

**COMPARATIVE MORPHOLOGY OF DISK FLORET
TRICHOMES OF *ENCELIA*
(ASTERACEAE: HELIANTHEAE)**

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THESIS: COMPARATIVE MORPHOLOGY OF DISK FLORET TRICHOMES
OF *ENCELIA* (ASTERACEAE: HELIANTHEAE)

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ABSTRACT

Disk florets of 19 *Encelia* taxa were examined with scanning electron microscopy to characterize disk floret trichome complement, density, and distribution on anthers, abaxial corolla lobes, and corolla tubes, to interpret their evolution in light of the phylogeny and ecology of the species, and to determine the utility of these characters for phylogenetic analysis and species delimitation. Trichomes are all multicellular, and include biseriate glands, biseriate achene hairs (*Zwillingshaare*), and narrow unicellular-based, straight uniseriate. Two trichome types present on leaves and other organs (curly uniseriate and broad, multicellular-based uniseriate) are absent on the florets, and one type (moniliform) occurs very rarely, observed on only two taxa. It is notable that the two trichome types which have proven useful in phylogenetic analysis of the genus (moniliform and broad-based uniseriate) are absent or rare on disk florets. Extensive intra-taxon and intra-population variability in trichome complement precludes the use of these characters in phylogenetic analysis using cladistic parsimony. However analysis of trichome density (represented by trichome counts for particular trichome types on particular organs) using multivariate statistical techniques yields results consistent with existing species boundaries, hypotheses of hybrid origin for two of the taxa, and the existence of two major clades within the genus.

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Introduction

The genus *Encelia* (Asteraceae: Heliantheae) consists of 14 species of perennial xerophytic shrubs and one herbaceous perennial, inhabiting coastal and desert regions of southwestern North America and coastal regions of western South America, including the Galápagos Islands. All species, with one exception, are interfertile obligate outcrossers, and some taxa are believed to be of homoploid hybrid origin (Clark, 1998).

Encelia is one of three related genera forming a clade known as the “*Encelia* alliance”, which is composed of *Encelia* and its sister group, a clade composed of the two sister genera *Enceliopsis* and *Geraea* (Clark and Sanders, 1986). The alliance is diagnosed by caducous paleae and flattened black achenes with ciliate, unpigmented margins (Blake, 1913; Clark and Sanders, 1986). *Encelia* is diagnosed by achenes with a constricted apical notch that lack prominent awns, a cartilaginous crown, and squamellae, all of which are present in *Enceliopsis* and *Geraea* (Blake, 1913; Abrams, 1960; Sanders and Clark, 1987).

Preliminary phylogenetic analysis of the genus based on morphological, biochemical, and molecular characters (Intercistronic Transcribed Spacer sequences of nrDNA) strongly supports the existence of two major clades within the genus. The “*frutescens* clade” comprises *E. actoni*, *E. frutescens*, *E. ravenii*, and *E. resinifera*, whereas the “*californica* clade” comprises *E. californica*, *E. conspersa*, *E. densifolia*, *E. farinosa*, *E. halimifolia*, *E. palmeri*, and *E. ventorum* (Clark, 1986). The former is marked by erect fruiting heads with expanded paleae (Clark, 1986) and the absence of resin ducts (Proksch and Clark, 1987), and the latter is marked by ultraviolet-reflecting adaxial ray corollas (Clark and Sanders, 1986) and a unique benzopyran-benzofuran dimer (Proksch and Clark, 1987). Relationships within the clades are more weakly supported or unresolved.

Among morphological characters, trichomes have proven useful in supporting species boundaries and phylogenetic hypotheses concerning both intergeneric and interspecific

relationships within the alliance. In their study of *Enceliopsis capitulum* morphology, Sanders and Clark (1987) provided evidence based on trichomes that supported the monophyly of the genus (whose members all share a dense white pubescence on leaves and the peduncle); species boundaries among *E. argophylla*, *E. covillei*, and *E. nudicaulis* (which differ in four trichome characters); and the placement of *Encelia nutans* within *Encelia* (as trichome complement of this species differs from that of *Enceliopsis*, and its trichomes are more similar in appearance to those found in *Encelia*). Nishida (1988) cited trichome evidence as a synapomorphy in support of the monophyly of *Geraea*, showing that the two *Geraea* species share the same trichome complement on leaves and capitula. In *Encelia*, Clark (1986) demonstrated that leaf trichomes are useful in support of the hypothesis of two clades within the genus.

Trichomes are very important adaptive features within the alliance, and are essential to survival in the xeric environments inhabited by its members. For example, trichomes form a thick, reflective pubescence on leaves of several taxa in *Encelia*, (including *E. farinosa*, *E. palmeri*, *E. canescens*, *E. actoni* and *E. ravenii*), as well as all three *Enceliopsis* species. Leaf pubescence has been observed to account for reflectance values ranging from 8% to 71% in different taxa (Ehleringer, Bjorkman, and Mooney, 1976; Ehleringer and Bjorkman, 1978; Ehleringer, 1981). The shading provided by this pubescence reduces leaf temperatures, thus preventing leaf damage in drought when water is unavailable for transpirational cooling (Ehleringer and Clark, 1987). It also maximizes net carbon gain in such conditions (Ehleringer and Mooney, 1978; Ehleringer, 1980; Ehleringer and Werk, 1986). Similar patterns of pubescence have been noted on both North and South American species, with coastal species in both regions tending to have glabrous, green leaves, and inland species inhabiting more xeric habitats, tending to have more pubescent, whitish leaves (Ehleringer et al., 1981). *E. densifolia* has a wetttable pubescence that increases in reflectivity when dry (Harrington and Clark, 1989). Additionally, glandular trichomes are thought to produce secondary compounds which function to discourage predation (Proksch and Rodriguez, 1983).

Floral trichomes have been examined in *Geraea* (Nishida and Clark, 1988) and *Enceliopsis* (Sanders and Clark, 1987), but not in *Encelia*. Trichomes have been examined on most other parts of *Encelia*, including leaves (Clark et al., 1980; Ehleringer and Cook, 1987), as well as peduncles, paleae, and phyllaries (Charest, 1988). On the leaves, Clark et al., (1980) described three different types of multicellular trichomes: biseriate glandular trichomes, moniliform trichomes, and uniseriate trichomes. The uniseriate trichomes may have broad, multicellular bases, or narrow, unicellular bases. The narrow unicellular-based trichomes can have tips which are either straight or curly. In her study of peduncle, phyllary, and paleae trichomes, Charest (1988) observed all of these trichome types.

The purpose of this research is to characterize disk floret trichome complement, distribution, and density in *Encelia*, to determine whether these characters support existing species boundaries, to determine if they can be used in phylogenetic analysis to resolve relationships within the genus, and to reach conclusions pertaining to the evolution of these characters in light of the phylogeny and ecology of the species.

Materials and Methods

Samples of vegetative and floral organs from both wild and cultivated specimens were collected, preserved, and stored in vials by previous researchers. These specimens were fixed in 10% formaldehyde in water or in formalin/ethanol/acetic acid (FAA), and placed in 70% ethanol for long-term storage. These vials were then selected and cataloged for use in this study based on the presence of capitula with mature disk florets.

Specimens were dehydrated in an ethanol series proceeding from 70% to 100%, in ten percent increments, with two changes in 100%. Twelve to fifteen florets from each population were selected under a dissecting scope based on the visibility of anthers (Fig. 1), abaxial corolla lobes (Figs. 1 and 2), and corolla tubes (Fig. 2). The florets were then separated from their achenes and subtending paleae with dissecting tools to facilitate observation of corolla tubes with scanning electron microscopy (SEM). Multiple populations were available for several of the *Encelia* taxa, while only one was available for others (Table 1). Whenever possible, florets were taken from different capitula, but in some cases, only one capitulum from a given population was available. After selection, the florets were placed in plastic specimen holders for critical point drying, and were then dried in a Ted Pella CO₂ critical point dryer. After drying, the florets were removed from the specimen holders and arranged on a dry paper towel for observation. The best 10 or 12 florets were then selected for further study. These florets were then mounted on aluminum specimen stubs with conductive colloidal silver paint. Typically, five or six florets fit on a single stub, and each population was represented by two stubs holding between 10 and 12 florets.

Following mounting, the stubs were placed in a BioRad SEM Coating System sputter coater and coated with gold particles under a 10 Pa vacuum at about 23 mA for about 500s. The florets were observed and photographed in an ISI WB-6 scanning electron microscope. Most of the observation and photography was carried out at low or medium magnification (usually ranging from 9 to 1500 ×), at an accelerating voltage of 10 or 15

kV. Trichome complement, distribution, and density were observed on anthers, abaxial corolla lobes, and tubes of each floret. Any trichomes visible on an organ were identified, counted, and their distribution noted. Trichome counts for corolla tubes represent only those trichomes that were visible through SEM, not all those on the floret. The trichome counts on tubes probably represent approximately 50% of those on the floret. In cases in which there were numerous trichomes (i.e. greater than 15), the absolute number was often impossible to determine due to high density and overlap. In these cases, a rough count was taken, and rounded up or down to the nearest ten—as the relative density of trichomes among populations was thought to be of greater importance than the absolute number. A total of 352 individual florets from 34 populations and 19 taxa were observed.

Polaroid Polapan 55 film, which yields both a positive and negative, was used to photograph the specimens; both were processed according to package directions. A suite of photographs was taken of each population depicting the areas of interest on representative specimens.

Principal components analysis (PCA) and multivariate analysis of variance (MANOVA) were carried out on the trichome data (Appendix A) using the SAS System for Windows, release 6.12 (1989-1996, the SAS Institute). Individual florets (omitting those with missing data) were used as observations for these analyses. Two different PCAs were performed, one to examine taxa, and one to examine the clades (i.e. omitting observations for certain taxa, including hybrids.) Two different MANOVAs were used to analyze the clades; one which included all data pertaining to each clade, and one which omitted the two greatest outliers (*E. actoni* (156) and *E. resinifera* (195)), as visible from the PCA.

Results

Trichome types--Four different types of multicellular trichomes were observed in the taxa examined: biseriate glands, uniseriate trichomes, moniliform trichomes, and biseriate achene hairs (*Zwillingshaare*).

Biseriate glandular trichomes were present on florets of all taxa, except for one population of *E. canescens* (273). These trichomes consist of two rows of cells forming a stalk, supporting an enlarged, generally globose or irregularly-shaped gland (Fig. 3). Their appearance varies such that some appear sessile with a shortened stalk, while others appear fairly elongate, with as many as seven or eight cells, in two rows, supporting the gland. Glands were found on the anthers of many of the taxa (Fig. 4). These measure (average, followed by one standard deviation) $59.4 \pm 10.3 \mu\text{m}$ long by $38.4 \pm 3.6 \mu\text{m}$ wide. They were found mainly in or near the furrow in the center of the abaxial lobe of the terminal appendage of the anther, and they number from 0 to 17 per anther. Glands were found on abaxial corolla lobes of many taxa, with shapes ranging from sessile (Fig. 5) to elongate (Fig. 6). The shape of the gland itself is variable, ranging from nearly spherical (Fig. 7) to irregular (Fig. 3). Glands on corolla lobes measure $62.9 \pm 16.8 \mu\text{m}$ long by $37.1 \pm 6.8 \mu\text{m}$ wide, and are thus comparable in size to anther glands. They number from 0 to 20 per lobe and were found in much greater concentration toward the center of the lobe than near the periphery. Glands were found on corolla tubes of all taxa (except for one population of *E. canescens* (273)), and are generally more elongate (Fig. 8) than either anther glands or corolla glands. They measure $76.7 \pm 22.2 \mu\text{m}$ long by $25.5 \pm 5.0 \mu\text{m}$ wide. They were usually found distributed fairly evenly over the surface of the tube (Fig. 9). They number from 0 to 100 per half-tube (i.e. the portion of the tube visible under scanning electron microscopy). Glands were sometimes found on the throat. In these cases, they were always continuous with either corolla glands from above or tube glands from below and were never found in isolated groups. They appeared to be spill-over from either the lobes or tube.

Uniseriate trichomes were present on florets of most taxa. These trichomes, as the name implies, are composed of a single row of cells, and all have narrow, unicellular bases (unlike the multicellular bases found on some *Encelia* leaf uniseriate trichomes; Clark et al., 1980; Ehleringer and Cook, 1987). These are usually quite long and narrow, and may have a jointed appearance (Fig. 10). Uniseriate trichomes were found on the abaxial corolla lobes of some taxa (Fig. 11). They measure $224.7 \pm 69.6 \mu\text{m}$ long by $24.1 \pm 7.5 \mu\text{m}$ wide and number from 0 to 80 per lobe. Uniseriate trichomes were also found on corolla tubes (Fig. 10). The trichomes are generally evenly distributed over the surfaces of these organs and are generally longer than on corolla lobes, measuring $388.7 \pm 164.4 \mu\text{m}$ long by $21.9 \pm 4.7 \mu\text{m}$ wide. They number from 0 to 50 per half-tube. Uniseriates were sometimes found on the throat, and like glands, were always continuous with trichomes of the same type from either corolla lobes above, or tubes below, and were never found in isolated groups. Unlike glands however, uniseriates sometimes extend down the sutures of the corolla lobes in long lines of one or more across. This occurs in *E. actoni* (Fig. 12) and *E. frutescens* subsp. *frutescens*.

Biseriate achene hairs (*Zwillingshaare*) were found on some of the taxa, and only on the corolla tubes (Fig. 13), where they may represent spill-over from the ovaries. These long, thin, biseriate trichomes have a bifurcated tip and are very smooth and non-jointed along their length (Fig. 14). They measure an average of $348.1 \pm 105.5 \mu\text{m}$ long by $14.7 \pm 3.5 \mu\text{m}$ wide and number from 0 to 80 per half-tube.

There are thus six disk floret trichome characters in *Encelia*: anther glands, corolla glands, corolla uniseriates, tube glands, tube uniseriates, and tube *Zwillingshaare*. In addition to this, two other trichome characters were observed—moniliform trichomes and anther uniseriates. A single moniliform trichome was found on an abaxial corolla lobe of a single floret of *E. californica* (123) (Fig. 15). One population of *E. canescens* (267) was also observed to have low numbers of moniliforms on corolla lobes (Fig. 16). These trichomes consist of a row of globose cells supporting an elongate gland with a globose tip. Anther uniseriates were observed occurring in ones, twos, or threes on some florets

from a single population of *E. canescens* (273) (Fig. 17). These uniseriates are similar to the ones observed elsewhere on the florets. These two cases were interpreted as exceptional, however, because of their extremely low frequencies.

Results by taxon—No distinguishing features were observed in *E. actoni*, *E. asperifolia*, *E. californica*, *E. conspersa*, *E. densifolia*, *E. frutescens* subsp. *frutescens*, *E. farinosa* var. *farinosa*, *E. farinosa* var. *phenicodonta*, *E. ventorum*, and *E. virginensis*. Taxa observed to have distinguishing features are described below. The features described refer to taxa (or populations where specified) and not to individual florets. (Complete data collected in this study, consisting of trichome counts on corolla lobes, tubes, and anthers are contained in Appendix A.)

E. canescens: The three populations examined are very different, and this taxon is exceptional for three reasons. First, it is the only taxon in which a population (273) was observed to lack glands. Second, it is the only taxon with a population in which uniseriate trichomes were observed on anthers (273) (Fig. 17). Third, it is one of only two taxa with a population (267) in which moniliform trichomes were observed on abaxial corolla lobes (Fig. 16); it shares this character with one floret of *E. californica*.

E. farinosa var. *radians*: This is the only taxon observed to have a complement of only tube glands, thus lacking the other five characters. The lack of uniseriates is especially notable because this variety also has few or no uniseriates on its leaves, peduncles, and phyllaries.

E. frutescens subsp. *glandulosa*: This is the only taxon observed to have a complement of every trichome type except anther glands.

E. halimifolia: This is the only taxon observed to have a complement of anther glands, corolla uniseriates, tube glands, and tube uniseriates.

E. palmeri: This is the only taxon observed to have a complement of corolla uniseriates, tube glands, and tube uniseriates.

E. ravenii: This is the only taxon observed to have a complement of anther glands, corolla glands, tube glands, and tube uniseriates.

E. resinifera: This is the only taxon with a population (195) having high numbers of *Zwillingshaare*.

E. resinifera subsp. *tenuifolia*: This is the only taxon observed to have a complement of anther glands, corolla glands, corolla uniseriates, and tube glands.

Statistical Analysis--The results of the first principal components analysis, which was performed to examine taxa, are represented by a plot of principal components 1 and 2 (Fig. 18), principal components 1 and 3 (Fig. 19), and an expanded view of the central area of the plot of principal components 1 and 2 (Fig 20). The first three components account for 69.8% of the variance. Other plots based on this analysis include those of taxa of putative hybrid origin and their parents (Figs. 21, 22, 23, and 24). The other principal components analysis, which was performed to examine the clades, is represented by figures 25 and 26. The first three components in this analysis account for 76.4 % of the variance.

The multivariate analyses of variance (MANOVA) were carried out to test the hypothesis that the *frutescens* clade and the *californica* clade are distinct based on trichome data. (The same data used in the PCA analysis of the two clades were used in this analysis as well.) The results of both MANOVAs (Wilks' Lambda = 0.436; F = 46.633; $\alpha < 0.0001$ for the one including all the data pertaining to the two clades, and Wilks' Lambda = 0.418; F = 46.321 $\alpha < 0.0001$ for the one omitting the two outliers) confirm this. These results strongly reject the hypothesis that there is no significant difference between the

centroids of the two putative clades, thus confirming their distinctness based on these characters.

Discussion

Comparisons with Enceliopsis and Geraea and cauline and foliar trichomes in

Encelia—The trichome complement of disk florets is restricted in comparison to other organs in the genus. Two types of trichomes found on leaves, peduncles, phyllaries, and paleae are absent on the disk florets: broad multicellular-based uniseriate trichomes and curly (narrow unicellular-based) uniseriate trichomes. It is notable that the two trichome types that have proven phylogenetically useful in previous studies (Clark, 1986)—the moniliform trichomes and the broad-based uniseriate trichomes—are either rare or absent.

Biseriate achene hairs (*Zwillingshaare*) are present on corolla tubes of several of the *Encelia* taxa in low numbers and in one population of *E. resinifera* (195) in very large numbers (as many as 80 per half tube). In *Enceliopsis* and *Geraea*, and elsewhere in *Encelia*, these trichomes have been observed only on achenes. Since these trichomes constitute the cilia on achenes of all three genera, it is possible that their presence on corolla tubes of some of the *Encelia* taxa represents a spill-over from the achenes.

Aside from the presence of *Zwillingshaare* on corolla tubes, and the rare occurrence of uniseriate trichomes on anthers and moniliform trichomes on corolla lobes, trichome complement, distribution, and density on disk florets in *Encelia* is the same as in both *Enceliopsis* and *Geraea*. Like *Encelia*, both *Geraea* (Nishida and Clark, 1988) and *Enceliopsis* (Sanders and Clark, 1987) have biseriate glandular trichomes on anthers (on the abaxial surface of the terminal appendage, in or near the central furrow) in low numbers, and on abaxial corolla lobes and corolla tubes in higher numbers. Narrow unicellular-based, straight uniseriate trichomes are also found on abaxial corolla lobes and corolla tubes in densities comparable to those found in *Encelia*.

Unlike *Encelia*, both *Enceliopsis* and *Geraea* have the same trichome types (uniseriates and biseriate glands) on disk florets as on other organs. Since biseriate glands and

uniseriates are the only trichome types found in the latter two genera, these genera do not display a restricted trichome complement on disk florets with respect to their other organs, as *Encelia* does.

Phylogenetic utility of disk floret trichomes—Even a cursory examination of the trichome data (Appendix A) reveals extensive intra-taxon and intra-population variability in trichome complement. Eight of nine taxa in which multiple populations were examined displayed at least one difference in complement (out of the possible six trichome characters explained above) among their populations. Five of these taxa displayed two or more such differences among their populations. Variability in complement is also common within populations. Of the total 34 populations examined, 28 display at least one difference in complement among their individuals, and 21 display two or more.

Because of this variability, trichome complement of disk florets is a phylogenetically uninformative character if used in a cladistic parsimony analysis using binary characters to represent presence and absence. For this type of analysis, it is necessary to have characters that are constant within the taxa to be classified, and variable among them. These characters are variable not only within taxa, but also within populations (i.e. among individuals), and thus their variability is present at the wrong level to be useful in classification based on parsimony. While it is possible to transform continuous data into discrete data by a number of methods (e.g. gap coding) to give polymorphic characters, this was not done for two reasons. First, while discrete gaps (i.e. between disjunct distributions) sometimes exist in the trichome counts, these gaps are not consistent throughout the taxa examined, and second, such a transformation is not justified in light of the fact that trichome counts (i.e. density) display variability within taxa (and in some cases, populations) as does trichome complement.

Species boundaries and delimitation—Although it is necessary to use discrete characters in cladistic parsimony analysis, this is not a requirement for exploring species boundaries.

In this study, trichome complement is unsuited for this purpose for the same reason it is unsuited for use in phylogenetic analysis (i.e. high intra-taxon and intra-population variability).

In this case, an examination of trichome counts (as opposed to trichome complement) proves much more useful in exploring species boundaries. For example, *Encelia farinosa* has three different varieties (*E. farinosa* var. *farinosa*, *E. f.* var. *phenicodonta* and *E. f.* var. *radians*). Considering only complement of these three taxa could lead one to conclude that *radians* is a different species, because it differs from the other two varieties by three characters. While this in itself would be a perfectly acceptable conclusion based only on these characters, the high inter-population and intra-population variation within both *E. farinosa* var. *farinosa* and *E. farinosa* var. *phenicodonta*, each of which have populations differing by three characters, would prohibit their use for this purpose. However, if principal components analysis (PCA—a method designed to analyze continuous data) is used on this data set, the three varieties all cluster together tightly in the plots of the first and second principal components (Figs. 18 and 20), and of the first and third principal components (Fig. 19). In terms of continuous data (trichome counts), these three varieties are very similar and cluster together, which is consistent with other data supporting the contention that they are conspecific.

In the principal components analysis, most of the individual florets form relatively small clusters representing their respective species. While the smaller clusters (e.g. *E. conspersa*, *E. densifolia*, *E. palmeri*, *E. ravenii*, and *E. resinifera* ssp. *tenuifolia*) often represent species from which only one population was sampled (in two cases because only one population of the species is known) this is not always the case. For example, in this study, *E. farinosa* is represented by three varieties and a total of seven populations. This species, with its seven populations, clusters much more tightly than either *E. actoni* or *E. resinifera*, which are each represented by two (widely divergent) populations. Additionally, *E. asperifolia*, with three populations, forms a fairly small cluster.

Although a few species (including *E. actoni*, *E. canescens*, *E. frutescens*, and *E. resinifera*) cluster only loosely, this analysis is very consistent with existing species boundaries, because of the fairly small clusters for most species, and the fact that none of even the large clusters are so large that they appear to represent only random noise (i.e. their individuals are not randomly distributed over the entire plot).

Evolutionary Implications—In addition to their consistency with existing species boundaries, the PCA results also shed light on some other important evolutionary hypotheses.

Species thought to be of hybrid origin (Clark, 1998) cluster between their putative parents. *E. asperifolia* clusters almost exactly between its putative parents, *E. frutescens* ssp. *glandulosa* and *E. californica*, in plots of principal components 1 and 2 (Fig. 21) and principal components 1 and by 3 (Fig. 22). *E. virginensis* likewise clusters between its putative parents (*E. frutescens* ssp. *frutescens* and *E. actoni*) in plots of these same components (Figs. 23 and 24). Inasmuch as intermediacy between putative parents is one line of evidence of hybrid origin, this analysis provides additional evidence for the hybrid origin of both species. Similar results were obtained by Clark (1998) in his study of *E. virginensis* and its putative parents in which eight morphological variables were measured, and analyzed by principal coordinate analysis.

Figures 25 and 26 clearly show that the *californica* clade and the *frutescens* clade cluster separately, with the majority of the *californica* clade scoring low on PC 1, and the *frutescens* clade scoring high on PC 1 and PC 2. The MANOVA analyses confirm that the centroids of the two groups are significantly different. This is consistent with other evidence that these two groups are clades.

Conclusion

Four different types of multicellular trichomes were observed on disk florets of 19 *Encelia* taxa (represented by 34 populations and 352 individual florets). These included biseriate glandular trichomes, narrow unicellular-based uniseriate trichomes, biseriate achene hairs (*Zwillingshaare*), and (rarely) moniliform trichomes. The two phylogenetically informative trichome types present on leaves and other organs—the broad multicellular-based uniseriate trichomes and moniliform trichomes—were either absent from florets, or were present in very low numbers on very few taxa. Trichome complement is highly variable, exhibiting extensive variability within taxa and populations, thus making it phylogenetically uninformative. However analysis of trichome data with multivariate statistical methods yields results consistent with existing species boundaries, hypotheses of hybrid origin of two taxa, and the distinctness of the two clades of the genus.

As other authors have investigated trichomes on leaves, stems, peduncles, phyllaries, and paleae, this study completes our knowledge of trichome complement, density and distribution in the genus. Although disk floret trichome characters are not phylogenetically useful, the multivariate analyses of trichome counts reveal that they do have a role in elucidating our knowledge of the evolution of *Encelia*.

Literature Cited.

- Abrams, L. and R. S. Farris. 1960. Flora of the Pacific states. Vol 4. Bignoniaceae to Compositae (By R. S. Farris). Stanford University Press, Stanford, CA.
- Blake, S. F. 1913. A revision of *Encelia* and some related genera. Proc. Amer. Acad. Arts 49: 346-396.
- Charest, N. A. 1988. A scanning electron microscopic study of the peduncle, phyllary, and palea trichomes of *Encelia* (Asteraceae: Heliantheae). M.S. Thesis, California State Polytechnic University, Pomona. vii + 83 p.
- Clark, C. 1986. The phylogeny of *Encelia* (Asteraceae: Heliantheae). Amer. J. Bot. 73: 757.
- . 1998. Phylogeny and adaptation in the *Encelia* alliance (Asteraceae: Heliantheae). Aliso 17(2): 89-98.
- , and D. L. Sanders. 1986. Floral ultraviolet in the *Encelia* alliance (Asteraceae: Heliantheae). Madroño 33:130-135.
- , W. C. Thompson, and D. W. Kyhos. 1980. Comparative morphology of leaf trichomes of *Encelia* (Compositae: Heliantheae). Botanical Society of America, Misc. Ser., Publ. 158.
- Ehleringer, J. R. 1980. Leaf morphology and reflectance in relation to water and temperature stress. In N. Turner and P. Cramer [eds.], Adaptations of plants to water and high temperature stress, 295-308. Wiley-Interscience, New York.

- . 1981. Leaf absorptances of Mohave and Sonoran Desert plants. *Oecologia* 49: 366-370.
- , and O. Bjorkman. 1978. Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa*. *Oecologia* 36: 151-162.
- , O. Bjorkman, and H. A. Mooney. 1976. Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. *Science* 192: 376-377.
- , and C. Clark. 1987. Evolution and adaptation in *Encelia* (Asteraceae), pp. 221-248. *In* L.D. Gottlieb and S. K. Jain [eds], *Plant evolutionary biology*. Chapman & Hall, London.
- , and C. S. Cook. 1987. Leaf hairs in *Encelia* (Asteraceae). *Amer. J. Bot.* 74(10): 1532-1540.
- , and H. A. Mooney. 1978. Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. *Oecologia* 37: 183-200.
- , H. A. Mooney, S. L. Gulmon, and P. Rundel. 1981. Parallel evolution of leaf pubescence in *Encelia* in coastal deserts of North and South America. *Oecologia* 49:38-41.
- , and Werk 1986. Modifications of solar radiation absorption patterns and the implications for carbon gain at the leaf level, pp. 57-82. *In* T. Givnish [ed.], *On the economy of plant form and function*. Cambridge University Press, London.
- Harrington, D. F., and C. Clark. 1989. Reduction in light reflectance of leaves of *Encelia densifolia* (Asteraceae) by trichome wetting. *Madroño* 36: 180-186.

- Nishida, J. 1988. Systematics of *Geraea* (Asteraceae: Heliantheae). M.S. Thesis, California State Polytechnic University, Pomona. vi + 31 p.
- , and C. Clark. 1988. Scanning electron microscopic study of the trichomes of *Geraea* (Asteraceae: Heliantheae). *Amer. J. Bot.* 75(6), part 2: 196-197.
- Proksch, P. and E. Rogriguez. 1983. Chromenes and benzofurans of the Asteraceae, their chemistry and biological significance. *Phytochemistry* 22: 2335-2348.
- , and C. Clark. 1987. Systematic implications of chromenes and benzofurans from *Encelia* (Asteraceae). *Phytochemistry* 26: 171-174.
- Sanders, D. and C. Clark. 1987. Comparative morphology of the capitulum of *Enceliopsis* (Asteraceae: Heliantheae). *Amer. J. Bot.* 74(7): 1072-1086.

TABLE 1. Collections of *Encelia taxa* used in this research.

Numbers are accession numbers. Vouchers are deposited at DAV or CSPU.

***Encelia actoni* Elmer**

- 156 United States. California. Inyo Co.: 17.9 km S of Scotty's Castle on road S to Furnace Creek. 30 April 1980.
- 171 United States. California. Riverside Co.: W of Aguanga.

***Encelia asperifolia* (S. F. Blake) Clark & Kyhos**

- 106 México. Baja California: ca. 16.1 km N of Millers Landing. 29 March 1978.
- 129 México. Baja California: Rosarito. 12 May 1978.
- 132 México. Baja California: Millers Landing, behind dunes. March 1978.

***Encelia californica* Nutt.**

- 115 México. Baja California: Mex Hwy 1, 32.2 km N of Nueva Chapala. 6 May 1978.
- 123 México. Baja California: Sand dunes E of Bahía San Quintín. 5 May 1978.
- 166 United States. California. Ventura Co.: CA Hwy 126, 7.4 km E of Piru. 18 May 1980.

***Encelia canescens* Lam.**

- 267 Perú. Lima: Valley of R. Chillón, 28.5 km from Lima. 12 February 1974.
- 273 Perú. Arequipa: Minas de Acarí. 19 January 1979.
- 268 Perú. La Libertad: Virú Valley on S side of Rio Virú, 14 km E of Virú on sandy wastes. 6 February 1974.

***Encelia conspersa* Benth.**

- 189 México. Baja California Sur: San Carlos, N of town on old dunes. 26 March 1981.

***Encelia densifolia* Clark & Kyhos**

- 184 México. Baja California Sur: Picachos de Santa Clara, 21.9 km NW of San Ignacio-Abreojos road at a point 24.7 mi NE of Punta Abreojos. 24 March 1981.

Encelia farinosa* Torrey & A. Gray var. *farinosa

- NM United States. California. San Bernardino Co.: N slope of Newberry Mountains, S of Newberry Springs.

- 18 United States. California. D. W. Kyhos collection.

***Encelia farinosa* Torrey & A. Gray var. *phenicodonta* (S. F. Blake) I. M. Johnston**

- 183 México. Baja California Sur: San Ignacio Pemex. 23 March 1981.
- 186 México. Baja California Sur: S of Bahía Concepción at Microondas Rosarito. 25 March 1981.
- 190 México. Baja California Sur: S end of Bahía Concepción, near beach. 27 March 1981.
- 202 México. Baja California Sur: Mex Hwy 1, 16.1 km NW of San Bartolo. 22 March 1982.

***Encelia farinosa* Torrey & A. Gray var. *radians* Brandege**

- 203 México. Baja California Sur: Road to La Ribera, 1.1 km E of Mex Hwy 1. 22 March 1982.

***Encelia frutescens* A. Gray**

- 154 United States. California. Inyo Co.: Wildrose Canyon, 22 km SW of the jct of Trona-Wildrose Rd. with the road to the charcoal kilns. 30 April 1980.

- 174 México. Baja California: Road to Cañon de Guadalupe, 11.3 km S of Mex Hwy 2. 20 February 1981.
- 214 United States. California. San Bernardino Co.: Poniente Road (to Kane Springs), 2.6 km S of I-15 access road SE of Newberry Springs in Newberry mountains. 14 May 1983.

***Encelia frutescens* A. Gray ssp. *glandulosa* C. Clark**

- 153 México. Baja California: Mex Hwy 5, 14.6 km N of San Felipe. 21 March 1980.

***Encelia halimifolia* Cav.**

- 143 México. Sonora. Near Puerto Libertad. A. Johnson 4128.

***Encelia palmeri* Vasey & Rose**

- 118 México. Baja California Sur: Mex Hwy 1, ca. 24.1 km S of Guerrero Negro. 7 May 1978.

***Encelia ravenii* Wiggins**

- 164 México. Baja California: 13.5 km W of Mex Hwy 5 and San Felipe on road to Valle de San Felipe. 19 April 1980.

Encelia resinifera* C. Clark subsp. *resinifera

- 195 United States. Arizona: Coconino Co.: Az Hwy 64, 21.4 km W of jct with US Hwy 89 at Cameron. 14 June 1981.
- 198 United States. Arizona: Coconino Co.: US Hwy 89A, 6.4 km N of jct with US Hwy 89 at Bitter Springs, S of Navajo Bridge. 16 June 1981.

***Encelia resinifera* C. Clark subsp. *tenuifolia* C. Clark**

- 200 United States. Arizona. Mohave Co.: Tuweep Valley, at end of road overlooking canyon (Toroweap Pt.) and in the watercourse below. 17 June 1981.

***Encelia stenophylla* E. L. Greene**

- 109 México. Baja California Sur: 15 km S of San José de Castro. 8 May 1978.

***Encelia virginensis* A. Nels.**

- 196 United States. Arizona. Yavapai Co.: Z Hwy 179, 17.4 km S of Sedona. 15 June 1981.
- 216 United States. California. San Bernardino Co.: On Black Canyon Road, 5.6 km N of jct with Essex Road in Colton Hills area. 14 May 1983.

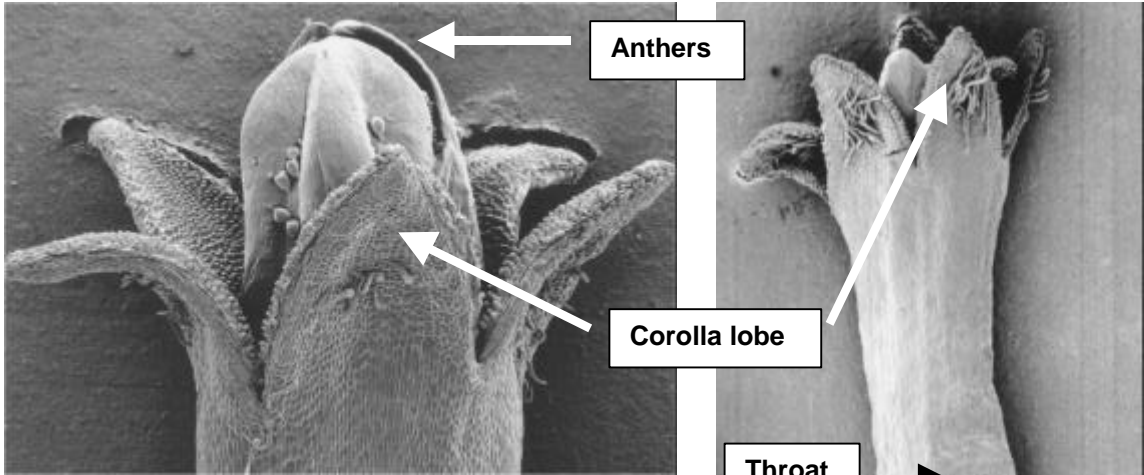


Figure 1. Upper portion of an *E. halimifolia* (143) floret showing anthers and corolla lobes (46x)

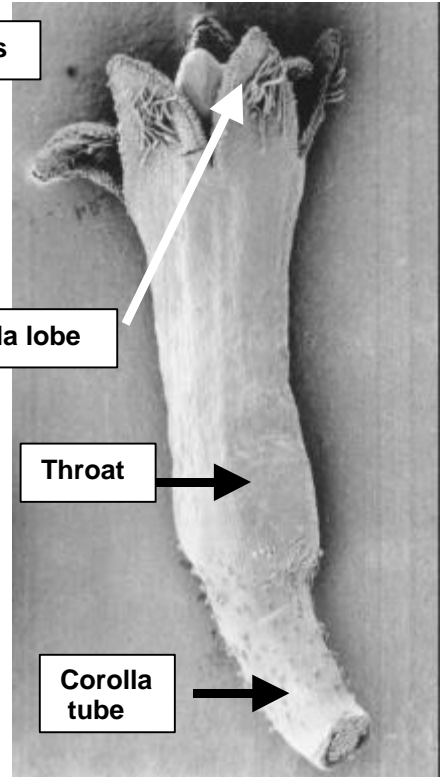


Figure 2. An entire *E. californica* (123) floret (28x)

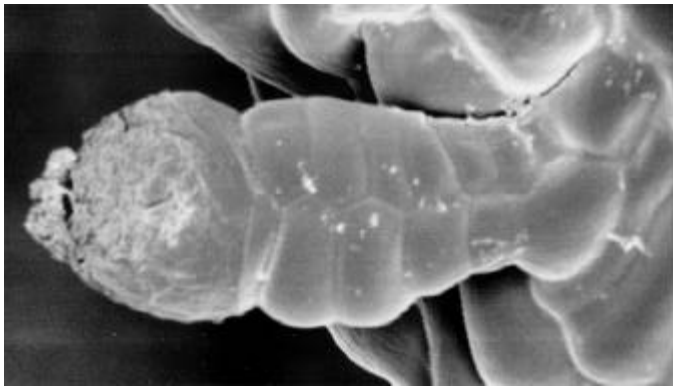


Figure 3. An *E. densifolia* (184) gland (1152x)



Figure 4. *E. halimifolia* (143) anther glands (558x)

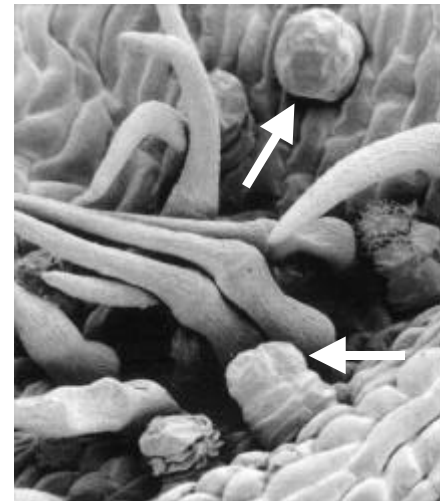


Figure 5. *E. asperifolia* (129) corolla glands (385x)

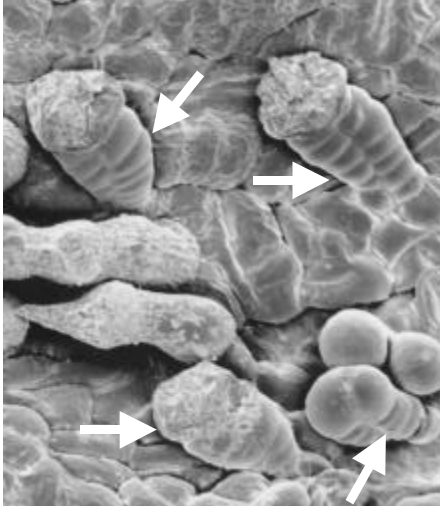


Figure 6. Elongate corolla glands on *E. densifolia* (184) (499x)



Figure 7. *E. canescens* (268) corolla gland with spherical tip (1503x)

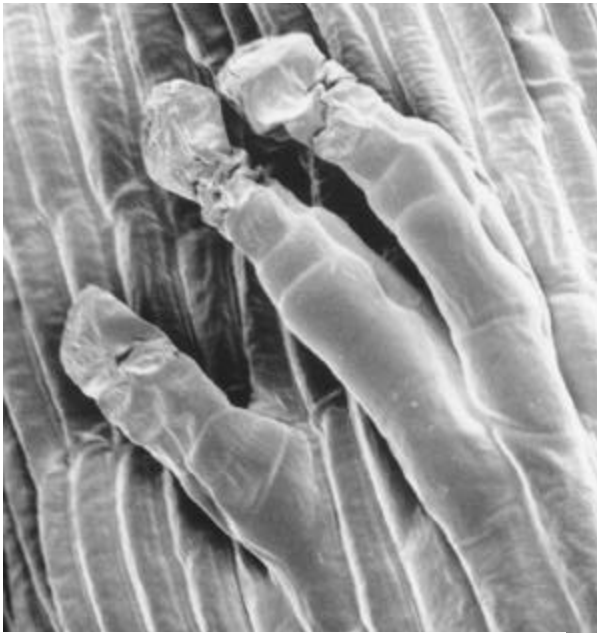
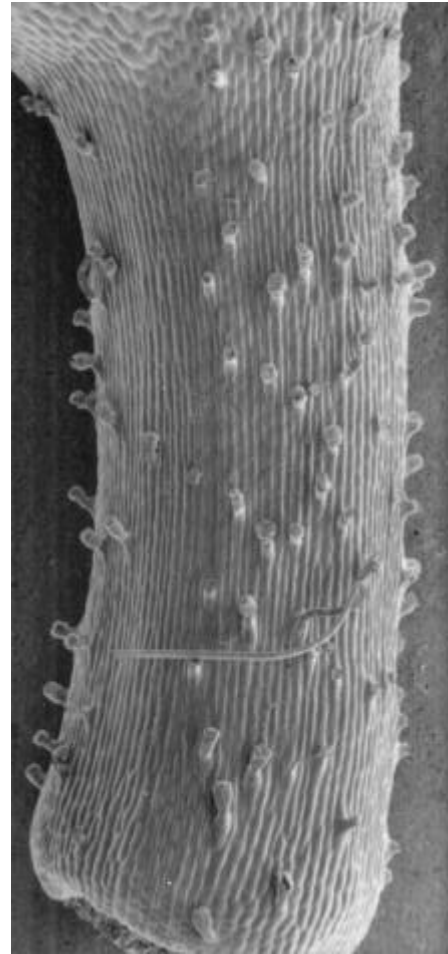


Figure 8. *E. densifolia* (184) tube glands (748x)

Figure 9 (right). *E. farinosa* var. *phenicodonta* (202) tube with glands (83x)



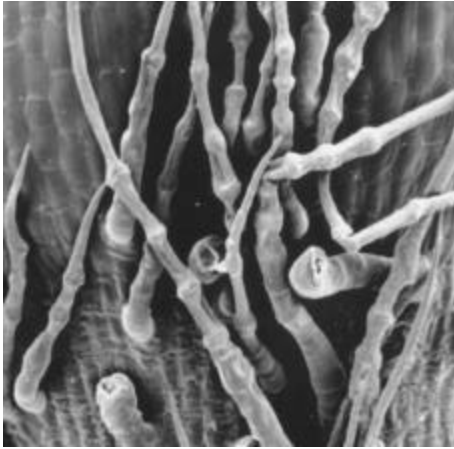


Figure 10. *E. frutescens* (214) tube uniseriate (228x)

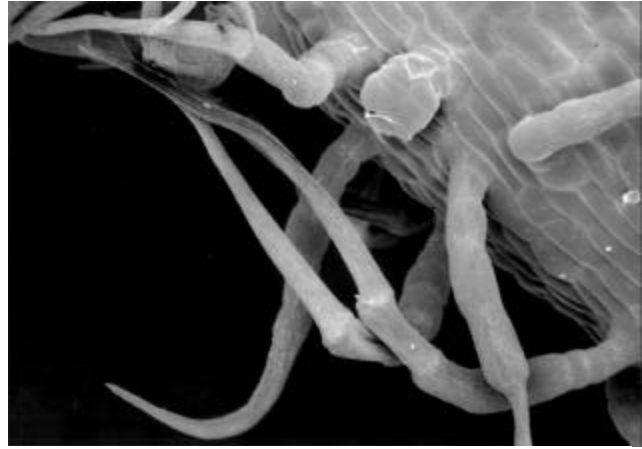


Figure 11. *E. frutescens* (174) corolla uniseriate (214x)

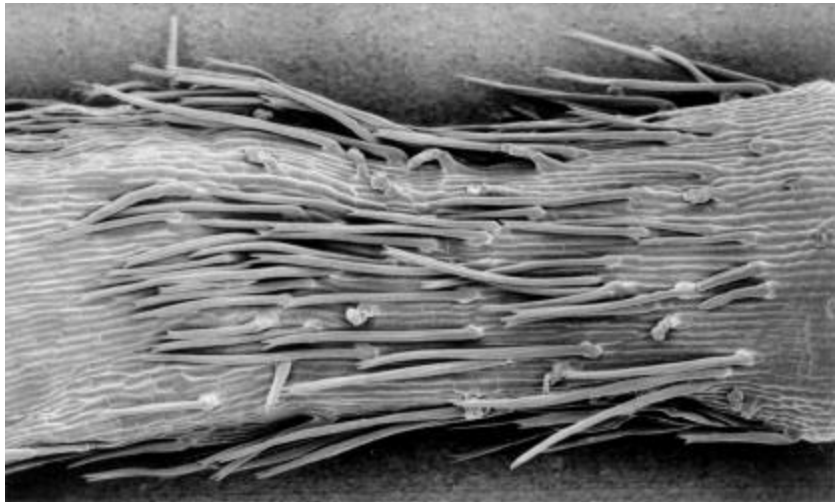


Figure 13. *E. resinifera* (195) tube Zwillingshaare (90x)

Figure 12 (left). *E. actoni* (156) uniseriate on throat, extending down from corolla lobes (18x)

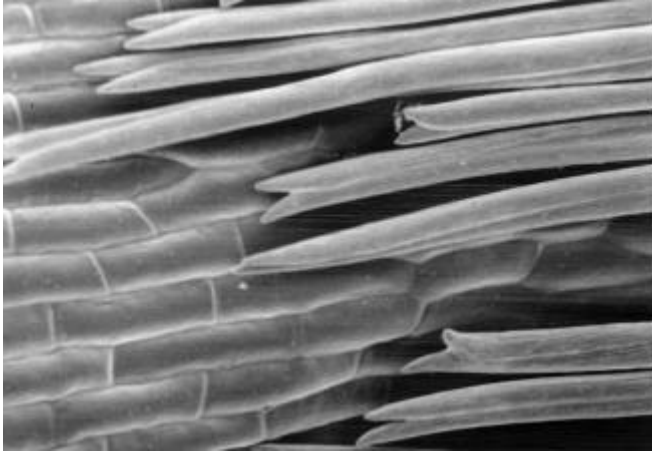


Figure 14. *Zwillingshaare* trichomes on an *E. resinifera* (195) tube (393x)

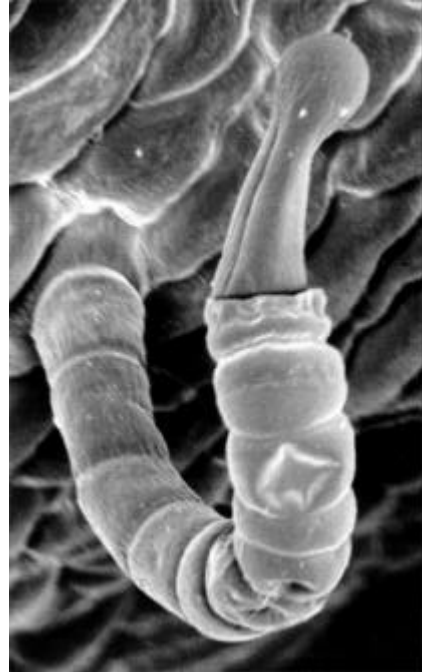


Figure 15. Moniliform trichome on an *E. Californica* (123) corolla lobe (1357x)

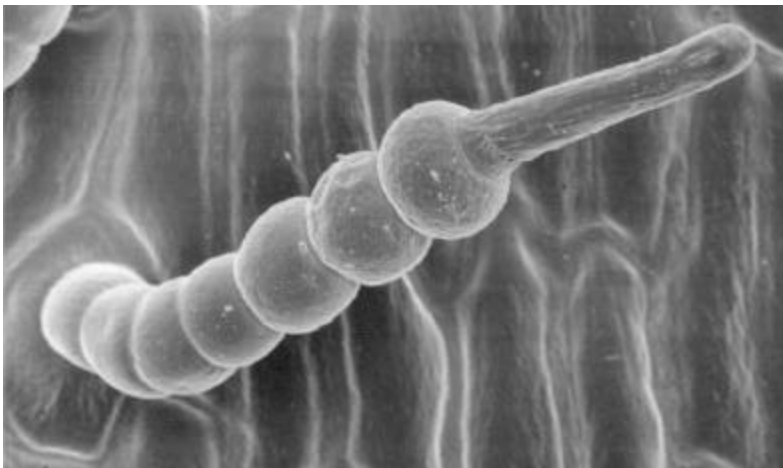


Figure 16. Moniliform trichome on an *E. canescens* (267) corolla lobe (1003x)

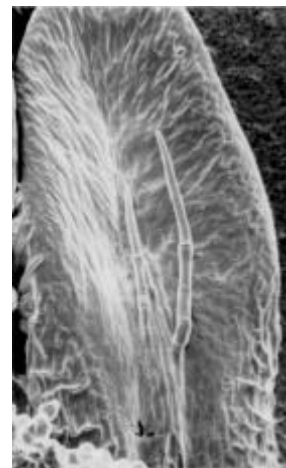


Figure 17. Anther uniseriate on *E. canescens* (273) (109x)

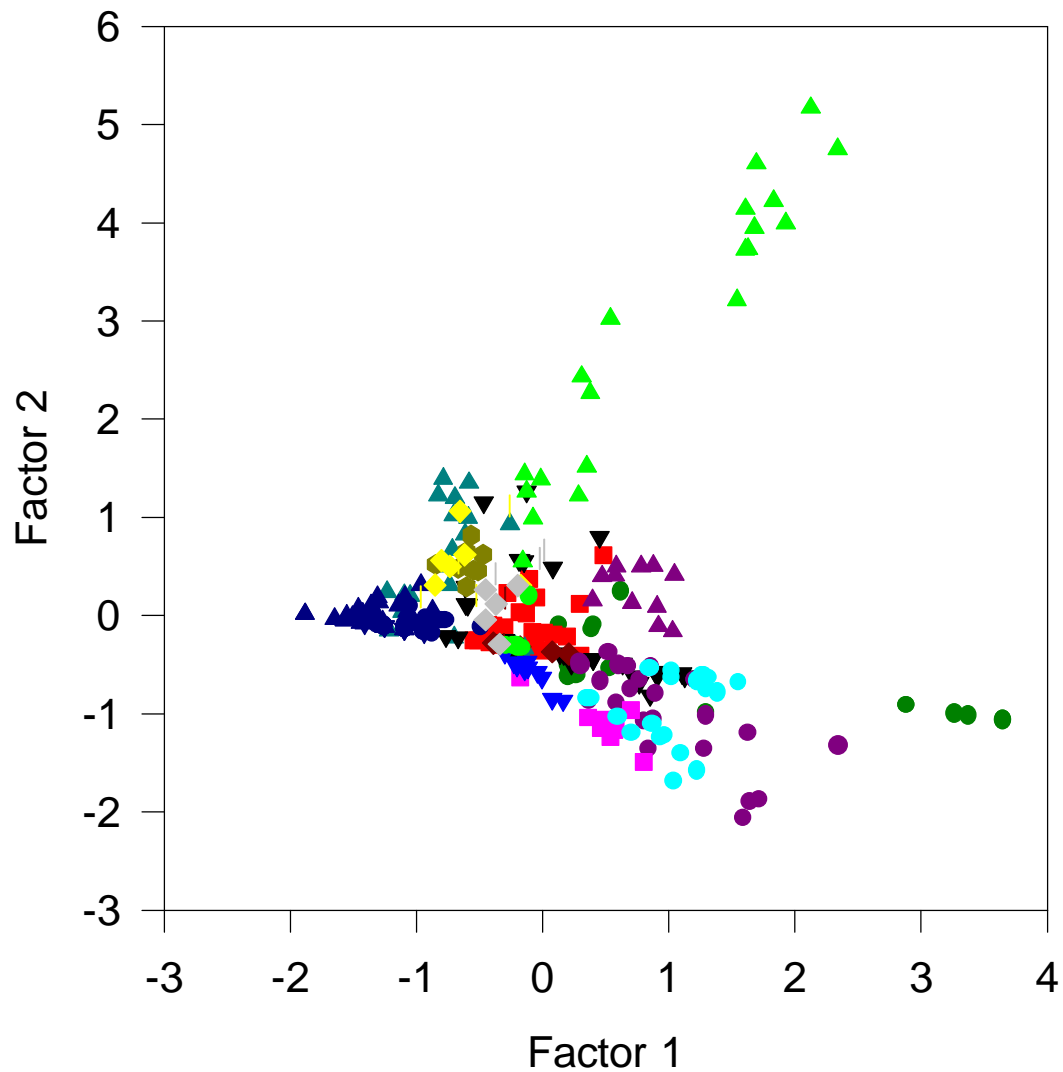


Figure 18. Plot of principal components 1 and 2 (accounting for 54.3% of the variance). (*E. actoni*, dark green; *E. asperifolia*, red; *E. californica*, blue-gray triangles; *E. canescens*, black; *E. conspersa*, dark red; *E. densifolia*, olive-green; *E. farinosa*, dark blue triangles; *E. frutescens*, purple circles; *E. glandulosa*, purple triangles; *E. halimifolia*, magenta squares; *E. palmeri*, medium-blue triangles; *E. ravenii*, yellow; *E. resinifera*, light green triangles; *E. stenophylla*, open squares; *E. tenuifolia*, light green circles; *E. ventorum*, gray; *E. virginensis*, light blue circles.)

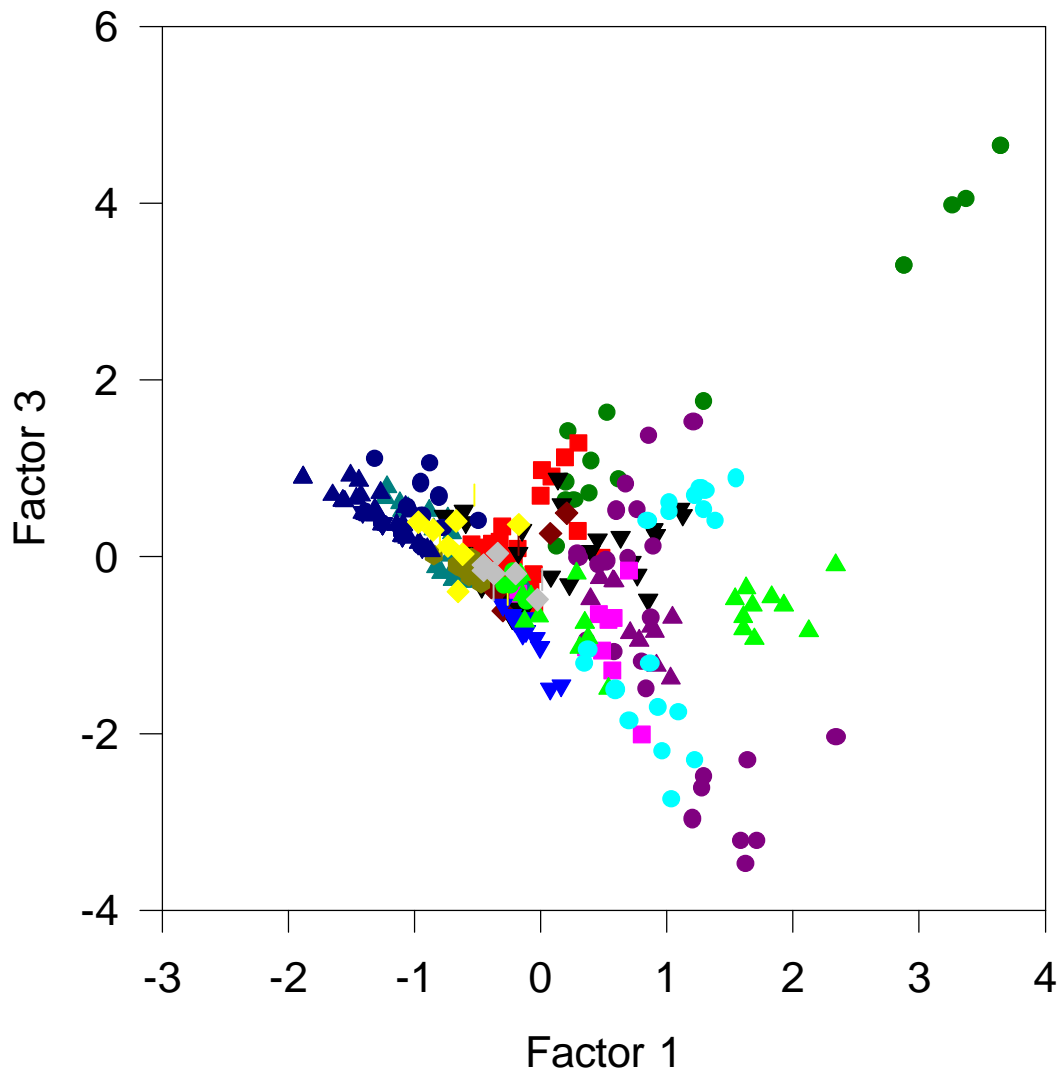


Figure 19. Plot of principal components 1 and 3 (accounting for 38.6% of the variance). (*E. actoni*, dark green; *E. asperifolia*, red; *E. californica*, blue-gray triangles; *E. canescens*, black; *E. conspersa*, dark red; *E. densifolia*, olive-green; *E. farinosa*, dark blue triangles; *E. frutescens*, purple circles; *E. glandulosa*, purple triangles; *E. halimifolia*, magenta squares; *E. palmeri*, medium-blue triangles; *E. ravenii*, yellow; *E. resinifera*, light green triangles; *E. stenophylla*, open squares; *E. tenuifolia*, light green circles; *E. ventorum*, gray; *E. virginensis*, light blue circles.)

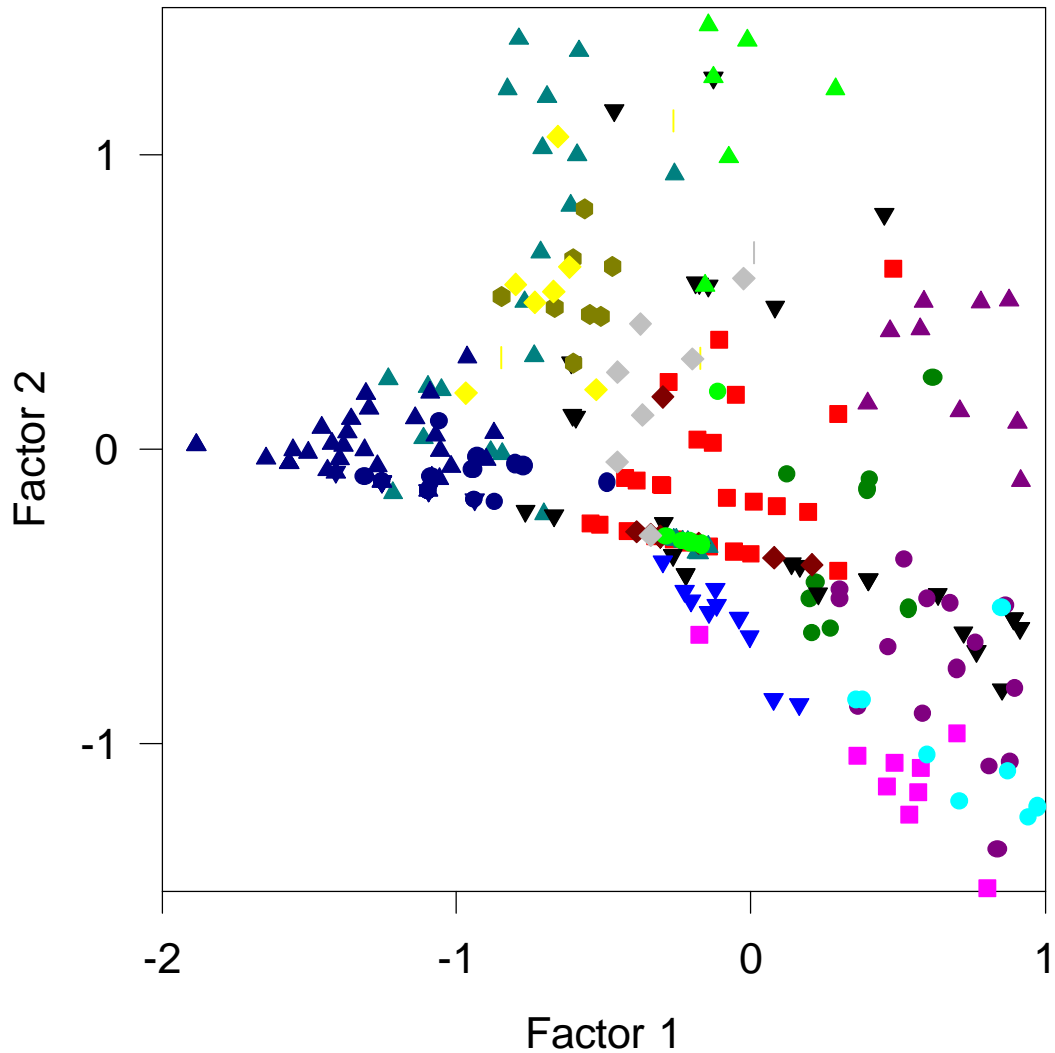


Figure 20. Enlarged central area from the plot of principal components 1 and 2 (accounting for 54.3% of the variance). (*E. actoni*, dark green; *E. asperifolia*, red; *E. californica*, blue-gray triangles; *E. canescens*, black; *E. conspersa*, dark red; *E. densifolia*, olive-green; *E. farinosa*, dark blue triangles; *E. frutescens*, purple circles; *E. glandulosa*, purple triangles; *E. halimifolia*, magenta squares; *E. palmeri*, medium-blue triangles; *E. ravenii*, yellow; *E. resinifera*, light green triangles; *E. stenophylla*, open squares; *E. tenuifolia*, light green circles; *E. ventorum*, gray; *E. virginensis*, light blue circles.)

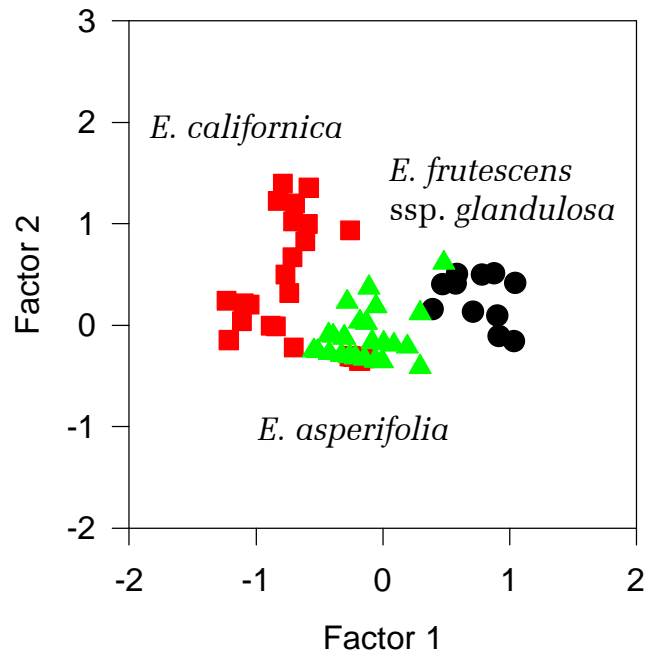


Figure 21. Plot of principal components 1 and 2 (accounting for 54.3% of the variance) showing *E. asperifolia*, a species of putative hybrid origin (green), and its parents *E. californica* (red), and *E. frutescens ssp. glandulosa* (black).

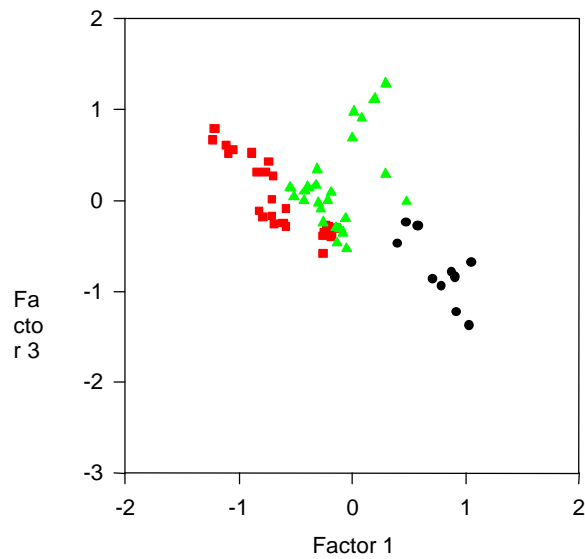


Figure 22. Plot of principal components 1 and 3 (accounting for 38.6% of the variance) showing *E. asperifolia*, a species of putative hybrid origin (green), and its parents, *E. californica* (red), and *E. frutescens ssp. glandulosa* (black).

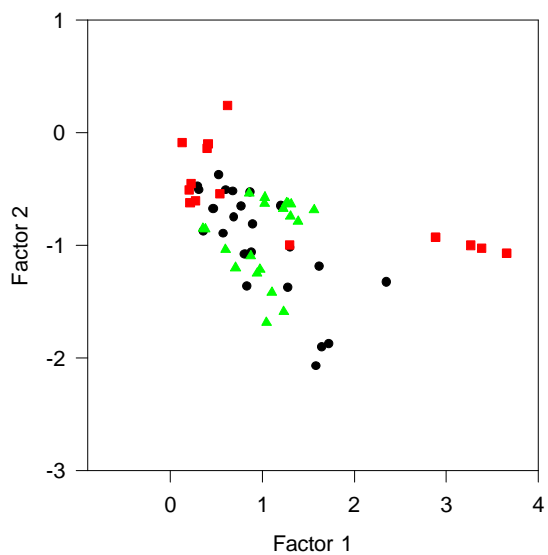


Figure 23. Plot of principal components 1 and 2 (accounting for 54.3% of the variance) showing *E. virginensis*, a species of putative hybrid origin (green), and its parents *E. actoni* (red), and *E. frutescens ssp. frutescens* (black).

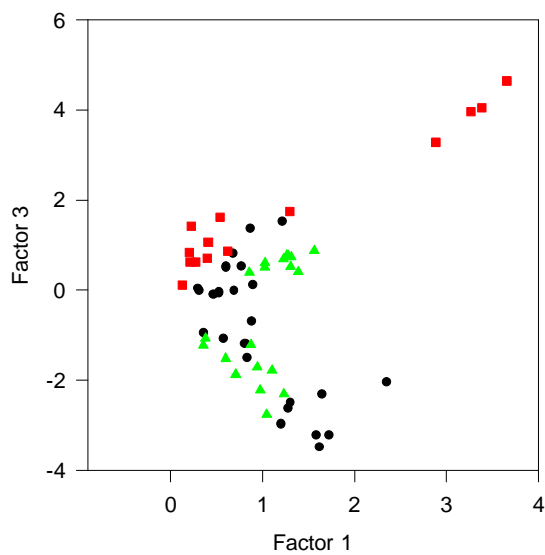


Figure 24. Plot of principal components 1 and 3 (accounting for 38.6% of the variance) showing *E. virginensis*, a species of putative hybrid origin (green), and its parents *E. actoni* (red), and *E. frutescens ssp. frutescens* (black).

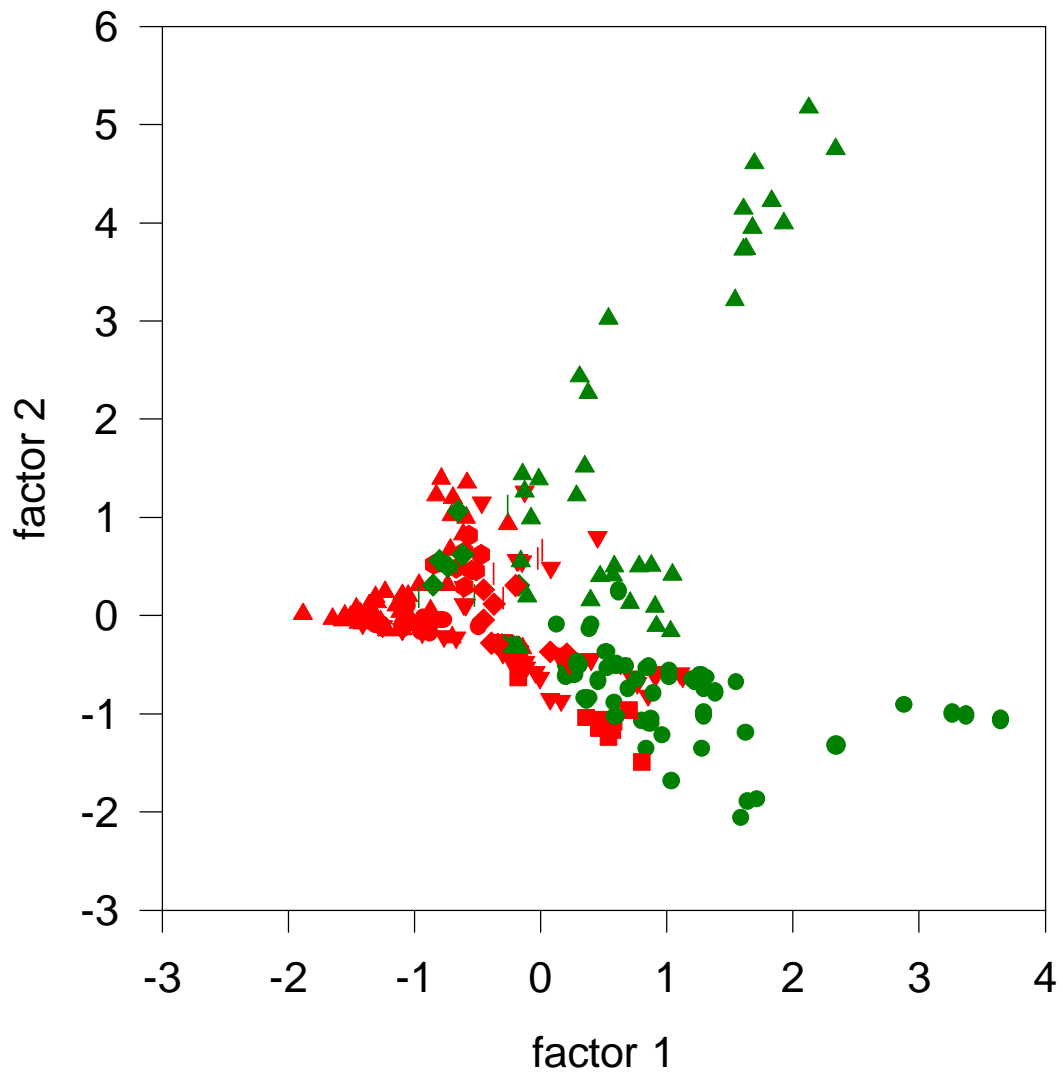


Figure 25. Plot of the first two principal components (accounting for 60.5% of the variance) showing the *frutescens* clade (*E. actoni*, *E. frutescens*, *E. glandulosa*, *E. ravenii*, *E. resinifera*, and *E. resinifera* ssp. *tenuifolia*) in green, and the *californica* clade (*E. californica*, *E. conspersa*, *E. densifolia*, *E. farinosa*, *E. halimifolia*, *E. palmeri*, and *E. ventorum*) in red.

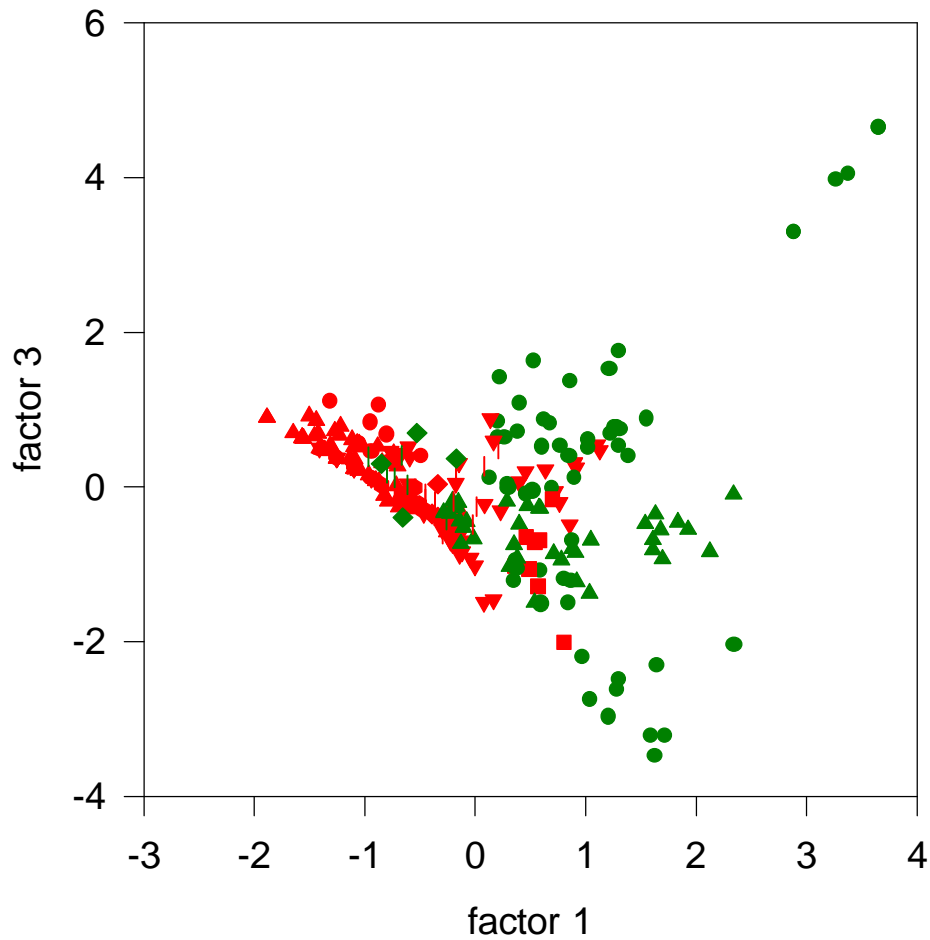


Figure 26. Plot of the first two principal components (accounting for 39.9% of the variance) showing the *frutescens* clade (*E. actoni*, *E. frutescens*, *E. glandulosa*, *E. ravenii*, *E. resinifera*, and *E. resinifera* ssp. *tenuifolia*) in green, and the *californica* clade (*E. californica*, *E. conspersa*, *E. densifolia*, *E. farinosa*, *E. halimifolia*, *E. palmeri*, and *E. ventorum*) in red.