction of fruit setting seed when hummingbirds were excluded to be related to larger flower diameters and shorter flowers. With no pollinator exclusion, fruits setting seed were related to larger diameters and nectar fructose. For seeds per fruit, multiple regression gave similar, but less clear, results. We conclude that *P. pseudospectabilis* is pollinated by both bees and hummingbirds, with other pollinators not to be excluded as possible contributors. We found no hard evidence of selective forces currently at work.

In pollination biology, the weight of many observations has led to recognition of unique syndromes of morphological and nectar chemistry characteristics among plant taxa primarily pollinated by a given animal group (e.g., Baker and Baker 1983b, Faegri and van der Pijl 1971, Grant and Grant 1968). The chemical composition of floral nectars, especially the sugar composition, has received attention in this regard (Baker and Baker 1983a). However, demonstration of the selective forces implied by such adaptation is needed for insights to be more than pedagogical aphorisms.

Studies to assess the interaction of floral nectar sugars and reproductive fitness in natural populations are needed. The large western genus *Penstemon*, Scrophulariaceae, has a wide range of nectar-sugar composition and floral morphology and is pollinated by several classes of animals. It is an excellent taxon for experimental pollination biology (Baker and Baker 1983a, Crosswhite and Crosswhite 1981).

*Penstemon pseudospectabilis* M. E. Jones occurs from southeastern California to southwestern New Mexico in the desert or in open woodlands. The flower color has been variously described: pink (Jepson 1925), deep pink to rose-purple (Kearney and Peebles 1969), and pink, bearing darker guidelines in the throat (Martin and Hutchins 1981). The flowers are borne on numerous branching shoots arising from the basal rosette. The corolla tube is ampliate, reaching its greatest diameter at about three-fourths of the length distal of the receptacle. Nectaries are on the abaxial surfaces of the upper two free stamens near their bases (Straw 1966). The flower opening is large enough to accommodate some bees.

Straw (1956) describes some morphological and colorimetric characteristics leading to floral isolation in four penstemon species, and, in comparison, the flower of *P. pseudospectabilis* appeared to have some features of both bee- and hummingbird-pollinated species. It seemed appropriate, therefore, to test for mixed pollination systems. We here report on results of an exclusion experiment providing initial data on floral morphology, nectar-sugar composition, seed set, and the plant's dependence on different pollinators.

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## MATERIALS AND METHODS

A population of *P. pseudospectabilis* in Cave Creek Canyon of the Chiricahua Mountains in Cochise County, Arizona, at about 1,370 m elevation was used for this study (Voucher: UTEP 22491). This population occurs within an open woodland on a south-facing slope and adjacent riparian and roadside areas of Cave Creek 150 m west of a U.S. Forest Service station. There appears to be little day-to-day human disturbance, but part of the population is within a fenced pasture occasionally used for horses or mules.

On 2 May 1987 we selected 20 plants for the study, each separated by at least 2 m. On each plant we chose three shoots for study. We made flower measurements and nectar collections. The following measurements were taken on one to six mature flowers on each stem using a millimeter ruler: length, corolla diameter at 25%, 50%, 75%, and 100% of the axis of the flower, and diameter of the opening. Nectar samples were taken from mature flowers on most shoots. Nectar was removed with a micropipette and placed on a filter paper disk to dry. We then stripped opened flowers from the shoots. The three treatments for our experiment were: (1) exclusion of all flying pollinators, (2) exclusion of hummingbirds, and (3) no exclusion to allow access by all pollinators. (The available pollinating fauna at Cave Creek, of course, varies greatly in numbers and composition during the season.)

On each plant one shoot was enclosed with an inverted half-gallon milk carton with cutout sides over which a stocking was stretched (Radford et al. 1974). The stocking was drawn over the base and gently tied shut. Another was enclosed in a 30-cm diameter, 50-cm-tall cage of 5-cm chicken wire. The cages were supported by metal stakes driven into the ground. A third shoot was marked but left entirely open. After the exclosures were completed we remained to observe visitation by potential pollinators. No attempt was made to observe nocturnal activity of moths. The following day we again observed visitors, took additional nectar samples, and left the cages in place. On 19 June 1987 we returned, removed the cages, and harvested the shoots.

For each collected shoot the total number of fruits and the number of fruits with seed were counted. Starting from the bottom of the shoot, the first 10 fruits with seed were removed and dissected and seeds were counted.

Our methodology for sugar analysis by High-performance Liquid Chromatography (HPLC) was the same as in previous studies (Freeman et al. 1983, 1984, 1985, Reid et al. 1985). The paper disks holding dried nectar were placed in Parafilm (R) pouches with 20 μl of water. After five minutes eluted sugars were removed by squeezing the pouch and collecting the solution with a microsyringe. The sample was then injected into the chromatograph for analysis.

A Rainin Instrument Co. liquid chromatograph with a Knauer refractive index detector and an Alltech amino bonded silica column (5 μm particle size and 150 mm long) was used. The solvent was an acetonitrile: water (75:25 v/v) system flowing at 2.0 ml/min. For calibration, regressions based on response to sugar standards were established. The standard solution contains fructose, glucose, and sucrose, each 10% by mass, injected in volumes of 1, 2, 3, and 4 μl. The response of this system to sugar mass was nearly linear, and second order, least squares regressions were used for calibration curves. Regression coefficients of 0.9995 (d.f. = 1, P < .02) are obtained. A BASIC program by Reid computed relative percent by mass of each sugar from the chart responses.

Results were placed in data files and analyzed using Number Cruncher Statistical Software (Hintze 1985) and a BASIC program by Reid for the Kolmogorov-Smirnov test of normality (Sokol and Rohlf 1983). In comparing the floral tube and opening diameters to other variables such as nectar composition or seed set, we tested both diameter and diameter squared (proportional to area).

## RESULTS AND CONCLUSIONS

The floral measurements are summarized in Table 1. Principal components analysis (Hintze 1985) of the floral measurements reveals that the first component (44% of the variance) is a positive correlate relation among five floral dimensions: length, diameter at 25% and 75% of length, opening, and end diameter. Thus, when one of these is larger, all are larger. The second component (19% of variance) is the independent variation of the diameter at 50% of length.

TABLE 1. Summary measurements for 59 *Penstemon pseudospectabilis* flowers. Pearson product-moment correlation coefficients significant at the P < .05 level are given. The standard deviation is indicated by s.d., range of measurements by ( ), and nonsignificant correlations are shown as n.s.

| # | Measurement | Mean (mm) | s.d. (mm) | Correlation with measurement # | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 6 | 5 | 4 | 3 | 2 |
| 1 | Length, 1 | 25.6 (22.0–28.0) | 1.5 | .293 | .437 | n.s. | n.s. | .426 |
| 2 | Dia. at 25% of l | 4.0 (3.0–5.0) | 0.3 | .447 | .426 | .319 | n.s. | |
| 3 | Dia. at 50% of l | 7.4 (5.5–9.0) | 0.8 | n.s. | n.s. | n.s. | | |
| 4 | Dia. at 75% of l | 10.1 (8.0–12.0) | 0.8 | .582 | .399 | | | |
| 5 | Dia. of opening | 6.5 (5.0–9.0) | 1.0 | .433 | | | | |
| 6 | Dia. at end | 8.5 (6.0–11.0) | 1.0 | | | | | |

TABLE 2. Summary data on the nectar-sugar composition of 74 floral nectar samples from *Penstemon pseudospectabilis* expressed as relative percent by mass of each sugar. Pearson product-moment correlation coefficients significant at the P < .01 level are given. All are normal in their distributions by the Kolmogorov-Smirnov test.

| Sugar | Mean % | s.d. % | Correlation coefficient | |
|---|---|---|---|---|
| | | | Sucrose | Glucose |
| Fructose | 11.7 | 4.4 | −0.955 | 0.881 |
| Glucose | 13.8 | 4.3 | −0.958 | |
| Sucrose | 74.5 | 8.3 | | |

Summary nectar-sugar data are given in Table 2. Freeman et al. (1984) found typical hummingbird nectar to contain 15% fructose, 11% glucose, and 74% sucrose, and gave 95% confidence limits for that property. The nectar of *P. pseudospectabilis* is similar and falls well within the domain of hummingbird nectars. While Freeman et al. (1984) showed hummingbird nectars often have somewhat more fructose than glucose (%F/%G = ca 1.4), the nectar of *Penstemon* studies here contained less (%F/%G = 0.8). This has been true of several *Penstemon* species studied (C. E. Freeman, unpublished data). The frequency distribution of percent composition by mass was normal (P < .01) by the Kolmogorov-Smirnov test (Sokol and Rohlf 1983).

Little data are available on the frequency distribution of nectar-sugar composition in different taxa, and it may be worth noting that such distributions are not always normal. The moth-pollinated species *Ipomopsis longiflora* has a skewed, nonnormal distribution in sucrose composition with a maximum above 90% sucrose (Freeman et al. 1985). An *Agave neomexicana* population at the upper limit of its altitudinal range has dispersed, nonnormal sugar compositions (Reid et al. 1985). The normality of distribution in *P. pseudospectabilis* does not rule out strong selective forces from pollinator preference, but it certainly provides no evidence for their presence. Indeed, present data on preference (Baker and Baker 1983a) and its elasticity with different food and water stresses does not permit more than the roughest of inferences. For example, Allen and Neill (1979) found that several hummingbird species regularly visit *Agave havardiana* for its abundant low-sucrose nectar (Freeman et al. 1983, Reid et al. 1985) in arid Big Bend National Park, Texas.

Twelve of the nectar samples were taken in the morning, and 41 were collected in late afternoon. Since the composition distributions were normal, we compared these for each sugar by ANOVA. Morning fructose was different from evening (F = 6.42, P = .001). However, glucose (F = 1.32, P = .26) and sucrose (F = 3.53, P = .06) were not significantly different. Temporal change in composition could affect pollinator activity, and this possibility requires further, more precise examination.

There were some significant correlations between floral morphology and sugar composition despite the relatively small variance in both these data sets. The area of the opening in the floral tube correlated with fructose (r = .517, P = .023), glucose (r = .541, P = .017), and sucrose (r = −.549, P = .015). The tube area 75% distal from the receptacle correlated

TABLE 3. Summary data on seed set in the *Penstemon pseudospectabilis* population. Treatment: A = shoot covered with stocking, B = shoot caged with 5-cm chicken wire, C = no exclosure. ( ) encloses the range.

| Treatment | A | B | C |
|---|---|---|---|
| Mean fruits with seed/shoot | 1.47 | 14.22 | 22.17 |
| | (0–4) | (1–32) | (8–34) |
| Mean total fruits/shoot | 25.23 | 31.17 | 35.33 |
| | (7–51) | (19–44) | (23–49) |
| Fruits with seed/total fruits | 0.06 | 0.45 | 0.63 |
| | (0.0–0.16) | (0.05–0.74) | (0.0–0.94) |
| Mean seeds/fruit with seed | 19.11 | 48.13 | 72.50 |
| | (0.0–66.0) | (18.9–86.0) | (54.6–102.6) |

with fructose (r = .471, P = .042), glucose (r = .458, P = .048), and sucrose (r = −.463, P = .046). Correlations with the end diameter and area were similar but somewhat lower and not significant at the 5% level. These relationships indicate that a larger, more open flower will have a lower sucrose composition.

We saw *Xylocopa* sp., small bees, flies, and several species of hummingbirds visit the flowers. The bees were not deterred by the 5-cm chicken wire, but the hummingbirds would not approach closer than a few dm. The *Xylocopa* approached the flowers from somewhat below and grasped the corolla with their forelimbs. Hanging briefly like a trapeze artist, they then forced their way partly into the floral tube, remained a few seconds, and left. In neither May nor June did we observe robbing or see torn flowers (Inouye 1983). Since *Xylocopa* lay eggs on large (1 cm) pollen pellets, it may be that pollen gathering was their primary objective in visiting these flowers.

Of the 20 plants originally selected, 2 appeared to have been disturbed, and the stocking on 1 additional shoot had a large hole. Thus, we determined seed set on 17 plant shoots excluding all pollinators, and 18 for those with a hummingbird exclusion cage and those with no exclusion.

It became apparent, upon examination of fruits, that the fresh fruit size was related to seed count. For 187 fruits we both counted seeds and measured greatest fruit diameter with a micrometer. After examining several relationships, we concluded that diameter cubed (Dcubed) gave the best correlation with number of seeds (N) for fruits greater than 2 mm: N = 0.8 + 0.569*Dcubed (mm), r = .902, d.f. = 185, P < .001. No fruit smaller than 2 mm diameter contained seed. This relationship may be convenient in larger

studies. Counting more than 23,000 seeds was a significant fraction of the effort in this work.

The seed set data were expressed in terms of seeds per fruit with seed, seeds per fruit, and fruits with seed per fruit to correct for differences in flowers per shoot (Table 3). In summary, 6% of flowers set seed with all flying pollinators excluded, 45% with hummingbirds excluded, and 63% with no exclusion. With all flying pollinators excluded, there were fewer than 2 seeds per fruit, 23 seeds with hummingbirds excluded, and 46 with no exclusion. The seed set with all flying pollinators excluded might be by selfing, through pollination by ants or other climbing insects, or our activities. Most of the seed set is from insect and hummingbird pollination. Overall, 580 (36%) of the 1,624 fruits set seed, and these had a mean seed set of 60, well below the maximum observed of 192. Clearly, the flowers in this test were not saturated by pollinators.

The seed set data were nonnormal in distribution, and we used nonparametric statistics to test differences among the treatments. We used both the Wilcoxon matched pairs test (assuming pairing since each plant tested had all three treatments) and the Mann-Whitney two sample test (assuming there was no significance to the pairing). Both tests showed all three treatments to be significantly different (P < .01) in all comparisons (fruits with seed/fruits, seeds/fruit with seed, and seeds/fruit). Thus, the effect of exclusion is highly significant.

Our final, and most speculative, analysis was to use multiple linear regression to explain two of the derived results, fruits with seed/fruits and seeds/fruit. We used the six measures of floral morphology and the percent by mass of each sugar as independent variables. The results are given in Table 4,

TABLE 4. Summary results of multiple linear regression analysis of *Penstemon pseudospectabilis* fruits with seed/ fruits and seeds/fruit with the three treatments in comparison with the morphological and sugar composition data. The first four variables selected and the cumulative regression coefficient are given. The sign of the correlative relationship between each independent variable and the dependent variable is shown by + or −. Treatment: A = shoot covered with stocking, B = shoot caged with 5-cm chicken wire, C = no exclosure.

| Variable analyzed: fruit with seed/total fruits | | | |
|---|---|---|---|
| Treatment = | A | B | C |
| Independent variable | | | |
| 1 | +sucrose | +dia. at 75% of l | +dia. end |
| 2 | +dia. opening | +dia. at 25% of l | +dia. opening |
| 3 | −length | +dia. at 50% of l | +fructose |
| 4 | +dia. at 25% of l | −length | +dia. at 75% of l |
| Regression coefficient | 0.779 | 0.816 | 0.687 |
| Significance, P | < .001 | < .001 | < .01 |
| | | | |
| Variable analyzed: seeds/fruit | | | |
| Treatment = | A | B | C |
| Independent variable | | | |
| 1 | +dia. at 25% of l | +dia. at end | −length |
| 2 | −glucose | −length | +dia. at 75% of l |
| 3 | −dia. at 50% of l | +dia. at 75% of l | +fructose |
| 4 | +dia. opening | +fructose | +dia. at 25% of l |
| Regression coefficient | 0.561 | 0.712 | 0.766 |
| Significance, P | < .05 | < .01 | < .01 |

and, while they are statistically significant, they must be regarded as preliminary. There was less than 1% difference between using diameters and areas in the regressions, and no difference in the selection of variables.

Fruits setting seed on the stocking-covered shoots were positively related to sucrose. The other three variables, length (negatively correlated) and two diameters (positive), are not inconsistent with this. Fruits setting seed with hummingbirds excluded positively related to three areas (and diameters) and negatively correlated with length. This hints that bees are more effective pollinators of shorter, more open flowers. With no exclusion, the most significant variables were three measures of area near the distal end of the flower, and fructose. The positive correlation for fructose is also not inconsistent with the preference of hummingbirds as discussed above.

Results for seed set using multiple regression are somewhat similar, but occasionally cryptic. For the stocking-covered shoots, seed set was positively related to diameter at opening and at 25% of length. It was negatively related to diameter at 50% of length and glucose. The negative glucose correlation parallels one of positive sucrose. The hummingbird-excluded shoots were again supportive of a shorter, broader flower, with an additional positive correlation for fructose. Finally, the

regression results for no exclusion are similar to those for hummingbird exclusion.

The results from this small experiment are remarkably supportive of the generalizations of pollination biology. They do not form an irrefutable chain of evidence, but they do lend detail to the complex mosaic of assertions made during recent decades. The data also support an assertion that careful experimentation in pollination biology will be numerically productive in testing and refining the rich insights of Herbert and Irene Baker and many others.

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