The Threat of Increasing Hybridization of an Endangered Plant Species, *Purshia subintegra*, in the Verde Valley, Arizona

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Abstract: The existence of the endangered shrub *Purshia subintegra* may be threatened by hybridization with the more common *P. stansburiana*. In the Verde Valley, the range of the rare *P. subintegra* overlaps with the range of *P. stansburiana*, allowing introgression to occur. Although *P. subintegra* and *P. stansburiana* have many distinguishing characteristics, there are plants in the Verde Valley that have a combination of characteristics, making them difficult to identify. Using discriminant function analysis, we found that the parents and introgressed forms had detectable morphological differences that could be used to classify individuals, but only to 87 percent accuracy. Examining phenological and ecological differences increased our ability to detect introgressed forms. *Purshia subintegra, P. stansburiana*, and the introgressed forms overlap in their time of flowering in the spring, but *P. stansburiana* and plants showing characteristics of introgression can also bloom again in the summer. Comparing the presence of both parents and the introgressed forms in the Verde Valley in undisturbed limestone soil, roadsides, and washes, we found a significant difference in the habitat distributions of *P. subintegra, P. stansburiana*, and introgressed forms. *Purshia subintegra* showed no habitat preference; however introgressed forms occurred more frequently along washes and roadsides than in undisturbed habitat and *P. stansburiana* occurred in washes and on undisturbed limestone soil, but not along roadsides. Increased road construction and human disturbance in the rare *P. subintegra* habitat may favor the spread of introgressed forms along roads and increase the likelihood of more introgression. This has grave implications for the conservation of this rare species.

Arizona cliffrose, *Purshia subintegra* (Kearney) Henrickson, is a rare soil endemic that was listed as an endangered species in 1984 (U.S. Fish and Wildlife Service 1984). It is known from four disjunct populations across central Arizona (Figure 1) in the upper Sonoran desert on Tertiary lakebed limestone deposits: Burro Creek in Mohave County (Kearney 1943), Bylas in Graham County (Pinkava et al. 1970), near Horseshoe Lake in Maricopa and Yapavai Counties, and in the Verde Valley near Cottonwood, Yapavai County (Anderson 1986). The largest, healthiest, and most morphologically variable population grows in the Verde Valley outside of Cottonwood, where its range overlaps with the more common cliffrose, *Purshia stansburiana* (Torr.) Henrickson, and there is introgression or hybridization (Anderson 1986, Reichenbacher 1994, U.S. Fish and Wildlife Service 1994).

The morphological variability of *P. subintegra* has led to confusion regarding its taxonomy and the degree to which introgression occurs in the species. Like other members of the genus, *Purshia subintegra* forms fertile hybrids in nature (Stutz and Thomas 1964, Koehler and Smith 1981), and it is often difficult to determine the taxonomic identity of the shrubs. Three scenarios to explain the variability of *P. subintegra* morphology have been suggested. The first hypothesis is that the population at Bylas is a rare form (named *P. pinkava*) and the other three populations are hybrid races of *P. pinkava* x *P. stansburiana* (Schaack 1987a, 1987b). The second hypothesis poses that *P. stansburiana* once extended further south, possibly overlapping with the range of *P. subintegra*. The variability in the Burro Creek and Horseshoe Lake populations is due to past introgression with *P. stansburiana*, whereas in the Verde Valley the phenotypic variability is due to past and current hybridization with *P. stansburiana*, which extends down into the desert floor from the surrounding foothills (Anderson 1986). The third hypothesis is that the four populations are *P. subintegra*, but gene exchange may have occurred in the past between *P. subintegra* and *P. stansburiana* in the Verde Valley, resulting in plants that are difficult to classify (Reichenbacher 1994). Genetic studies have neither supported nor refuted any of these hypotheses (Phillips et al. 1988). Currently, the Arizona cliffrose recovery plan recognizes that *P. subintegra* is phenotypically plastic in response to climatic and environmental conditions and forms an introgressing hybrid

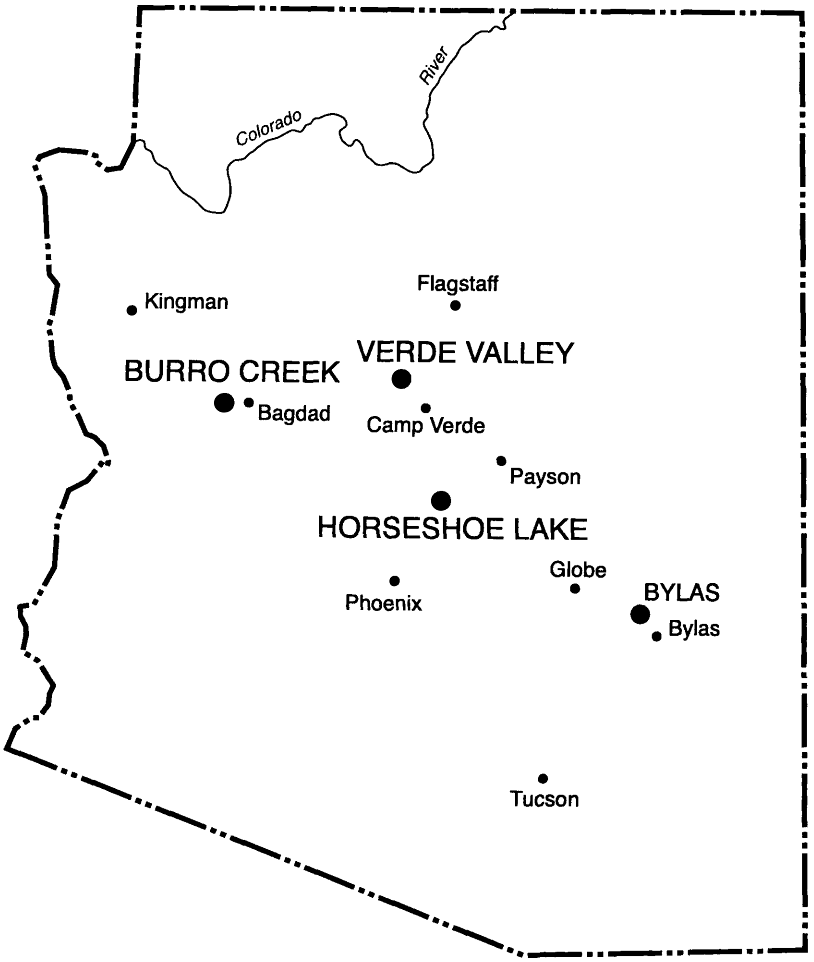

Figure 1. Map of Arizona with the four populations of Arizona cliffrose. Map is modified from U.S. Fish and Wildlife Service (1994).

swarm with *P. stansburiana* in the Verde Valley (U.S. Fish and Wildlife Service 1994).

The Verde Valley population is threatened by habitat destruction, road construction, and land sales. A planned new road will bisect the population, destroy about 10 percent of the known individuals, and degrade available and potential habitat. Development along the road will most likely lead to the additional loss of *P. subintegra* habitat. In addition, roads can fragment the population, increase disturbance and human use of the area, alter the physical and chemical environment, and provide corridors for weed invasion (reviewed in Trombulak and Frissell 2000, Parendes and Jones 2000). Roadsides have been associated with the introduction and spread of hybrids within the genus *Purshia* (Koehler and Smith 1981) and other genera (Brochmann 1984, Bramwell 1990).

We are concerned about the impact of disturbance associated with road building on the genetic diversity and integrity of *P. subintegra* in the Verde Valley. It is possible that the road construction and associated disturbance may create corridors for introgressed forms, bringing them spatially closer to stands of "pure" *P. subintegra* and therefore impacting the population dynamics of *P. subintegra*. To address these concerns we first determined which morphological characters could be used to distinguish between *P. subintegra*, *P. stansburiana*, and introgressed forms. To determine if roads would favor introgressed forms, we examined the habitat preferences of *P. subintegra*, *P. stansburiana*, and introgressed forms in washes, roadsides, and limestone outcrops.

Methods

Because of discrepancies in classification of the introgressed forms, our first goal was to determine a set of characters to identify plants in the field. We grew putative *P. subintegra*, *P. stansburiana*, and the introgressed form from rooted stem cuttings in greenhouses at the Arboretum at Flagstaff. Using these greenhouse-grown plants and plants from Burro Creek and Horseshoe Lake, we determined easily identifiable morphological characteristics to use in the field to make identifications. To account for the phenotypic variation of *P. subintegra*, we selected 57 plants for inclusion in the analysis: 16 *P. subintegra*, 10 *P. stansburiana*, and 11 introgressed forms that represented the known range of plants in the Verde Valley, and 5 plants each from the Burro Creek and Horseshoe Lake populations. Note that *P. stansburiana* does not currently grow in the vicinity of the Burro Creek or Horseshoe Lake populations.

Characteristics from previous treatments that were known to be useful in identifying the *P. subintegra* complex were leaf shape, impressed-punctate glands on leaves, hypanthium-pedicel glands, hypanthium-pedicel length, and young twig pubescence (Table 1, Reichenbacher 1994, U.S. Fish and Wildlife Service 1994). We considered everything between the descriptions of *P. subintegra and P. stansburiana* to be the introgressed form. We used these descriptions to make the a priori determination of taxa from which we took cuttings in the field.

To measure the morphological characteristics of the greenhouse-grown plants, we used only the vegetative characteristics because floral characteristics were not available for all individuals when grown in the greenhouse (Table 2). We scored five leaves and the young stems for each plant. We analyzed our ability to identify the different taxa with discriminant function analysis (SYSTAT 8.0).

Table 1. Morphological characteristics used to distinguish *Purshia subintegra*, *P. stansburiana*, and introgressed form in the field. (Modified from Reichenbacher 1994, U.S Fish and Wildlife 1994.)

Morphological characteristics	*Purshia subintegra*	Introgressed form	*Purshia stansburiana*
Leaf shape	Entire to 1 to 2 lobes	1 to 5 lobes	3–5 deep lobes
Impressed-punctate glands on leaves	Absent	Absent/present	Present
Leaf pubescence	Loosely to densely arachnid-pubescent	Glabrous to densely pubescent	Glabrous
Young twigs	Tomentose, red-brown color	Tomentose to glandular	Red, not tomentose, and stalked glands
Hypanthium pubescence	Tomentose	Tomentose or with stalked glands	Stalked glands

Table 2. List of vegetative characteristics used in discriminant function analysis. The values of each morphological characteristic for each taxon as determined by the discriminant function analysis is listed.

Morphological characteristics	Values	*Purshia subintegra*	Introgressed form	*Purshia stansburiana*
Number of leaf lobes	Number of lobes; entire leaf is 1	1 to 3	1 to 3	3 to 5
Leaf pubescence	0 – no hairs to 5 – densely arachnid-pubescent	1 to 5	0 to 4	0 to 2
Impressed punctate glands on leaves	Presence/absence	Absent	Present or absent	Present
Stalk glands on young stems	Presence/absence	Absent	Present or absent	Present

Using phenological data, we refined our list of characteristics to easily identify these taxa in the field. We visited the Verde Valley throughout the year and recorded plant location, vegetative characters, and patterns of flowering, especially for plants that flowered out of their typical season. We used these factors to refine the determination of parental or introgressed status.

In May and June of 1999 we monitored *P. subintegra*, *P. stansburiana*, and introgressed forms in three different habitats in the Verde Valley: washes, disturbed roadsides with limestone soils, and undisturbed limestone soils. We located five sites of each habitat with *Purshia* and picked a random starting plant. We then walked through the habitat and determined the characteristics and taxa of the first 25 plants encountered. We determined taxa using the vegetative characteristics listed in Table 2 and a floral characteristic, the presence of stalked glands on the hypanthium. We analyzed the number of each taxa found in the five sites in each habitat with a chi-square test (Snedecor and Cochran 1989).

Results

Using the morphological characteristics in Table 1, the discriminant function analysis classified *Purshia subintegra, P. stansburiana*, and their introgressed forms with 87 percent accuracy (Table 3). Our identification of *P. subintegra* and *P. stansburiana* was correct, but identification of the introgressed forms was only 55 percent accurate. The analysis classified putative hybrids as *P. stansburiana* based on the presence of stem glands.

We found that leaf shape was an extremely plastic character and therefore not the best one to use to distinguish *P. subintegra* from *P. stansburiana* or the introgressed form. Cuttings collected from the field that had entire or slightly lobed leaves (our conservative criteria for being classified as *P. subintegra*) grew new leaves with two or more lobes in the greenhouse. We found that the best taxonomic characters were presence or absence of glands on the leaves, stems, and hypanthia, because they were less plastic.

To help resolve the identification of the introgressed form, we examined the phenological differences of plants in the Verde Valley (Figure 2). *Purshia subintegra* and *P. stansburiana* differ in their timing of flowering but have a 2-week period of overlap. *Purshia subintegra* blooms in early spring from April to late May, whereas *P. stansburiana* blooms from late spring (May to late June) until September, if water is available. Individual plants showing some, but not all of the morphological characteristics of *P. stansburiana* have two blooming periods: in the spring 2–3 weeks after *P. subintegra* begins to bloom and again in August and September in response to monsoon precipitation (Figure 2). We have observed plants that show characteristics of introgression and characteristics of *P. stansburiana* blooming only in late summer in the Verde Valley. The exact timing depends on the microsite and yearly climate. Thus, biseasonal flowering phenology is a useful character for determining whether a plant is introgressed.

We found a significant difference ($\chi^2 = 22.8$, df = 4, p = 0.0001) between the distribution of *P. subintegra, P. stansburiana*, and the introgressed forms in three habitats in the Verde Valley (Table 4). *Purshia subintegra* did not show a habitat preference, but *P. stansburiana* was more likely to be found in washes and was almost never found along roadsides. Roadsides and washes contained significantly more plants of introgressed form than expected.

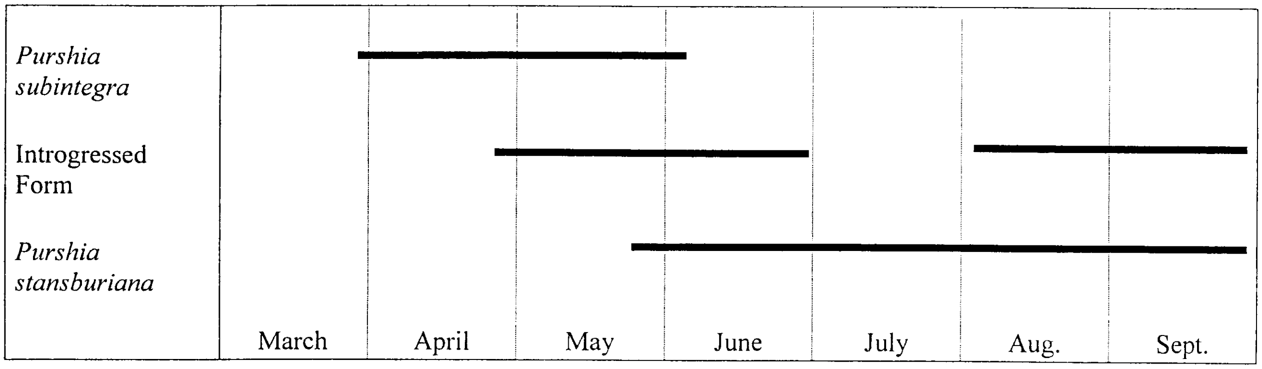

Figure 2. Timing of flowers in the Verde Valley.

Table 3. Results of the discriminant function analysis. Original identification was from the field-collected plants using characteristics in Table 1.

Original Identification	Post-hoc classification			
	P. subintegra	Introgressed form	*P. stansburiana*	Percent correct
P. subintegra	25	1	0	96
Introgressed form	2	6	3	55
P. stansburiana	0	0	10	100
	27	7	13	87

Table 4. The expected and observed values for the number of plants for each taxon and habitat using a χ^2 test. Numbers in italics indicate pairs with the greatest differences (standardized deviates greater than 1).

	Purshia subintegra		Introgressed form		*Purshia stansburiana*	
Habitat	Expected	Observed	Expected	Observed	Expected	Observed
Undisturbed	52	50	60	60	13	15
Wash	52	49	*60*	*52*	*13*	*24*
Road	52	56	*60*	*68*	*13*	*1*
Total		155		180		40

Discussion

In the Verde Valley the hybrid swarm and associated parental species are distributed in a non-random pattern. *Purshia subintegra* is distributed mostly on limestone outcrops, but also occurs naturally in first-order drainages and larger washes and along roads (Figure 3). *P. stansburiana* encounters *P. subintegra* along washes within the valley and on the southwestern edge of the range. The introgressed forms occur in all the habitats, but significantly more than expected were found along roadsides at the southwest edge of the *P. subintegra* range. One possible reason for this pattern is that roadsides are preferred habitat for introgressed forms.

Alternatively, the occurrence of introgressed forms along roadsides may be an artifact of roads being built through existing hybrid swarms rather than creating them. Roads have been built only on the edges of the distribution of Arizona cliffrose, where the two parent species overlap (Figure 3). The new road being built through the *P. subintegra* habitat will provide an opportunity to research these two alternate hypotheses.

Roads and other disturbances can cause the breakdown of ecological barriers between species by providing habitats and movement corridors for hybrids (Bramwell 1990, Levin et al. 1996), thereby increasing the rate of introgression. The proposed road bisecting the *P. subintegra* population could provide such a corridor for introgressed forms to make contact with the densest and most fecund stand of pure *P. subintegra*. In the center of the population on the undisturbed limestone outcrops, *P. subintegra* exists without close contact with the introgressed form or *P. stansburiana*. However, the proposed road differs from other roads in the area because it will bisect the population, crossing washes and other areas containing the introgressed form, thereby increasing the likelihood of introgression.

Increased introgression or hybridization has several consequences for the population dynamics of *P. subintegra*. When a rare species comes into contact with an abundant widespread species, genetic assimilation or swamping can occur, potentially causing outbreeding depression or a loss of unique gene combinations in the rare species (Rieseberg 1991, Levin et al. 1996). Our studies showed that hybrids had greater opportunities for pollen transfer and seed set, because they have two bouts of flowering and seed set. Because the flowering period of the introgressed forms overlaps with the flowering periods of *P. subintegra* and *P. stansburiana*, they likely will cross-pollinate with pure parental species (see Figure 2). Previous

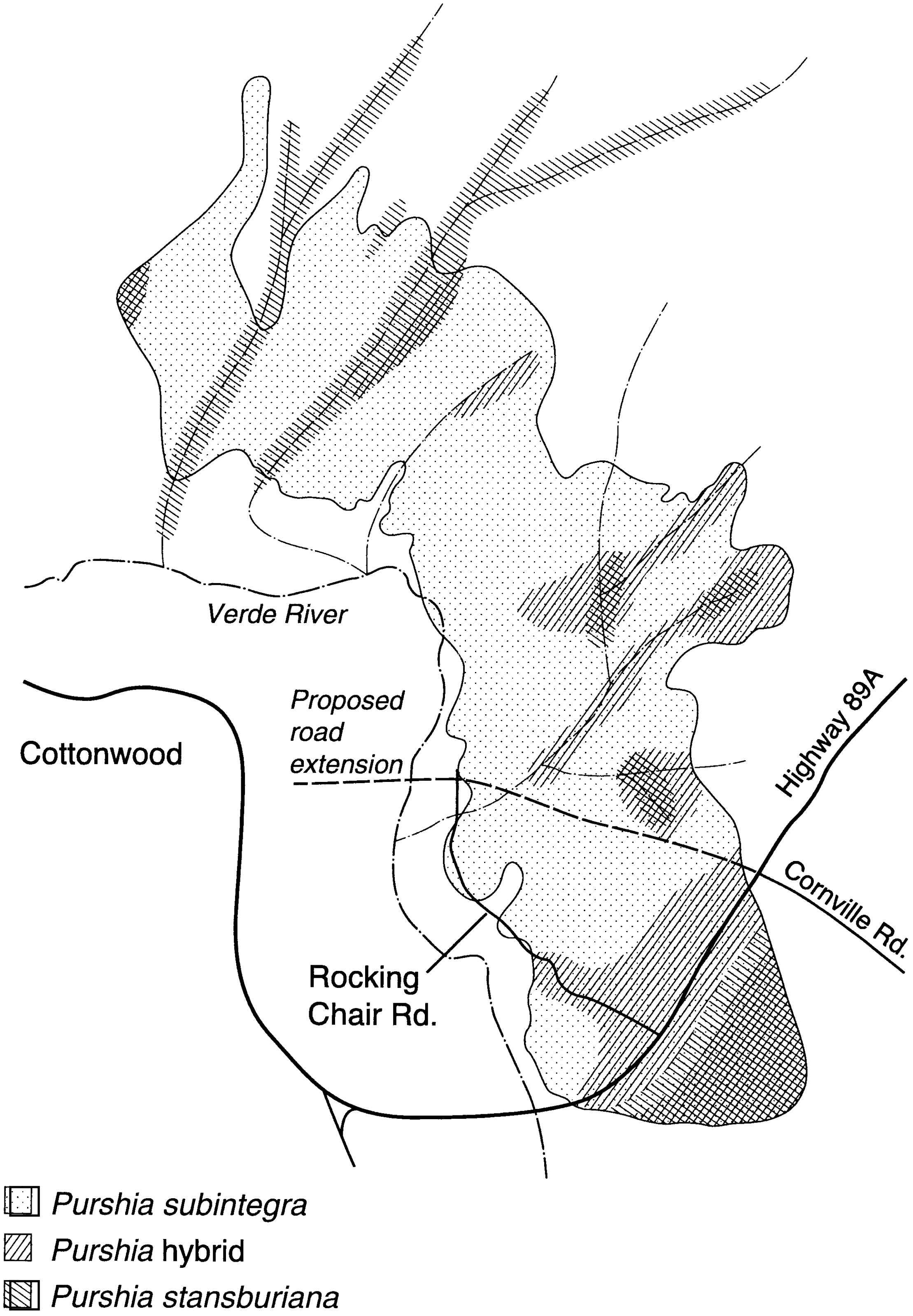

Figure 3. Map of *Purshia subintegra, P. stansburiana,* and the introgressed forms in the Verde Valley with the proposed Mingus Avenue extension. The distribution of each taxa is based on our results, U.S. Fish and Wildlife (1994), and Denham and Fobes (1994).

studies have shown that *P. subintegra* and the introgressed form can cross and produce fertile seed (Baggs and Maschinski this volume, Fitts et al. 1992); therefore through interference competition introgressed forms limit the chance for pure *P. subintegra* pollen to reach pure *P. subintegra* stigmata. In addition, a longer season of flowering and seed production gives introgressed forms a competitive advantage for responding to unpredictable rainfall and a greater seed rain. Our studies showed that introgressed forms grew on a wide range of habitats, suggesting that they are good competitors and may outcompete parental species for seedling microsites and limited resources. Conversely, *P. subintegra* grows in restricted habitats and has variable seed production that is positively correlated to the previous winter's precipitation (Baggs and Maschinski 2000). Following winter drought, *P. subintegra* is not likely to set flowers or seed in the spring and will lose a year of seed production.

Yet hybrid or introgression zones can be important for diversity and evolution (Stebbins 1959). Hybrid origins have been proposed for several rare species (Rieseberg 1991) including species of *Purshia* (Stutz and Thomas 1964). Introgression may in fact provide a mechanism to introduce genetic variability and new characters into a population of rare plants to allow its continued survival and adaptation into new environments and sites (Rieseberg 1991, Stebbins 1959), a positive aspect of hybridization in the management of rare species (Whitham and Maschinski 1996). This scenario has also been suggested for many species in the genus *Purshia* (Stutz and Thomas 1964, Anderson 1993). Yet there is concern about losing the unique characters of this rare species as a consequence of hybridization (Anderson 1993).

There are many possibilities for future research. We are continuing to research the genetic relationship of *P. subintegra*, *P. stansburiana*, and introgressed forms through common garden experiments and genetic analysis. In addition, the new road construction will allow us to examine the impacts of road construction on a rare soil endemic. Because roadsides can provide habitat for both *P. subintegra* and the introgressed forms, recruitment and establishment into the roadsides of *Purshia* can be monitored. The road will also bisect the population so that continued monitoring can help us understand the effect of the road on the population dynamics of *P. subintegra* in the Verde Valley.

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