A QUANTITATIVE ANALYSIS OF POLLEN VARIATION IN TWO SOUTHERN CALIFORNIA PERENNIAL *HELIANTHUS* (HELIANTHEAE: ASTERACEAE)

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ABSTRACT: *A quantitative analysis of pollen from Helianthus californicus, H. nuttallii var. nuttallii, H. nuttallii var. parishii, and H. "Newhall Ranch" is undertaken. Equatorial diameter, polar diameter, length and width of colpi, pore diameter, spine length, number of micropores at the base of spines, and number of spines in a direct line along the equator of the grain between apertures were measured for 34 collections of these taxa. Multivariate analysis of variance (MANOVA) of continuous variables reveals a significant difference among the taxa. Post hoc multiple pairwise comparisons show that the difference discovered using MANOVA is due largely to the differences between H. californicus and H. nuttallii (subsp. nuttallii and parishii do not differ). The Newhall Ranch population differed significantly in some traits with H. californicus; however, differed in others with H. nuttallii. This intermediate relationship is consistent with chromosome counts, which are also intermediate to the counts available for H. californicus and H. nuttallii. While not conclusive, these data are consistent with the hypothesis that the Newhall Ranch population is neither H. californicus nor H. nuttallii, but perhaps an intermediate polyploid, linking the two.*

KEYWORDS: *Helianthus californicus, Helianthus nuttallii subsp. nuttallii, Helianthus nuttallii subsp. parishii, palynology, quantitative analysis, rare taxa.*

INTRODUCTION

California has seven native, perennial species of sunflower (Helianthus L.). Four of these occur in southern California: Helianthus californicus DC., H. gracilentus A. Gray, H. niveus (Benth.) Brandeg., and H. nuttallii Torr. & A. Gray (Munz 1974). Most of these species are easily recognized. For example, H. gracilentus and H. niveus differ from the other native perennial species in that they possess phyllaries that are shorter than or subequal to the disk flowers; however they differ from one another in that H. niveus has densely white hairy leaves and is associated with dunes, whereas H. gracilentus has short scabrous (rough) hairs and is associated with chaparral. The differences between the two remaining southern California native species (H. californicus, H. nuttallii subsp. nuttallii and H. nuttallii subsp. parishii [A. Gray] Heiser), are much less profound. Indeed, in many morphological features there is substantial overlap between the two subspecies, including trichome distribution and type, phyllary length, and corolla length (Table 1). In spite of this, there is no question that H. californicus and H. nuttallii represent different species.

In their monograph on North American sunflowers, Heiser et al. (1969) note that Helianthus californicus is amply distinct from H. nuttallii based on its very long, reflexed phyllaries, although this feature may vary more than Heiser et al. indicate (M. Elvin, pers. comm.). They further note that the pollen of H. californicus is consistently larger than that of H. nuttallii. They suggest that pollen size is a good character to differentiate these closely related species. The two subspecies of H. nuttallii differ primarily in characteristics of their hairs (Table 1) and their geographic distribution in Southern California. Subspecies parishii, as noted above, has been presumed extinct, and no collections have been made since the mid 1930s.

The recent discovery of a perennial Helianthus, in a wet meadow on Newhall, Ranch, Los Angeles County, has generated both excitement and confusion. The small stand of six individuals is anomalous morphologically, being intermediate between the two subspecies of H. nuttallii, and somewhat similar to some populations of H. californicus. There has been some speculation that this small stand may represent an extant population of Helianthus nuttallii subsp. parishii. It remains unclear if this small population represents H. nuttallii subsp. nuttallii, H. nuttallii subsp. parishii, H. californicus, or an entirely different taxon.

Studies of cytology of Helianthus have reported different chromosome numbers for Helianthus californicus and H. nuttallii. The diploid chromosome number for H. californicus has been reported to be 2n= 102 (Heiser et al. 1969), whereas that of H. nuttallii subsp. nuttallii is 2n= 34 (Heiser et al. 1969). It is important to note that the chromosome count for H. nuttallii is based on material collected in the eastern portion of its range (Heiser et al 1969), rather than California. Therefore, the chromosome number for H. nuttallii subsp. nuttallii in California has not been characterized. In addition there have never been chromosome counts made for H. nuttallii subsp. parishii.

A recent investigation of the cytology of the Helianthus growing on Newhall Ranch was conducted at Rancho Santa Ana Botanic Garden (RSABG: Soza 2003). Chromosome counts from root tips of germinating seeds revealed a count of 2n= 68. This represents a count previously unreported for either H. nuttallii or H. californicus. Unfortunately, it is not known if the unusual chromosome number is characteristic of the Newhall Ranch population, of H. nuttallii subsp. parishii, or of H. nuttallii as a whole in Southern California.

It has been recognized that there is a high correlation between pollen size and number of duplicate copies of chromosomes present in the genome (ploidy level) (Dermin 1930). If the previous counts of H. nuttallii (X= 34) represent the base number, then the count of the population near Newhall is doubled (2X), and the count for H. californicus is tripled (3X). It is likely that an increase in pollen size is associate with the doubling and tripling of the diploid genome. If that were true, it would be possible to discriminate between the X, 2X, and 3X individuals by comparing pollen. This is consistent with the use of pollen size as a character to distinguish H. californicus from H. nuttallii by Heiser et al. (1969).

The purpose of this study is to quantitatively examine the pollen of Helianthus californicus, H. nuttallii subsp. nuttallii and H. nuttallii subsp. parishii and to compare pollen from the Helianthus population found on Newhall Ranch (hereafter referred to as H. "Newhall Ranch") to the above listed taxa. These comparisons are designed to determine if there are qualitative differences or if there are size differences in pollen grains among these three taxa in Southern California.

MATERIALS AND METHODS

Air dried samples of disk flowers were taken from herbarium specimens at RSABG Herbarium representing 9 collections of Helianthus californicus, 15 collections of H. nuttallii subsp. nuttallii and 9 collections of H. nuttallii subsp. parishii (Table 2). Pollen from the six individuals on Newhall Ranch was also sampled. Pollen from each sample was mounted onto an aluminum stub, using graphite adhesive. The stubs were desiccated for 24 hours in a vacuum oven, at 50° C. After desiccation, stubs were coated with gold, using a Pelco Auto Sputter Coater SC-7. Samples were examined using an ISI WB-6 scanning electron microscope, at 10kv. Photomicrographs were taken of the samples at magnifications ranging from 450X to 3,000X.

Measurement data were taken directly from the photomicrographs. Measures were made of three individual pollen grains from each sample for: equatorial diameter, polar diameter, length and width of colpi, pore diameter, spine length, number of micropores at the base of spines, and number of spines in a direct line along the equator of the grain between apertures. The three values from each sample were either averaged (continuous data) or the modal value was used (counts). All continuous measurements are reported in micrometers.

Continuous data (measurements) were analyzed using StatView 5.0.1 (1998, SAS Inc.) on a Macintosh G4 computer. On the basis of equality of variance F-tests, it was demonstrated that all continuous variables, except pore diameter, displayed unequal variances within the species/subspecies assignments. Log transformation appears to normalize the variances, based on subsequent equality of variance F-tests. Log-transformed data were analyzed using analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA), and Bonferroni corrected multiple pairwise t-tests.

Integer data (counts) were analyzed using the nonparametric Kruskal-Wallis test and, for comparative, heuristic purposes, parametric t-tests.

RESULTS

The pollen of Helianthus californicus (Fig. 1A), H. nuttallii subsp. nuttallii (Fig. 1B), H. nuttallii subsp. parishii (Fig. 1C), and H. "Newhall Ranch" (Fig. 1D) are characterized as being spherical, with three apertures. The apertures are compound, being composed of a somewhat linear thinned region of the pollen wall, referred to as a colpi, and a circular pore, more or less centrally located in the colpus. The entire surface of the pollen grain is ornamented with large

spines. The base of the spines is buttressed and possesses a series of small holes (micropores) in one or two series. Although the surface of the spines tends to be smooth, the remaining surface of the pollen grains may be smooth to irregularly and densely roughened. In general, the pollen of H. californicus, H. nuttallii and H. "Newhall Ranch" are quite similar in overall form.

Pollen among the sampled Helianthus differs in size (Table 3). The largest pollen is that of Helianthus californicus, with a mean size of about 25µ X 25µ. The smallest pollen is that of samples of H. nuttallii subsp. nuttallii, at about 20µ X 20µ. Results of MANOVA and ANOVA reveal a significant difference among H. californicus, H. nuttallii subsp. nuttallii, H. nuttallii subsp. parishii, and H. "Newhall Ranch" (F= 5.400; DF= 18; p= 0.0001; Table 4). Significant differences were found, using ANOVA, in all of the continuous variables except spine length and pore diameter (Table 4). Multiple pairwise comparisons using the continuous traits (Table 5) reveals that in some features, e.g., equatorial diameter, H. "Newhall Ranch" differs significantly from H. nuttallii subsp. nuttallii and H. nuttallii subsp. parishii, but in others, e.g., polar diameter the Newhall Ranch population differs significantly from H. californicus. However, in colpus width the Newhall Ranch population differs significantly from H. californicus, H. nuttallii subsp. nuttallii, and H. nuttallii subsp. parishii. Non parametric, Kruskal-Wallis tests do not detect a significant difference among the sampled taxa in either micropore number (H_3= 2.892; p= 0.4086), or the number of spines along the equator, between apertures (H_3= 5.514; p= 0.1378).

DISCUSSION

This study reinforces the observations of Heiser et al. (1969) that pollen of Helianthus californicus is significantly larger than that of H. nuttallii. The precise measurements we present are different than those reported by Heiser et al. (1969). These authors describe the pollen diameter of H. californicus as 36 microns, and the pollen of H. nuttallii as 21 microns; whereas, we found mean measures of ca. 25 (with a range of 21-30) microns and 20 (with a range of 16-24) microns, respectively. Part of the discrepancy may be due to the differences in methods of measurement. In the 1960s and 1970s it was common (as it still is) to place pollen into a glycerin-based mounting medium. Glycerin causes pollen grains to enlarge, if the preparation is given time. By contrast, our method requires that the pollen is dry and study (photography) takes place under a vacuum. Likewise, whereas measurements by Heiser et al. were likely to be made using an ocular micrometer and a compound microscope, our measures were based on scanning electron microscopy. We believe our measures are more accurate than the previous measures.

Because there has never been a chromosome count of Helianthus nuttallii subsp. parishii it has not been possible to determine if the count previously provided for H. nuttallii subsp. nuttallii differs from that found in H. "Newhall Ranch." Here we have contrasted pollen size between the two subspecies of H. nuttallii. We did not detect significant differences between H. nuttallii subsp. nuttallii, and H. nuttallii subsp. parishii for any of the measures of pollen we used. While

this does not prove that these two subspecies possess the same chromosome number, these data provide no evidence that their pollen differs in size, as might be expected if one were polyploid. Therefore we conclude that the two subspecies of H. nuttallii have the same sized pollen and likely the came chromosome number. By contrast, H. californicus has significantly larger pollen than both of the subspecies of H. nuttallii, as a reflection of its higher chromosome number.

Of particular interest to us are the relationships of pollen size of H. "Newhall Ranch" with respect to H. californicus, H. nuttallii subsp. nuttallii, and H. nuttallii subsp. parishii. The post hoc tests (Table 5) reveal a complicated pattern. For some traits the Newhall Ranch population differs significantly from H. californicus, but does not differ from H. nuttallii (e.g., polar diameter). However, in others, the Newhall Ranch population differs significantly from H. nuttallii in, but does not differ from H. californicus (e.g., equatorial diameter). In some traits, H. "Newhall Ranch" significantly differs from both H. californicus and H. nuttallii (e.g., culp width). The Newhall Ranch plants are apparently intermediate with respect to pollen size, but somewhat extreme with respect to features of the aperture and spines.

The intermediacy of pollen size is consistent with what is known concerning chromosome number. Helianthus nuttallii is considered a diploid, with a haploid chromosome number of 17. By contrast, H. californicus has a haploid chromosome number of 51; three times that of H. nuttallii. Helianthus "Newhall Ranch" has been shown to possess a haploid chromosome number of 34, twice that of H. nuttallii and the precise intermediate number. It is relevant to note that Heiser et al. (1969) suggested the origin of H. californicus likely involved hybridization, with the contribution of two copies of the genome from H. nuttallii subsp. parishii. The Newhall Ranch population could represent the stabilized intermediate entity, intermediate between H. nuttallii subsp. parishii and H. californicus. The Newhall Ranch population possesses the doubled chromosome number of H. nuttallii, as required by the Heiser et al. (1969) hypothesis.

We therefore conclude that the Newhall Ranch population of Helianthus does not represent an outlying population of Helianthus californicus. These two taxa differ in chromosome number and several pollen size traits. At the same time we also conclude that Newhall Ranch population is not likely to be H. nuttallii subsp. parishii. This is supported by precisely the same reasons. The Newhall Ranch population has pollen that significantly differs in several ways from H. nuttallii subsp. parishii, and the chromosome numbers for H. nuttallii (subsp. nuttallii) differ. Rather, we suggest that the Newhall Ranch population of Helianthus likely represents a unique entity.

It is not clear whether the six individuals present are the result of clonal/vegetative growth (ramets), or whether they are different genetic individuals. As a result it is not known if the Newhall population is truly a population or merely a clonal individual. However, viable seed are produced, suggesting some sexual reproductive capacity.

Further study may be necessary to elucidate the status of the unusual polyploid Helianthus. Chromosome counts of California populations of H. nuttallii subsp. nuttallii could verify some of the underlying assumptions we have made; e.g., that there is no variation in counts across the range of H. nuttallii. Genetic data also may likely be necessary to determine relationships among this complex group of Helianthus and shed light on the origin of H. californicus.

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Table 1. *Comparative features of the native, perennial Helianthus species of Southern California with yellow disk corolla lobes. The following abbreviations are used in the table: t- trichomes; l- length; w- width; Chrom- chromosome number*

	H. californicus	*H. nuttallii subsp. nuttallii*	*H. nuttallii subsp. parishii*
Stem t	*glabrous, glaucus*	*glabrous to scabrous*	*glabrous to tomentose*
Leaf l	*10-23 cm*	*10-20 cm*	*10-20 cm*
Leaf t	*scabrous*	*scabrous*	*scabrous to tomentose*
Phyllary l	*10-25 mm*	*8-16 mm*	*8-16 mm*
Phyllary w	*3-4 mm*	*2-3 mm*	*2-3 mm*
Phyllary t	*glabrous, ciliate*	*glabrous, strigose*	*tomentose*
Ray l	*20-30 mm*	*1.5-2.5 mm*	*1.5-2.5 mm*
Disk l	*6-8 mm*	*5-6 mm*	*5-6 mm*
Fruit l	*4.5-5.5 mm*	*3-4 mm*	*3-4 mm*
Chrom	*2n= 102*	*2n= 34*	*unknown*

Table 2. *Samples of Helianthus californicus, H. nuttallii subsp. nuttallii and H. nuttallii subsp. parishii used in the quantitative pollen study. Voucher data and the general location of the sampl source are provided. All vouchers are housed at the RSABG herbarium.*

Taxon	*Voucher*	*Location*
H. nuttallii subsp. nuttallii	*C. F. Baker s.n.*	*CO, Larimer Co., Laporte*
H. nuttallii subsp. nuttallii	*G. J. Goodman 6-738*	*UT, Uintah Co., 2 mi N Vernal*
H. nuttallii subsp. nuttallii	*V. Clayton 10487*	*UT, Utah Co., NW of Provo*
H. nuttallii subsp. nuttallii	*R. Long 1135*	*WY, Albany Co., 30 mi SW Laramie*
H. nuttallii subsp. nuttallii	*A. Kruckeberg 2541*	*WA, Yakima Co., between Mabton and Tappenish*
H. nuttallii subsp. nuttallii	*H. Mason 13492*	*CA, Lassen Co., Lower Lake*
H. nuttallii subsp. nuttallii	*V. Yoder 6268*	*CA, Inyo Co., Alabama Hills*
H. nuttallii subsp. nuttallii	*L. Benson 15033*	*CA, Inyo Co., 2 mi W Independenca*
H. nuttallii subsp. nuttallii	*M. E. Jones s.n.*	*CA, Inyo Co., Bishop*
H. nuttallii subsp. nuttallii	*F. Brooks 4252*	*CA, Inyo Co., N of Bishop*
H. nuttallii subsp. nuttallii	*W. Martin 20596*	*CA, Inyo Co., 2 mi W Bishop*
H. nuttallii subsp. nuttallii	*J. C. Roos s.n.*	*CA, San Bernardino Co., Victorville*
H. nuttallii subsp. nuttallii	*F. W. Peirson 4022*	*CA, San Bernardino Co., Lone Pine Canyon, San Gabriel Mtns.*
H. nuttallii subsp. nuttallii	*I. M. Johnston s.n.*	*CA, San Bernardino Co., Cushenbury Springs, San Bernardino Mtns.*
H. nuttallii subsp. nuttallii	*R. F. Thorne 55252*	*CA, San Bernardino Co., Cushenbury Springs, San Bernardino Mtns.*
H. nuttallii subsp. parishii	*L. M. Booth 1388*	*CA, Orange Co., Newport Lagoon*
H. nuttallii subsp. parishii	*F. W. Peirson 5247*	*CA, Orange Co., Wintersberg*
H. nuttallii subsp. parishii	*Dr. Hasse 1891*	*CA, Los Angeles Co., Cienega*
H. nuttallii subsp. parishii	*S. B. Parish 5125*	*CA, San Bernardino Co., near San Bernardino*
H. nuttallii subsp. parishii	*J. C. Roos 1202*	*CA, San Bernardino Co., E of San Bernardino*

Table 2 (continued)

Taxon	*Voucher*	*Location*
H. nuttallii subsp. parishii	*S. B. Parish 265*	*CA, San Bernardino Co., San Bernardino*
H. nuttallii subsp. parishii	*I. M. Johnston s.n.*	*CA, San Bernardino Co., 2 mi S San Bernardino*
H. nuttallii subsp. parishii	*W. G. Wright s.n.*	*CA, San Bernardino Co., San Bernardino*
H. nuttallii subsp. parishii	*S. B. Parish 1931*	*CA, San Bernardino Co., near San Bernardino*
H. "Newhall Ranch"	*J. M. Porter 13675*	*CA, Los Angeles Co., Newhall Ranch*
H. californicus	*M. A. Nobs 1755*	*CA, Inyo Co., 13.5 mi N Bishop*
H. californicus	*N. Wallace 541*	*CA, Kern Co., Piute Mtns.*
H. californicus	*P. A. Munz 11147*	*CA, Los Angeles Co., Bouquet Canyon*
H. californicus	*S. Boyd 9047*	*CA, Los Angeles Co., Agua Dulce Canyon*
H. californicus	*L. C. Wheeler 202*	*CA, Riverside Co., San Jacinto Mtns.*
H. californicus	*C. W. Tilforth 306*	*CA, Riverside Co., Santa Rosa Mtns.*
H. californicus	*F. W. Peirson 10426*	*CA, San Diego Co., 7 mi W Campo*
H. californicus	*P. C. Everett 9370*	*CA, San Bernardino Co., Cushenbury Springs, San Bernardino Mtns.*
H. californicus	*L. S. Rose 52058*	*CA, Solano Co., Manka Corners*

Table 3. *Summary statistics of quantitative variation in Helianthus californicus, H. nuttallii subsp. nuttallii, H. nuttallii subsp. parishii and the sunflower discovered on Newhall Ranch (H. "Newhall Ranch"). The means, in micrometers, and parenthetically, one standard error of the means are provided. The abbreviations diam, and # denote diameter and number, respectively.*

	H. californicus	*H. nuttallii nuttallii*	*H. nuttallii parishii*	*H. "Newhall Ranch"*
Polar diam.	24.8 (±0.946)	19.9 (±0.849)	21.3 (±0.446)	21.6 (±0.969)
Equatorial diam.	24.7 (±0.830)	19.9 (±0.653)	20.5 (±0.439)	23.7 (±1.326)
Colpus length	18.8 (±0.505)	15.9 (±0.758)	14.3 (±0.609)	17.2 (±1.612)
Colpus width	4.7 (±0.499)	3.8 (±0.414)	3.1 (±0.251)	2.0 (±0.229)
Pore diam.	4.5 (±0.263)	3.8 (±0.390)	4.1 (±0.218)	3.3 (±0.403)
Spine length	5.5 (±0.292)	5.1 (±0.119)	5.0 (±0.222)	3.3 (±0.296)
Micropore #	11.9 (±1.744)	9.6 (±0.945)	7.4 (±1.536)	8.8 (±0.648)
Spine #	5.4 (±0.294)	4.9 (±0.100)	7.4 (±0.125)	5.4 (±0.324)

Table 4. *Multivariate analysis of variance (MANOVA) comparing quantitative variation in pollen morphology of Helianthus californicus, H. nuttallii subsp. nuttallii, H. nuttallii subsp. parishii, and H. "Newhall Ranch" (TAXA). The upper portion of the table provides a summary of the MANOVA. The lower portion provides analysis of variance for each of the log transformed measurements.*

	DF	*F-value*	*p-value*
TAXA	18/91	5.400	<0.0001

Character	*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*p-value*
Polar diameter	3	0.241	0.080	6.216	0.0016
Equatorial diameter	3	0.321	0.107	9.721	<0.0001
Culp length	3	0.476	0.159	5.772	0.0025
Culp width	3	3.389	1.130	10.603	<0.0001
Spine length	3	0.147	0.049	2.006	0.1300
Pore diameter	3	6.618	2.206	2.245	0.0992

Table 5. *Homogeneous subsets, based on multiple pairwise comparisons tests, using Fisher's PLSD comparing continuous variation in pollen morphology of Helianthus californicus (CAL), H. nuttallii subsp. nuttallii (NUT), H. nuttallii subsp. parishii (PAR), and H. "Newhall Ranch" (NEW). For each character, taxa sharing the same value do not differ significantly; however, taxa with different values significantly differ. In some cases different pairwise test seem to conflict, and taxa can be members of two overlapping groups. The presence of a forward slash denotes the two groups.*

Character	*NUT*	*PAR*	*NEW*	*CAL*
Polar diameter	*1*	*1*	*1*	*2*
Equatorial diameter	*1*	*1*	*2*	*2*
Culp length	*1/2*	*1*	*2/3*	*3*
Culp width	*1/2*	*1*	*3*	*2*
Spine length	*1/2*	*1/2*	*1*	*1/2*
Pore diameter	*1/2*	*1/2*	*1*	*1/2*

Figure 1. *Representative pollen grains of: A) Helianthus californicus, B) H. nuttallii subsp. nuttallii, C) H. nuttallii subsp. parishii, and D) H. "Newhall Ranch". Note the scale bars to the lower left of each pollen grain, and that the pollen grains are not at the same magnification.*