

## THREE NATURALLY OCCURRING *PENSTEMON* HYBRIDS

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**ABSTRACT.**—Three wild *Penstemon* hybrids are documented herein: *Penstemon clevelandii* × *spectabilis*, *P. centranthifolius* × *spectabilis*, and *P. centranthifolius* × *eatonii*. Each putative hybrid was compared to its respective parental species using manifold floral and vegetative characters for which the parents differed. For each hybrid hypothesis, characters were counted as being intermediate or not intermediate. There were significantly more intermediate characters in all 3 cases (one-sided sign tests,  $P < 0.05$ ). These *Penstemon* species evidently do hybridize naturally, and for nearly all characters the hybrids are consistent with the view that the parents differ largely by additive genetic effects.

*Key words:* character-count method, hybridization, *Penstemon*.

One would think that hybridization in the wild should be limited in one way or another. If interspecific promiscuity were rampant, distinctions between the species involved would dissolve into continuous variation, and there would be no basis for recognizing them as distinct. Limited hybridization, nevertheless, is a widespread phenomenon in some groups of organisms. Among vascular plants, Rieseberg (1997) estimated the frequency of hybrid combinations to be about 11%. These hybrids are highly concentrated taxonomically in certain families and genera (Ellstrand et al. 1996). They are especially concentrated among perennials with outcrossing breeding systems (Grant 1981). Although a small amount of hybridization occurs between a great many species, it should be emphasized that even in groups that are prone to hybridization, one typically finds hybrid individuals at rates of one in thousands. There are, however, 2 types of situations in which hybrid swarms are more extensive: (1) in narrow transition zones where 2 species meet along a steep ecological gradient (e.g., an elevational gradient), and (2) in unusually disturbed sites where species meet that have been previously kept apart due to differences in ecology (e.g., in clear cuts).

Reportings of hybrids vary greatly in the amount of evidence presented for hybridity. Often no data are given, or only a brief typological description is provided. Furthermore, when the data are extensive, they are often summarized as hybrid indices or ordination

scatterplots that combine characters before assessing intermediacy; this does not allow the reader to judge whether a pattern of morphological intermediacy arose through hybridization (“a reticulate series” sensu Wilson 1992) or, for instance, through one species giving rise to a 2nd species that then gave rise to a 3rd species (“a phyletic series” sensu Wilson 1992). In the former case the hybrid would be expected to be intermediate in character after character. In the latter case the intermediate entity would be expected to resemble the species that gave rise to it in some characters and the species that it gave rise to in other characters. The character-count procedure of Wilson (1992) provides a statistical method for assessing the likelihood of hybridity.

The purpose of the present article is to document 3 cases of natural hybridization in the genus *Penstemon* (Scrophulariaceae). *Penstemon* hybrids have often been noted in passing. Keck (1937) described the case of *Penstemon centranthifolius* × *spectabilis*, also known as *P. × parishii*, which we will also analyze. Straw (1955, 1956b) formulated hypotheses about the diploid hybrid origins of several *Penstemon* species. Crosswhite (1965) compiled several reports of other *Penstemon* hybrids: *Penstemon barbatus* × *virgatus* (Keck 1960) was reported from the north side of the Grand Canyon; *P. barbatus* × *comarrhenus* (Bennet 1959) was collected from Segi Canyon, Arizona; *P. barbatus* × *glaber* (Viehmeyer 1958) was found near Flathead Lake, Montana; and

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*P. barbatus* × *strictus* (Welsh and Erdman 1964) was collected from Mesa Verde, Colorado. In all cases the number of hybrid individuals reported was very modest. The single case we know of with more extensive hybridization between *Penstemon* species is where the high-elevation *Penstemon davidsonii* meets various mid-elevation species (*P. newberryi*, *P. rupicola*, *P. cardwellii*) in many sites near timberline throughout the Sierra Nevada and Cascade mountain ranges (Clausen et al. 1940, S. Datwyler unpublished data, P. Wilson and S. Kimball personal observation).

#### MATERIALS, METHODS, AND RESULTS

We found the following putative hybrids in several locales in southern California. There were only a few individuals found of each type of hybrid among hundreds of parental individuals.

- A. Two individuals of *Penstemon clevelandii* × *spectabilis* (Fig. 1A) were found on rocky outcrops surrounded by chaparral, mixed in a *P. clevelandii* population, where *P. spectabilis* inhabit the roadside nearby (33:35N 116:31W, 4000 m, 6 June 1998, *Valenzuela* 74).
- B. *Penstemon centranthifolius* × *spectabilis* (Fig. 1B) were found in several sites. Three individuals were located in the Santa Monica Mountains near Mulholland Highway in a recently burned site (34:06N 118:42W, 400 m, 27 April 1998, *Wilson* 3520). Seven individuals were found along California State Route 74 in Riverside County, also on a recently burned hillside (33:37N 116:32W, 4000 m, 6 June 1998, *Valenzuela* 75).
- C. One individual of *Penstemon centranthifolius* × *eatonii* (Fig. 1C) was found growing along a dry wash among a large population of *P. eatonii* with a few scattered *P. centranthifolius* individuals near Burns Canyon Road in the San Bernardino National Forest (34:12N 116:34W, 4500 m, 7 June 1998, *Valenzuela* 79).

We tested these hypotheses of hybridity using the character-count procedure of Wilson (1992). First, for each putative hybrid we listed vegetative and floral characters by which the parents differed (Tables 1–3). Up to 50 indi-

viduals of each parental form were scored, as were as many hybrids as we found. For quantitative characters we measured several flowers on the small number of hybrid plants that we found, which was admittedly an act of pseudo-replication. For all characters the question was asked, “Is the hybrid intermediate between the 2 parental extremes?” For each putative hybrid the number of intermediate characters was significantly more than the number of non-intermediate characters ( $P < 0.05$ ); thus, we reject the null hypotheses that the pattern of diversity arose through a series of phyletic steps. For *P. centranthifolius* × *eatonii*, we included differences in the anther morphology of the parents. Figures 2A–C show how the hybrid’s anthers open to an intermediate degree. In addition, the pollen of this inter-subgeneric hybrid was poorly developed (Fig. 2D): 42% of 577 grains from the hybrid plant were smaller than normal and football shaped, compared to 8% of 310 grains from *P. centranthifolius* and 0% of 299 grains from *P. eatonii*.

Notice that by the character-count procedure, one’s sample size is the number of characters, not the number of plants. The number of plants may affect one’s accuracy in evaluating the characters. For instance, if more hybrids had been measured, the average for mouth asymmetry in Table 1 might have been intermediate. On the other hand, maybe it would have remained non-intermediate. Regardless, significance is judged based on the number of characters that are and are not intermediate. A related concern about the character-count procedure might be that we judged intermediacy as simply “+” or “–” when clearly error variance might allow for a sample’s + to belie a large population’s –. This is justified on the assumption of the null hypothesis that such sampling error is likely to give as many +’s as –’s; the possibility of a false + in one character is canceled out by the equal possibility of a false – in another character. On average, such sampling errors count against the type II error rate, not the type I error rate. A final assumption of the procedure is that the characters are free to behave independently as would be the case if they were mainly determined by separate genetic elements. Although we know nothing about pleiotropy among the characters we used, we tried to avoid characters that seemed to be redundant aspects of some more inclusive character. For example, we did not use

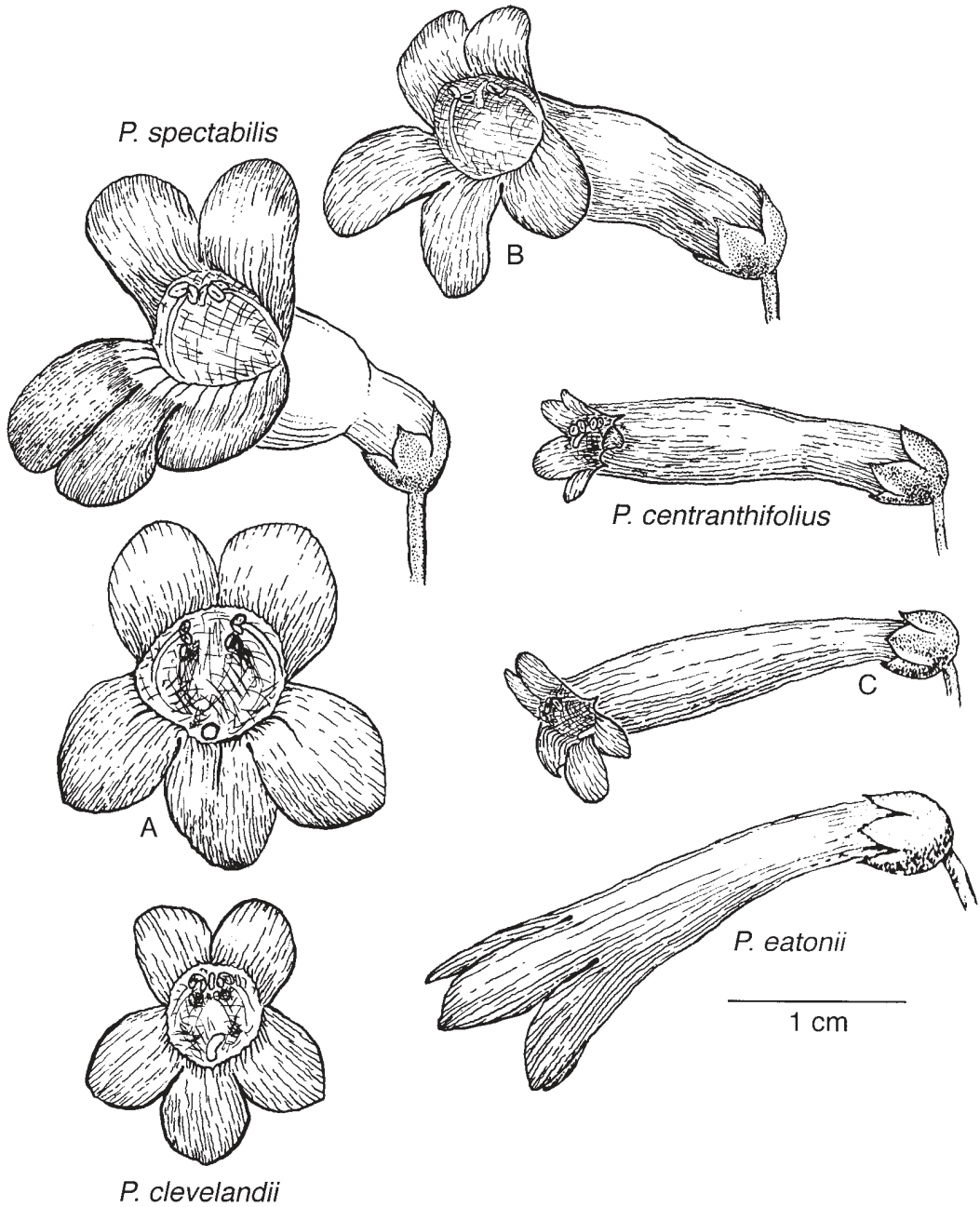


Fig. 1. Putative hybrids and parents: A, *Penstemon clevelandii* × *spectabilis*; B, *Penstemon centranthifolius* × *spectabilis*; C, *Penstemon centranthifolius* × *eatonii* (traced from photographs).

TABLE 1. Count of characters as intermediate (+) or not (–) for hybridity hypothesis A. Means  $\pm$  standard errors (sample size). L-U = difference between the upper and lower semi-circumferences at the mouth. W/L = anther width/anther length. L-S = difference in length between a long and a short stamen.

Character	<i>P. clevelandii</i>	<i>P. clevelandii</i> $\times$ <i>spectabilis</i>	<i>P. spectabilis</i>	Intermediate?
Pedicle length	7.724 $\pm$ 0.378 (15) <sup>a</sup>	9.129 $\pm$ 0.500 (17) <sup>a</sup>	13.752 $\pm$ 1.10 (14) <sup>a</sup>	+
Sepal shape (W/L)	0.609 $\pm$ 0.016 (48)	0.690 $\pm$ 0.014 (4) <sup>a</sup>	0.898 $\pm$ 0.017 (50)	+
Flower color	reddish purple	magenta	blue-purple	+
Corolla length	17.592 $\pm$ 0.190 (48)	19.50 $\pm$ 0.540 (4) <sup>a</sup>	22.072 $\pm$ 0.217 (50)	+
Mouth asymmetry (L-U)	0.860 $\pm$ 0.088 (48)	1.00 $\pm$ 0.408 (4) <sup>a</sup>	0.542 $\pm$ 0.149 (50)	–
Circumference at 3/4 of corolla	15.033 $\pm$ 0.326 (48)	20.725 $\pm$ 2.072 (4) <sup>a</sup>	23.185 $\pm$ 0.248 (50)	+
Stamens (L-S)	2.583 $\pm$ 0.103 (48)	2.750 $\pm$ 0.250 (4) <sup>a</sup>	5.425 $\pm$ 0.127 (50)	+
Staminode vestiture	hairy	sparsely hairy	glabrous	+
Staminode length	10.283 $\pm$ 0.220 (48)	12.12 $\pm$ 0.663 (5) <sup>a</sup>	17.537 $\pm$ 0.255 (49)	+
				8:1 ( <i>P</i> < 0.05)

<sup>a</sup>Flower measurements pseudoreplicated by examining several flowers within fewer plants.

TABLE 2. Count of characters as intermediate (+) or not (–) for hybridity hypothesis B. Means  $\pm$  standard errors (sample size). L-U = difference between the upper and lower semi-circumferences at the mouth. W/L = anther width/anther length. L-S = difference in length between a long and a short stamen.

Character	<i>P. centranthifolius</i>	<i>P. centranthifolius</i> $\times$ <i>spectabilis</i>	<i>P. spectabilis</i>	Intermediate?
Leaf margin	entire	few small teeth	coarsely toothed	+
Foliage surface	strongly glaucous	weakly glaucous	not glaucous	+
Outer corolla color	deep red	magenta	blue-purple	+
Inner corolla color	red	rose with magenta veins	white with purple veins	+
Outer corolla and calyx vestiture	glabrous	sparsely glandular puberulent	glandular puberulent	+
Circumference at 3/4 of corolla	11.124 $\pm$ 0.198 (9)	16.300 $\pm$ 0.249 (30) <sup>a</sup>	23.185 $\pm$ 0.248 (50)	+
Mouth asymmetry (L-U)	2.616 $\pm$ 0.097 (50)	1.759 $\pm$ 0.199 (30) <sup>a</sup>	0.542 $\pm$ 0.149 (50)	+
Stamens (L-S)	2.070 $\pm$ 0.148 (50)	3.759 $\pm$ 0.410 (30) <sup>a</sup>	5.425 $\pm$ 0.127 (50)	+
Anther shape (W/L)	1.063 $\pm$ 0.029 (50)	0.481 $\pm$ 0.019 (28) <sup>a</sup>	0.378 $\pm$ 0.008 (48)	+
Staminode length	13.110 $\pm$ 0.246 (48)	14.583 $\pm$ 0.294 (30) <sup>a</sup>	17.537 $\pm$ 0.255 (49)	+
				10:0 ( <i>P</i> < 0.05)

<sup>a</sup>Flower measurements pseudoreplicated by examining several flowers within fewer plants.

multiple lengths of various flower parts, since these might all be aspects of floral tube length.

## DISCUSSION

The main purpose of this paper has been to give the morphological evidence supporting our interpretation of 3 kinds of hybrids found in the wild. In each case the pattern of intermediacy was what one would expect of  $F_1$  or  $F_1$ -like hybrids with little or no dominance for the characters studied. Because the hybrids we studied are of some further interest, we will continue by discussing hypotheses concerning the genetic basis for species differences in *Penstemon*, a scenario by which a few

$F_1$  hybrids might go on to form an independent species that retains its  $F_1$ -like characters such as has been suggested for the origin of *P. clevelandii*, and the mechanisms that might limit hybridization between the parental species that we studied.

We found morphological intermediacy in 27 of 30 characters. Such an abundance of intermediate characters is contrary to Rieseberg's (1995) summarization that hybrids are no more likely to display intermediate character states than parental ones. Rieseberg said that hybrids are a mosaic of parental (dominant), intermediate (underdominant), and extreme (overdominant) characters, but our results suggest that in *Penstemon* morphological divergence is

TABLE 3. Count of characters as intermediate (+) or not (–) for hybridity hypothesis C. Means  $\pm$  standard errors (sample size). L-U = difference between the upper and lower semi-circumferences at the mouth. W/L = anther width/anther length. L-S = difference in length between a long and a short stamen.

Character	<i>P. centranthifolius</i>	<i>P. centranthifolius</i> $\times$ <i>eatonii</i>	<i>P. eatonii</i>	Intermediate?
Basal leaves	few	intermediate	dense	+
Pedicel length	14.548 $\pm$ 0.880 (24) <sup>a</sup>	8.943 $\pm$ 0.499 (15) <sup>a</sup>	4.698 $\pm$ 0.286 (22) <sup>a</sup>	+
Sepal shape (W/L)	0.872 $\pm$ 0.021 (50)	0.720 $\pm$ 0.037 (4) <sup>a</sup>	0.495 $\pm$ 0.020 (6)	+
Flower color	deep red	red	red-orange	+
Circumference at 3/4 of corolla	7.594 $\pm$ 0.223 (9)	7.123 $\pm$ 0.095 (4) <sup>a</sup>	13.885 $\pm$ 0.506 (6)	–
Mouth asymmetry (L-U)	2.616 $\pm$ 0.097 (50)	1.825 $\pm$ 0.118 (4) <sup>a</sup>	3.040 $\pm$ 0.341 (6)	–
Shape of anther (W/L)	1.063 $\pm$ 0.029 (50)	0.404 $\pm$ 0.004 (4) <sup>a</sup>	0.359 $\pm$ 0.010 (6)	+
Extent of anther dehiscence	entirely dehisced (100%)	distal ~83%	distal 75%	+
Toothing along anther opening	none	occasional toothing	papillate-toothed	+
Staminode length	13.110 $\pm$ 0.246 (48)	14.575 $\pm$ 0.697 (4) <sup>a</sup>	15.958 $\pm$ 0.581 (6)	+
Difference in lower and upper lips	0.348 $\pm$ 0.143 (9)	–0.580 $\pm$ 0.259 (4) <sup>a</sup>	–1.937 $\pm$ 0.274 (6)	+
				9:2 ( <i>P</i> < 0.05)

<sup>a</sup>Flower measurements pseudoreplicated by examining several flowers within fewer plants.

largely by additive alleles. We use “additive” in the sense of a quantitative geneticist to mean with neither dominance nor epistasis (Falconer and Mackay 1996). In  $F_{1S}$  the strictly additive value would be the average of the parental values. This is not precisely the case for many of our characters, but given the small number of hybrid flowers that we measured, much of the deviation from the mid-parent values might be due to error variance. Having studied only a few hybrids without their individual pedigrees, we cannot be conclusive in our suggestion that the parents differ mainly by additive factors. It is possible that the characters are polygenic, with some loci dominant for one parent and other loci dominant for the other parent. Also, there could be epistatic differences that might show themselves in subsequent  $F_{2S}$  and backcrosses. We are currently in the process of artificially producing various crosses to further assess the genetic basis for differences between *P. centranthifolius* and *P. spectabilis*. As of this writing, the simplest interpretation of our results is that we found  $F_{1S}$  and the parents differ largely in terms of additive genetic elements.

There is some additional evidence that divergence in *Penstemon* is by many genes of equal additive effect (cf. *Mimulus*; Bradshaw et al. 1995). In 1955, Straw suggested that *P. clevelandii* might have originated through the stabilization of *P. centranthifolius*  $\times$  *spectabilis*.

Straw thought that distinct ecological preferences arose through independent segregation, and so these ecological characters would have a non-additive genetic basis; nevertheless, Straw’s reason for thinking that *P. clevelandii* was of hybrid origin was its intermediacy in morphology. For a number of characters, this morphological intermediacy holds up to statistical analysis (P. Wilson and G. Aldridge unpublished data). Moreover, Wolfe et al. (1998a, 1998b) have reported that *P. clevelandii* shows complementarity of molecular markers, that it is positioned between its putative parents in terms of genetic distance, and that it is somewhat depleted of private alleles. If *P. clevelandii* is really the stabilized hybrid derivative of *P. centranthifolius*  $\times$  *spectabilis*, then the morphological differences between the parents that remain intermediate in *P. clevelandii* must be based on many genes of nearly equal additive effect; otherwise, when some loci became fixed for one parent and others fixed for the other, *P. clevelandii* would have come to resemble one parent in some characters and the other parent in other characters or else would have taken on a novel morphology as the result of novel gene combinations (as in Grant 1966, Rieseberg et al. 1996).

*Penstemon clevelandii*’s derivation through hybridization would have involved a series of rare events. We have studied *Penstemon* for 4 field seasons, and the individuals reported on

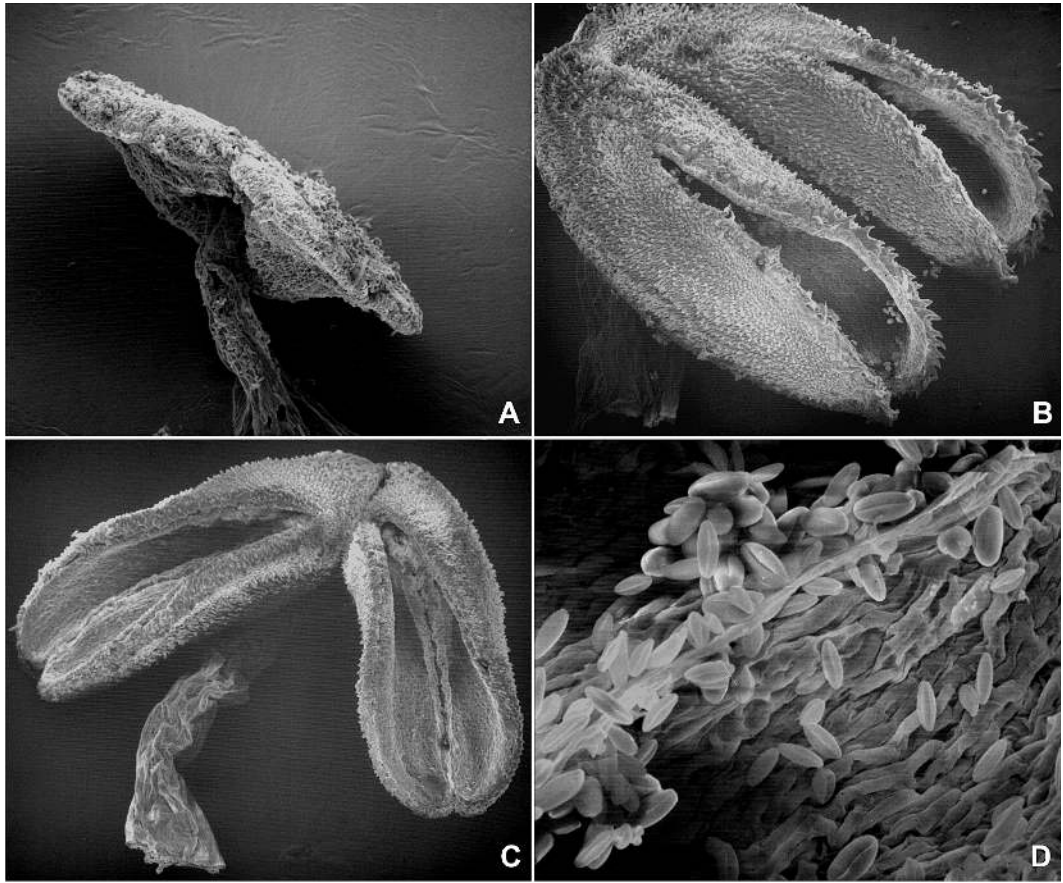


Fig. 2. Scanning electron micrographs of anthers and pollen: A, *Penstemon centranthifolius*; B, *Penstemon eatonii*; C, the putative hybrid; D, pollen of the putative hybrid with many small undeveloped grains.

herein are all the hybrids we have found except for those involving *P. davidsonii*. When  $F_1$ s do form, one would expect the vast majority of subsequent breeding would be with the parental species; i.e., they would backcross. These backcrossed individuals would themselves mostly backcross. Introgression of genes might proceed, as has been suggested for transfer of chloroplasts and nuclear genes from *P. centranthifolius* to other species in the section *Peltanthera* (Wolfe and Elisens 1994), but only very rarely would  $F_1$ s be isolated and allowed to breed into a pure species like *P. clevelandii*.

This raises the mechanistic question of why *Penstemon* hybrids are so rare. In the case of *P. centranthifolius* and *P. spectabilis*, reproductive isolation is compounded by several factors. Straw (1956a, 1956b) thought it was mainly a difference in pollinators. Hummingbirds visit

the red tubular flowers of *P. centranthifolius*, while bees and wasps visit the blue-flowered *P. spectabilis* (Thomson et al. 2000). George (1974) confirmed that *P. centranthifolius* and *P. spectabilis* have different primary pollinators; however, she emphasized that there was considerable opportunity for interbreeding via inconstant pollinations. This suggests that there must be other isolating barriers besides pollinator specificity. Chari and Wilson (2001) found additional barriers to interbreeding in the form of pollen-stylar incompatibility, poor interspecific seed set, a slight infertility of  $F_1$ s, and a small amount of hybrid breakdown. The cumulative effect of all these barriers is considerable, although still not enough to account for the rarity of *P. × parishii*; thus, other factors may also be involved. The other 2 pairs of parental species that we studied each share a

common type of pollinator, but they rarely occur together. In the case of *P. clevelandii* and *P. spectabilis*, it is probably because of ecological differences, *P. clevelandii* being more of a desert species. In the case of *P. centranthifolius* and *P. eatonii*, habitat differences may also be important; in addition, the hybrids are probably largely sterile judging from the shrunken pollen grains we found. Thus, an array of isolating mechanisms may act together in maintaining the integrity of the parental species.

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