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Studies in *Sidalcea* Taxonomy

Abstract

The objectives of this study were to investigate taxonomic relationships among the four species of *Sidalcea* growing in Oregon's Willamette Valley, and among various populations of *Sidalcea nelsoniana*. These relationships were assessed by examining pollen with the scanning electron microscope, and performing principal component analysis (PCA) on gross morphological features of the plants. Pollen morphology was of limited use in making intra- and inter-specific comparisons. Chromosome number was determined for six populations of *S. nelsoniana*; it was identical for all populations ($n = 10$). PCA was useful in segregating the four *Sidalcea* species. However, a PCA of 73 specimens of *S. nelsoniana* revealed no distinct sub-taxa; this information is useful in making management decisions for this Category 2 candidate species.

Introduction

Sidalcea is comprised of herbaceous annuals and perennials native to western North America (Roush 1931, Hitchcock 1957). The four species found in the Willamette Valley of Oregon, *S. nelsoniana* Piper (Nelson's checker-mallow), *S. cusickii* Piper (Cusick's checker-mallow), *S. campestris* Greene (meadow sidalcea), and *S. virgata* Howell (rose checker-mallow), are all perennials that bloom in late spring to mid-summer and are characterized by a gynodioecious breeding system. In a gynodioecious species individual plants are either pistillate (the flowers are male-sterile) or hermaphroditic (the flowers are perfect). Often morphological differences, such as overall plant size or flower size (Willson 1983), can be found between these two types of individuals. The existence of these two different morphological types within a species has resulted in frequent misidentification of these species. Therefore, the primary goal of this study was to clarify species relationships by examining selected taxonomic and morphologic characteristics of these species.

The taxonomy of *S. nelsoniana*, a western Oregon endemic, has been a concern to federal agencies as it is a candidate species for federal listing as a Category 2 species (U.S. Fish and Wildlife Service 1985). It is found in the northern Coast Range in both the Nestucca River and the Wilson River drainages, and in the Willamette Valley from Benton and Linn Counties north to Washington County (Halse and Glad 1986). The

existence of *S. nelsoniana* in these two different habitats has led to speculation that there may be two separate taxa. This paper also examines this hypothesis.

The proposed construction of a water supply reservoir at Walker Flat in the Coast Range, west of Carlton, would inundate an area which currently supports a large population of *S. nelsoniana* (Glad *et al.* 1987). Determining the taxonomic status of the *S. nelsoniana* at Walker Flat would help federal agencies assess the significance of managing this population.

Materials and Methods

Relationships among the four species of *Sidalcea*, and within *S. nelsoniana*, were determined by examination of pollen with a scanning electron microscope (SEM), and principal component analysis (PCA) of selected morphological characteristics. In addition, chromosome counts were made for six *S. nelsoniana* populations, two from the Coast Range and four from the Willamette Valley.

Air dried pollen collected from the four species of *Sidalcea* was dispersed on 15 mm diameter coverslips coated with a thin adhesive film (Mikrostick). Coverslips were attached to aluminum planchets using conductive silver paint. After mounting, specimens were coated with approximately 150 Å of 60:40 weight percent Au/Pd alloy in a Varian VE-10 vacuum evaporator at a vacuum of 1×10^{-5} Torr. Examination was made using an AMRAY 1000 A SEM, operated at 10 or 20 kV, at the Electron Microscope Facility, Department of Botany and Plant Pathology, Oregon State University. Images were

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recorded on Polaroid Type 55 positive/negative 4 x 5 format film.

For chromosome determination, floral buds from six populations of *S. nelsoniana* were collected in the field and fixed in a modified Carnoy's solution (4 chloroform: 3 ethanol: 1 glacial acetic acid, v/v/v). Acetocarmine squashes of pollen mother cells were obtained by using the technique of Snow (1963).

The morphological measurements for this study were obtained from 131 herbarium specimens of the four species of *Sidalcea* borrowed from the herbaria at Oregon State University, the University of Oregon, Washington State University, the University of Washington, Willamette University, and the United States Fish and Wildlife Service, Boise, Idaho. Of these specimens 73 were of *S. nelsoniana*, 22 of *S. campestris*, 21 of *S. virgata* and 15 of *S. cusickii*. Specimens were from throughout the species' distribution in the Willamette Valley. A list of the specimens, and their site localities, used in the PCA is available from the senior author on request.

The five morphological parameters listed in Table 1 were measured on all 131 herbarium specimens. Only those specimens which had all five morphological parameters were used in the PCA. These parameters were selected for the PCA because they are considered to be important traits in the latest monograph of the genus (Hitchcock 1957) and are traits emphasized in the latest regional flora (Hitchcock and Cronquist 1973). Sixteen morphological characters were initially examined (CH2M Hill 1986). Many of these characters were judged to be so similar among species as to be non-discriminating and were not included in the investigation (e.g., calyx pubescence, flower density, upper stem pubescence, style number). Other characters, while important taxonomically, are usually not found on most herbarium specimens (e.g., root type, fruit traits).

Examination of the herbarium specimens revealed that the flowers on the perfect-flowered plants tended to be larger than the flowers on the pistillate-flowered plants. The influence of sex on the parameters used in the PCA was tested using a factorial analysis of variance (ANOVA) with flower sex and species as the factors. The number of specimens of each species and flower type are indicated in Table 2. This analysis indicated a significant effect of flower sex and

TABLE 1. Definition of morphological parameters used in *Sidalcea* principal component analyses.

Abbreviation	Definition	Numeric Code
BASTPU	basal stem pubescence	1 = none 2 = simple 3 = forked or stellate
PETCOL	petal color	1 = white or pink 2 = red, lavender or purple
ADPETLG	adjusted petal length	millimeters
ADCALLG	adjusted calyx length	millimeters
CALCOL	calyx color	1 = green 2 = green with some purple 3 = purple

species on the length of the petals and calyces. Therefore, for the PCA, the length of the petals and calyces were adjusted for sex-related flower differences. For both petals and calyces, this adjustment was made separately for each species by adding the difference between the mean length of the perfect-flowered specimens and the mean length of the pistillate-flowered specimens to each of the pistillate-flowered individuals. Following this adjustment, the mean length of the pistillate-flowered individuals was equal to the mean length of the perfect-flowered individuals. This eliminated segregation of individuals based solely on the sex of the flower. Data on the five parameters listed in Table 1 for *S. nelsoniana*, *S. virgata*, *S. cusickii* and *S. campestris* were analyzed by a PCA computer program (SAS Institute Inc. 1985). The raw data from the specimens used in this analysis are summarized in Table 2.

Following the PCA of the four species, a PCA was performed on 73 specimens of *Sidalcea nelsoniana* alone, in order to examine the relationship between the specimens found in the Coast Range, particularly those from Walker Flat, and those of the Willamette Valley.

Results and Discussion

The pollen of all specimens of *Sidalcea* is typically malvaceous (Figure 1). The grains are 60-70 microns in diameter, are conspicuously spiny, have a granular surface and are pantoporate.

TABLE 2. Means and standard deviations of morphological parameters for *Sidalcea* species analyzed in the principal component analysis.

Species	Flower Sex	n	Parameter ¹				
			BASTPU	PETCOL	PETLG	CALLG	CALCOL
<i>S. nelsoniana</i>	Perfect	41	2.0 ± 0.3	2.0 ± 0.0	11.5 ± 2.0	5.5 ± 0.7	2.0 ± 0.2
	Pistillate	32	2.0 ± 0.2	2.0 ± 0.0	7.1 ± 1.3	4.7 ± 0.7	2.0 ± 0.2
<i>S. virgata</i>	Perfect	11	3.0 ± 0.0	2.0 ± 0.0	19.8 ± 3.6	8.7 ± 1.4	1.7 ± 0.5
	Pistillate	10	2.9 ± 0.3	2.0 ± 0.0	10.6 ± 1.5	6.3 ± 1.2	1.8 ± 0.4
<i>S. cusickii</i>	Perfect	8	2.5 ± 0.9	1.9 ± 0.4	14.5 ± 3.5	7.8 ± 2.0	1.9 ± 0.4
	Pistillate	7	3.0 ± 0.0	2.0 ± 0.0	9.7 ± 1.7	6.9 ± 0.8	1.8 ± 0.4
<i>S. campestris</i>	Perfect	15	2.1 ± 0.4	1.1 ± 0.2	17.3 ± 4.6	7.5 ± 1.3	1.7 ± 0.5
	Pistillate	7	2.0 ± 0.0	1.0 ± 0.0	10.2 ± 0.7	6.1 ± 0.7	1.6 ± 0.5

¹See Table 1 for definitions of parameters.

The smooth, sticky surface material covering at least part of the granular surface (Figure 1-C,E) is due to the presence of pollenkit (Dobson pers. comm.), which has been reported from other species of *Sidalcea* (Dobson 1988). Although the pollen of *S. campestris* exhibits some variability in size and shape of the spines, the pollen grains of all the taxa are very similar and do not appear to be useful taxonomically.

The number of chromosomes in all six of the *S. nelsoniana* populations that were examined were identical ($n = 10$). The specimens counted were *Halse 3178, 3312* (Yamhill County, Walker Flat), *Halse 3313* (Tillamook County, Devils Lake Fork of the Wilson River), *Halse 3289, 3293* (Benton County, Corvallis), *Halse 3240* (Polk County, Rickreall), *Halse 3291* (Marion County, Turner). This count is consistent with an earlier report (Kruckeberg 1957). *S. nelsoniana*, like *S. cusickii*, shows no signs of polyploidy, although polyploidy is found in both *S. campestris* ($2n = 60$) and *S. virgata* ($2n = 20$ or 40) (Kruckeberg 1957).

The results of the ANOVA indicate that there is a statistically significant difference ($p < 0.001$) among species for all of the five parameters listed in Table 1, indicating that all of them are useful in distinguishing among species. The ANOVA also indicates statistically significant ($p < 0.001$) differences in petal length and calyx length between the perfect-flowered and pistillate-flowered plants of the same species. There is also a significant difference attributable to the species-by-flower sex interaction for the petal length ($p < 0.01$) and the calyx length ($p < 0.05$). This in-

dicates that the magnitude of the effect of flower sex on petal and calyx length varies among species (Table 2).

The first two components of the PCA for the four species of *Sidalcea* account for 70.6 percent of the total variation, and are plotted in Figure 2. The eigenvectors for each component are listed in Table 3. In general, there is an observable separation between *S. nelsoniana* in the left-central section of the plot, *S. campestris* in the right-lower section, and *S. virgata* in the right-upper of the figure. *S. cusickii* is less clearly segregated from the other species, and intermingles extensively with *S. virgata* and somewhat with *S. nelsoniana*. These results suggest that, with the exception of *S. cusickii*, the characters used in the PCA are generally effective and appropriate for distinguishing between the four species of *Sidalcea* used in this study. The third, fourth and fifth components were examined (plots are not shown) and these also failed to provide a clearcut segregation between *S. virgata* and *S. cusickii*. However, *S. cusickii* and *S. virgata* have some distinguishing fruit and calyx characters not used in this study (e.g., calyx venation and shape, carpel reticulation).

One of the problems in identifying species of *Sidalcea* from the Coast Range and Willamette Valley is that the keys and species descriptions in both Hitchcock's monograph (1957) and local floras do not distinguish petal and calyx length differences associated with flower sex. Generally the petal length, and calyx length, is simply given as the combination of the lengths of both sexes; i.e., in *S. nelsoniana* petal length is given

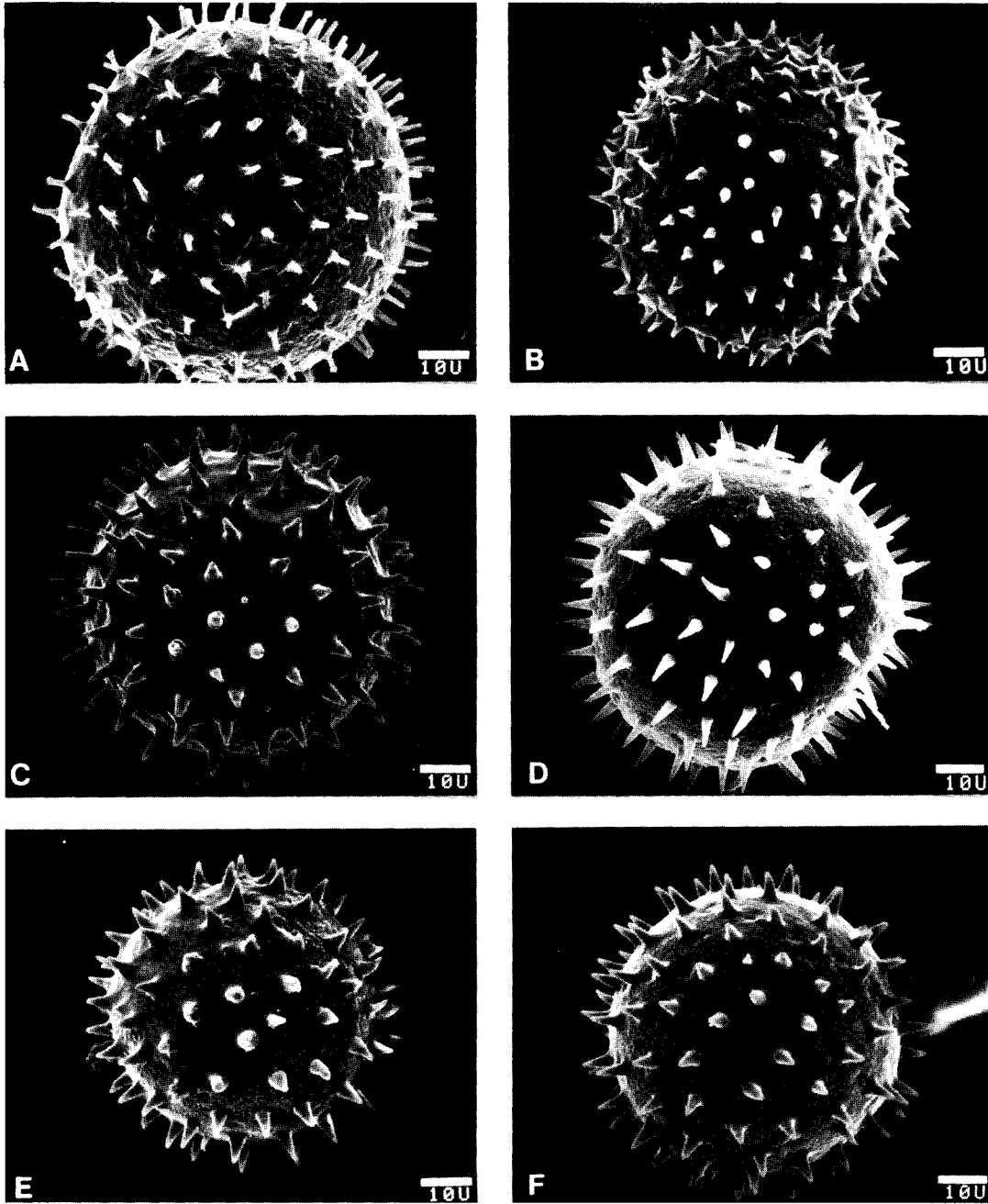


Figure 1. Scanning electron photomicrographs of *Sidalcea* pollen. A, *S. campestris*, Polk Co.: Highway 22, Rickreall, Halse 3299; B, *S. campestris*, Linn Co.: Cogswell-Foster, Halse 3455; C, *S. virgata*, Benton Co.: Smith Loop Road, south of Corvallis, Halse 3281; D, *S. cusickii*, Douglas Co.: Calapooia Valley, Cole s.n.; E, *S. nelsoniana*, Yamhill Co.: Walker Flat, Halse 3312; F, Polk Co.: Rickreall, Halse 3290. (Voucher specimens at Oregon State University).

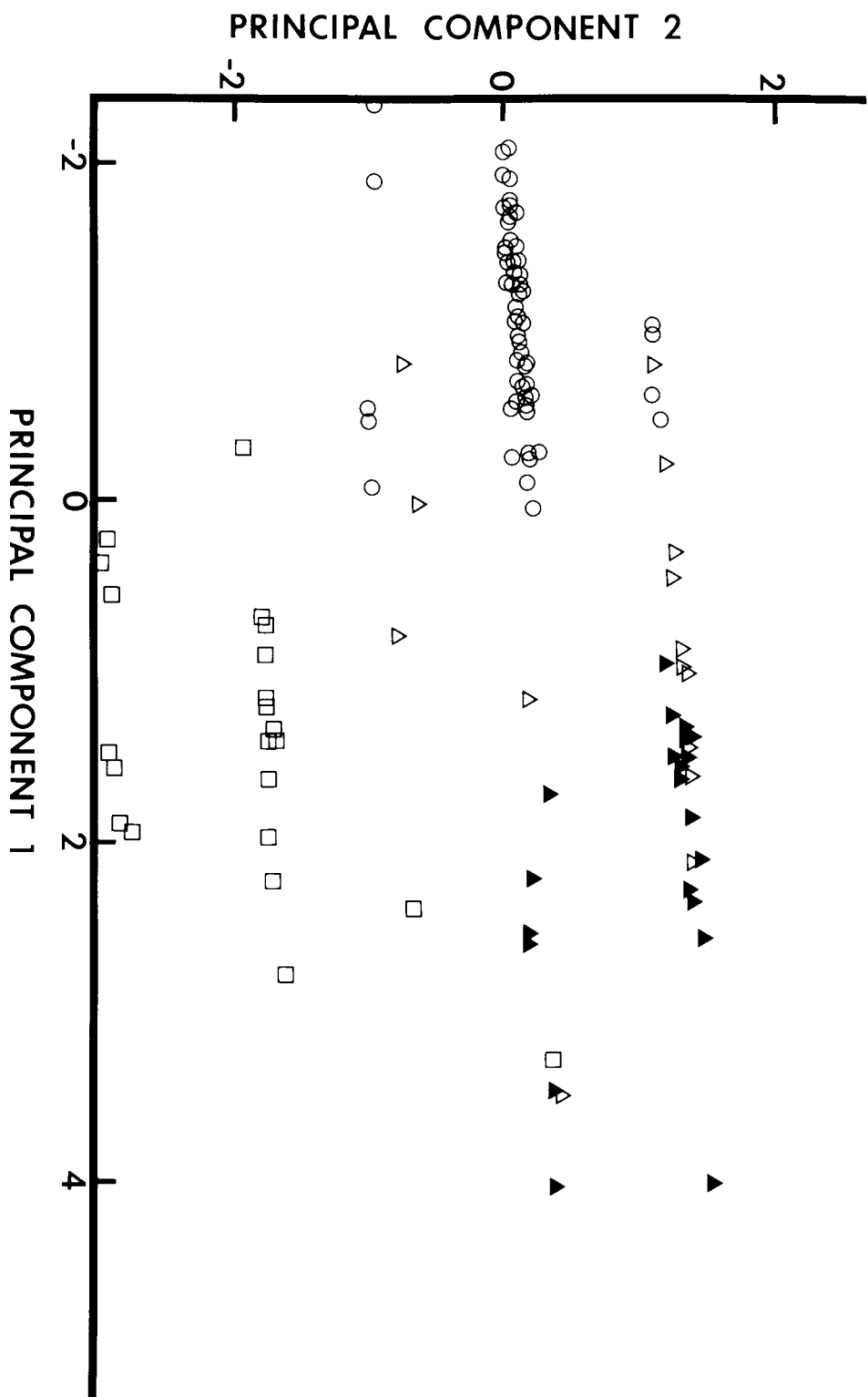


Figure 2. The first two principal components for specimens of *Stalcea* species. *S. nelsoniana* (○), *S. virgata* (△), *S. cusickii* (▽) and *S. campestris* (□).

TABLE 3. Eigenvectors for morphological parameters in each component of the principal component analysis of four species of *Sidalcea*.

Parameter ¹	Component				
	1	2	3	4	5
BASTPU	+0.397	+0.542	-0.259	-0.693	-0.032
PETCOL	-0.241	+0.743	-0.277	+0.539	+0.148
CALCOL	-0.275	+0.376	+0.864	-0.185	-0.043
ADPETLG	+0.599	+0.000	+0.271	+0.208	+0.724
ADCALLG	+0.591	+0.114	+0.189	+0.388	-0.672
Variance					
Explained	46.7%	23.9%	17.1%	9.3%	3.0%

¹See Table 1 for definitions of parameters.

1. Petals white to pink; basal stem pubescence simple; petals of perfect flowers 13-25 mm long, calyx 6-9 mm long; petals of pistillate flowers 9-12 mm long, calyx 5-7 mm long. *S. campestris*
1. Petals red, lavender or purple
 2. Basal stem pubescence simple; petals of perfect flowers 9-15 mm long, calyx 4.5-7 mm long; petals of pistillate flowers 5-9 mm long, calyx 4-6 mm long. *S. nelsoniana*
 2. Basal stem pubescence of forked to stellate hairs
 3. Petals of perfect flowers 11-19 mm long, calyx 6-10 mm long; petals of pistillate flowers 8-12 mm long, calyx 6-8 mm long; calyx usually prominently veined, lobes widened above base, ± ovate-lanceolate. *S. cusickii*
 3. Petals of perfect flowers 15-25 mm long, calyx 7-10 mm long; petals of pistillate flowers 9-13 mm long, calyx 5-7 mm long; calyx not prominently veined, lobes not widened above base, tapered evenly to the tip. *S. virgata*

The PCA using the five characters examined on 73 specimens of *S. nelsoniana* shows no component separation among geographical populations of this species. The first two components of the PCA for *S. nelsoniana* are plotted in Figure 3. The total variation in component 1 is similar in both Figure 2 and Figure 3. However, the overlap of the Willamette Valley and Coast Range populations in Figure 3 along the component 1 axis make it impossible to segregate these groups. The first two components account for 68.5 percent of the variation (Table 4). In Figure 3, the specimens from the Coast Range and the Willamette Valley are intimately intermixed, indicating that, based on the characters used in this PCA, the Willamette Valley and Coast Range populations of *S. nelsoniana* are not distinct morphologically. Similarly, the specimens from Walker Flat are closely intermixed with other Coast Range specimens, indicating that the population at Walker Flat is not a distinct taxon. The Coast Range populations are also not differentiated from the Willamette Valley populations

as 5-15 mm, rather than indicating that petal length in perfect flowers is 9-15 mm, while petal length in pistillate flowers is 5-9 mm. It is important to account for flower sex, as both perfect- and pistillate-flowered plants are common. Examination of 32 populations of *S. nelsoniana* indicated the average ratio of pistillate-flowered plants to perfect-flowered plants is 1.36:1 (CH2M Hill 1987). Incorrect identification can result from using a key that fails to distinguish differences in petal and calyx size associated with flower sex. A key which allows for these differences, as well as utilizing other primary characteristics, is presented below:

TABLE 4. Eigenvectors for morphological parameters in each component of the principal component analysis of *Sidalcea nelsoniana*.

Parameter ¹	Component				
	1	2	3	4	5
BASTPU	-0.205	+0.535	+0.807	+0.141	+0.000
PETCOL	-0.000	+0.000	+0.000	+0.000	+1.000
CALCOL	-0.010	+0.830	-0.557	+0.026	+0.000
ADPETLG	+0.702	+0.016	+0.044	+0.710	+0.000
ADCALLG	+0.682	+0.157	+0.189	-0.689	+0.000
Variance					
Explained	43.1%	25.4%	24.2%	7.3%	0.0%

¹See Table 1 for definitions of parameters.

when the third and fourth components are examined (plots are not presented). These results agree with electrophoretic analysis of both leaf and seed proteins from Coast Range and Willamette Valley populations of *S. nelsoniana* (CH2M Hill 1986) which also show no differences among geographic populations of *S. nelsoniana*.

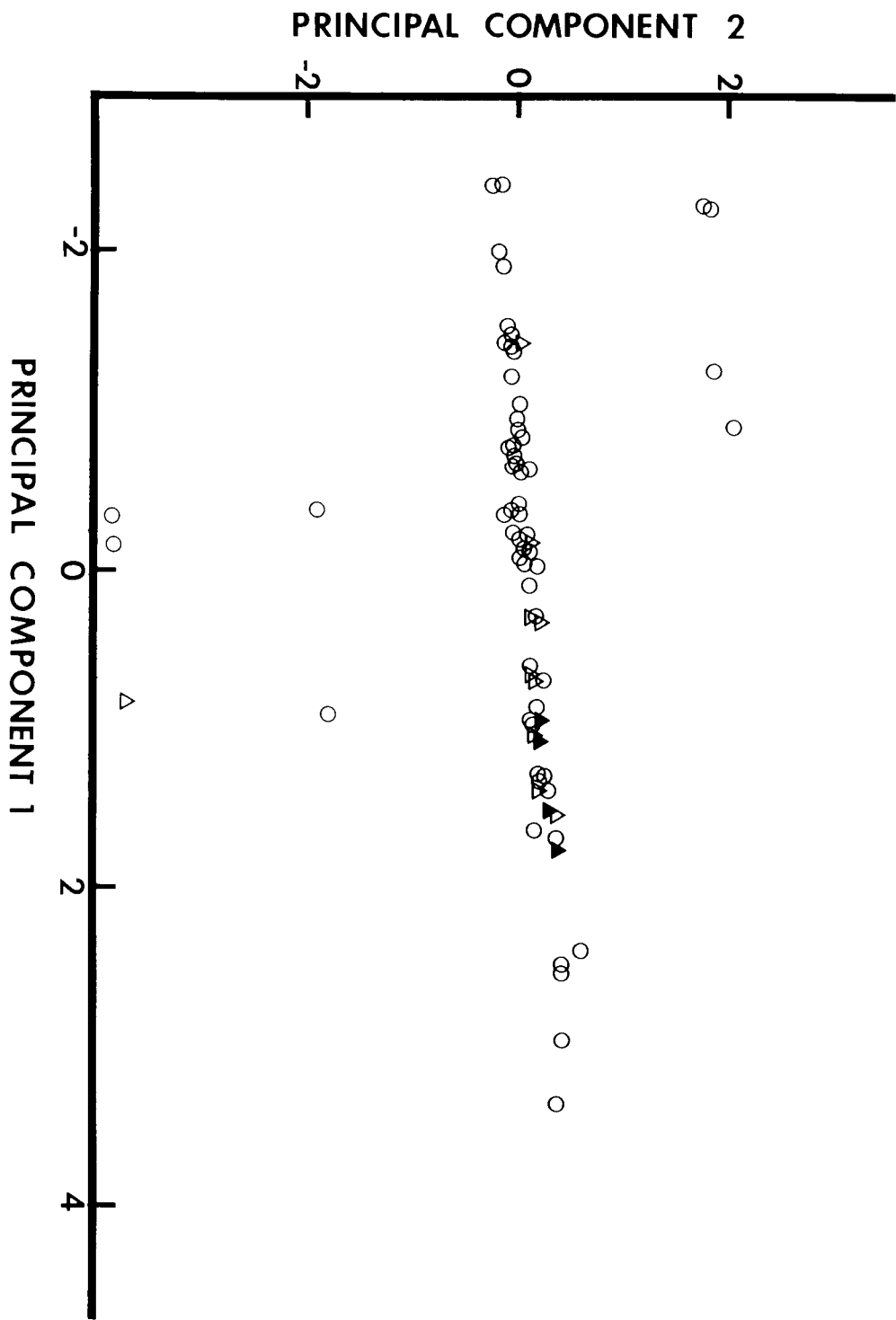


Figure 3. The first two principal components for specimens of *Sidalcea nelsoniana*, Willamette Valley (○), Coast Range (excluding Walker Flat) (△), and Walker Flat (▼).

An absolutely definitive statement on the genetic diversity between populations of *S. nelsoniana*, and in particular between those at Walker Flat and other locations, is beyond the scope of this study. However, to the extent that the taxonomic characteristics studied here are manifestations of the plants' genetic makeup, this study supports the hypothesis that the Walker Flat population is not genetically distinct from other populations, and that the loss of the Walker Flat population would not deplete the genetic diversity of the species.

In summary, analysis of pollen morphology and chromosome number, and the PCA of standard morphological traits have been used to confirm the species integrity of the four Willamette

Valley *Sidalcea*. Recent concerns over the taxonomic status of these species may have been influenced by oversight of the morphological variation within gynodioecious species. The analysis presented here also shows that *S. nelsoniana* is morphologically consistent throughout its range and so there is no basis for dividing the species into separate taxa.

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