Morphological and molecular characters of the endemic fawn-lily *Erythronium elegans* and its relationship to other Pacific Northwest fawn-lilies

Final Contract Report to
Bureau of Land Management, Salem District

June 2009
(updated October 2009)

by

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Erythronium elegans, Rocky Point

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BACKGROUND AND LITERATURE REVIEW

Erythronium elegans (Liliaceae), the elegant fawn-lily, is endemic to the northern Coast Ranges of Oregon. First described by Hammond and Chambers (1985) from Mt. Hebo in the Oregon Coast Range, it was state-listed as endangered in 1995. It is now known from seven localities in northwest Oregon, and continues to be listed as endangered in Oregon (Oregon Natural Heritage Information Center 2007). The population at Mt. Hebo (here treated as a single locality, but extending over much of the summit of Mt. Hebo) is the largest, with thousands of plants. Of the remaining populations, several contain <100 plants. The population ecology and habitat requirements of *E. elegans* have been studied by Guerrant (1999) in two populations, at Mt. Hebo and at Fanno Bog.

Hammond and Chambers considered *E. elegans* to be most closely related to the coastal pink fawn-lily *E. revolutum* and the southern Oregon Klamath fawn-lily *E. klamathense*. Two studies (Stockhouse and Oen 1988, Allen 2001) made use of isozymes to investigate relationships of *E. elegans* and related species. These reports showed that *E. elegans,* unlike most other western North American species, has a chromosome number of $2n = 48$ and is tetraploid.

Recent morphological and molecular work (Allen 2001, Allen et al. 2003, Allen 2008) has provided evidence that *E. elegans* is of probable hybrid origin, but is not closely related to *E. klamathense*. Proposed species contributing to *E. elegans* are the Oregon fawn-lily *E. oregonum,* the pink fawn-lily *E. revolutum,* and the avalanche lily *E. montanum*. *Erythronium oregonum* is found in forest openings in western Washington and Oregon, usually away from the coast; *E. revolutum* is a species of coastal to montane moist (often riparian) habitats. Both species overlap in range with *E. elegans,* and are known to hybridize with other species (Applegate 1935, Allen and Antos 1988, Mathew 1992). The avalanche lily is a montane to subalpine species, occurring from the Cascade Mountains of central Oregon north to B.C. (Allen et al. 1996, Allen and Robertson 2002), but not currently found within the range of *E. elegans*.

OBJECTIVES OF THIS PROJECT

The overall objective of this project was to provide new information on taxonomic, genetic and molecular features of *E. elegans,* and to determine how it is related to other western North American *Erythronium* species. The project included both morphological and molecular components. This information will assist in determining management priorities and strategies for populations of *E. elegans*.

Specific goals were to:

1. assess the taxonomic distinctness of *E. elegans* and identify its diagnostic features;
2. investigate further its hybrid origins and relationship to other western Oregon species;
3. report latest findings on genetic differences among *E. elegans* populations;
4. produce a revised key to Pacific Northwest *Erythronium* species (including all those considered as possible parent taxa of *E. elegans*).

METHODS AND MATERIALS

Populations of four species of *Erythronium* in Oregon (*E. elegans, E. klamathense, E. oregonum* and *E. revolutum*) were sampled for this project between 2006 and 2009. A number of previously collected samples from other populations of these species were also included. In addition, other species (*E. montanum*, *E. quinaultense, E. grandiflorum*, and several Californian species) were included in the phylogenetic analyses, which includes 15 of the 16 *Erythronium* species native to western North America. Localities of all sampled populations are given in Table 1.

Materials collected from each population included dried leaf samples, dried flowers, and a voucher herbarium specimen. Flowers for morphological analysis were collected from 1-10 plants per population, then disassembled and the flower parts taped onto 4 x 6 inch filing cards. These were labeled, allowed to dry, and stored for later measurement. Leaf material for DNA analysis was also collected from 1-10 plants per population. Leaf fragments (typically 1/2 to 1/3 of an individual leaf) were collected from individual plants, placed in coin envelopes with desiccated silica gel crystals until completely dry, then stored dry at room temperature until used.

Morphological Analyses

Individual pressed and taped flowers from populations of *E. elegans, E. oregonum, E. montanum*, *E. revolutum* and *E. quinaultense* were used for measurements of flower parts. *Erythronium* flowers have six tepals (arranged in an outer and inner series of three each), and six stamens (positioned opposite the tepals). The following characters were measured: tepal length, tepal width, anther filament length, anther filament width, and style length. Tepal length-width ratios were also calculated. For a given flower, all individual tepals and anther filaments were measured (and length-width ratios derived), then measurements were averaged separately for the inner and outer series in each flower, to give a single value per flower for each of these characters.

These data were used to assess differences in floral morphology between species and among the various populations. The measurements for all flowers were summarized separately for each species, to give means and variances for each trait. Characters of particular interest were plotted to assess differences and degree of species overlap. Principal components analyses were also carried out to assess overall degree of floral similarity among all of the five species measured, and between different species pairs.

Molecular Analyses

DNA was extracted from 1 to 4 leaf samples per population, using standard methods (Allen et al. 2003). Extracted DNA was then used for sequencing of four DNA regions: (1) the internal transcribed spacer (ITS) region of the nuclear ribosomal gene complex; (2) the chloroplast psbA-trnH spacer region; (3) the chloroplast trnS-G spacer region; and (4) the chloroplast accD-psaI spacer region. These DNA regions were chosen because sequencing of several preliminary samples showed that they contained useful variation, both within and among species.

Sequencing was done directly from DNA produced using the polymerase chain reaction (PCR). Methodologies and primers used are described by Allen et al. (2003), Shaw et al (2005) and Allen (2008). All DNA sequencing was done by Macrogen Inc.

For each DNA region, sequences from all samples were aligned using the sequence alignment programs ClustalX and Jalview. Phylogenetic analyses of the highly variable ITS region (including neighbour-joining, maximum parsimony and maximum likelihood) were carried out,

to show relationships among populations and species based on the nuclear genome. For the chloroplast spacer regions, data from the different DNAs were combined, and relationships among the sequence variants (haplotypes) found in each population and species were analyzed using the program TCS. These analyses yielded a haplotype network.

Table 1. *Erythronium* populations included in this study

Collection #	Locality
Pacific Northwest Species	
E. elegans	
1342	OR, Mt. Hebo summit #1 (near Ed Guerrant's sun plots)
1346	OR, Lincoln Co., Lost Prairie
1347	OR, Lincoln Co., S side of Saddle Bag Mt.
1351	OR, Polk Co., Fanno Ridge Bog, T8S R8W sec 14
1358	OR, Tillamook Co., Triangulation Point
1359	OR, Lincoln Co., Rocky Point
9919	OR, Mt. Hebo along rd E of summit
9920	OR, Polk Co., Fanno Ridge Bog
E. grandiflorum	
Grand1	OR, Wasco Co., Columbia Gorge E of Rowena
Mission1	BC, Mission Ridge NW of Lillooet
Nisk1	BC, Niskonlith Meadows near Chase
1344	OR, Tillamook Co., Mt. Hebo summit trail
1349	OR, Benton Co., Mary's Peak, N side of summit
2004	OR, Linn Co., Cone Peak trail just N of Iron Mt.
9628	BC, Blackwall Meadows, Manning Provincial Park
9901	OR, Wasco Co., Columbia Gorge E of Rowena
9914	WA, Whitman Co., Kamiak Butte
E. montanum	
9801	OR, Hood River Co., Lolo Pass NW of Mt. Hood
9802	OR, Hood River Co., N of Wahtum Lake
9803	WA, Skamania Co., Elk Pass E of Mt. St. Helens
9805	WA, Pierce Co., S of Cayuse Pass nr Mt. Rainier
9806	WA, Clallam Co., Hurricane Ridge, S side of Eaqle Peak
9807	WA, Grays Harbor Co., Mt. Colonel Bob
9808	WA, Clallam Co., above S Soleduck River W of Pine Mt.
E. oregonum	
1348	OR, Benton Co., Corvallis, Avery Park
1350	OR, Benton Co., Mary's Peak, roadside below summit meadow
1352	OR, Benton Co., South Fork Alsea River, T15S R8W sec 6 nw 1/4
1353	OR, Benton Co., Mary's Peak
1354	OR, Polk Co., Condenser Peak, T7S R8W sec 14 sw 1/4
9902	OR, Josephine Co., Wolf Creek
UVic01	BC, Victoria, University of Victoria campus

Collection #	Locality
E. quinaultense	
9104	WA, Jefferson Co., N of Quinault Lake, Higley Peak
9105	WA, Jefferson Co., Matheny Ridge, rd #2140
9703	WA, Jefferson Co., Manor Ridge, T25N R11W sec 23
E. revolutum	
1302	WA, Clallam Co., Beaver Creek
9702	B.C., SW Vancouver Island, Harris Creek (San Juan valley)
9905	OR, Douglas Co., Kentucky Creek
9906	OR, Lane Co., Sweet Creek
9907	OR, Tillamook Co., Mt. Gauldy
9910	OR, Tillamook Co., Alder Creek
9911	OR,Tillamook Co., Neahkahnie Mt.
9912	WA, Pacific Co., Upper Naselle River
9918	OR, Clatsop Co., Saddle Mt.
Southern Oregon/California species	
E. klamathense	
Erklam1	CA, Siskiyou Co, Castle Lake
1355	OR, Klamath Co., Rogue River NF, below Coldsprings Trailhead, T35S R5E sec 12
E. californicum	
1243	CA, Placer Co., Mosquito Ridge Rd.
E. citrinum	
Elliott	OR, Jackson Co., Elliott Creek E of Applegate Lake
Josephine	OR, Josephine Co., lower Josephine Creek
roderickii	CA, Trinity Co., Scott River
E. helenae	
1241	CA, Napa Co., near Mt. St. Helena
E. hendersonii	
9903	OR, Jackson Co., Wards Creek Road E of Rogue River
9904	OR, Jackson Co., Fielder Creek Rd W of Rogue River
E. multiscapideum	
1240	CA, Butte Co., W of Magalia
E. purpurascens	
1247	CA, Nevada Co., Grouse Ridge
E. taylori	
Shevock 13281	CA, Tuolumne Co., Pilot Ridge
E. tuolumnense	
Tuol1	Cultivated nursery stock

Note: Collection numbers are those of G. Allen unless otherwise indicated. Collections were contributed by G. Allen, R. Exeter, D. Loewen and J. Shevock.

RESULTS

Results of morphological studies

Size and shape features of the flowers of five *Erythronium* species (*E. elegans* and the species considered most closely related to it) are summarized in Table 2 below. Each species shows considerable variation in flower size, which probably reflects mainly differences in microsite and growth conditions. However, several floral traits showed distinct differences among species, especially tepal widths, tepal length-width ratios and filament widths.

Table 2. Size ranges of selected floral structures in *Erythronium elegans* and related species.

Flower traits	*E. elegans*	*E. montanum*	*E. oregonum*	*E. quinaultense*	*E. revolutum*
Outer tepals					
Length (mm)	25-41	17-48	35-47	30-52	29-54
Width (mm)	7-16	6-15	7-18	8-16	4-10
Length/width ratio	2.7-4.8	2.8-6.2	3.0-5.0	2.6-4.65	3.9-7.5
Inner tepals					
Length (mm)	26-42	24-47	32-60	34-55	28-60
Width (mm)	8-17	7-17	8-18	8-15	5-11
Length/width ratio	2.4-4.2	2.2-4.5	3.0-5.0	2.4-4.8	3.6-6.5
Outer anther filaments					
Length (mm)	8-14	10-18	11-19	12-17	14-21
Width (mm)	0.7-1.5	0.4-0.8	0.8-2.0	0.9-3.8	1.8-3.5
Inner anther filaments					
Length (mm)	6-12	8-16	9-15	10-15	11-19
Width (mm)	0.7-1.5	0.4-0.8	0.8-2.0	0.9-3.8	1.6-3.2
Style					
Length (mm)	8-17	10-22	12-18	8-17	10-20

Anther filament width is a useful character for distinguishing these species (Fig. 1). The filaments are narrow and linear in *E. montanum*, and exceptionally wide in *E. revolutum* (sometimes exceeding 3.0 mm in width). Anther filaments in *E. elegans* are wider than those of *E. montanum* but generally narrower than those of *E. oregonum* and *E. quinaultense*.

Tepal shape also shows species-specific differences (Fig. 2). The tepals of *E. elegans* are very similar in shape to those of *E. montanum*, both species having tepals relatively wide for their length (with low length-width ratios). The tepals of *E. oregonum* and *E. quinaultense* are somewhat narrower, and those of *E. revolutum* the narrowest of all five species.

Fig. 1. Anther filament length vs. width in *Erythronium elegans* and four related species.

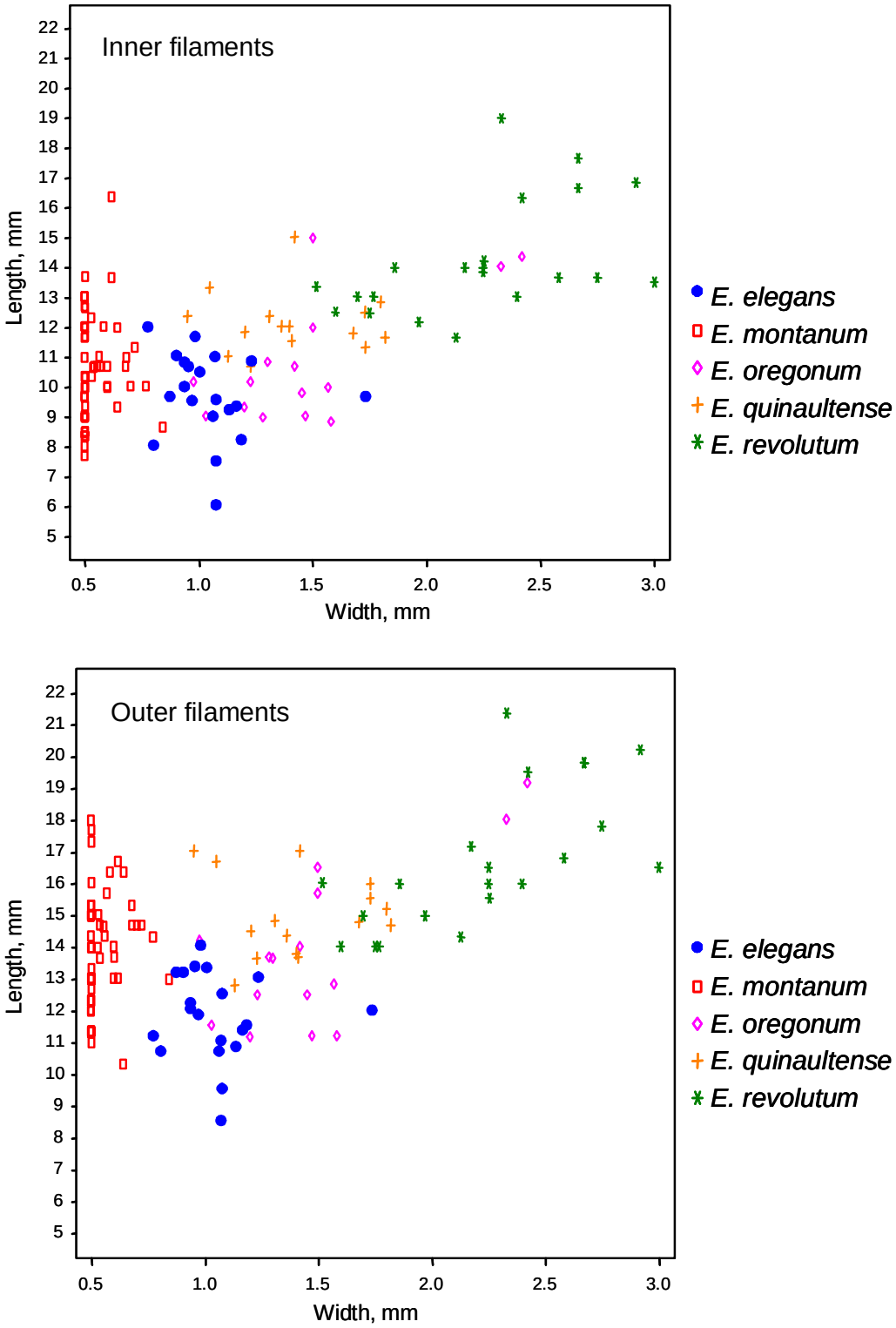
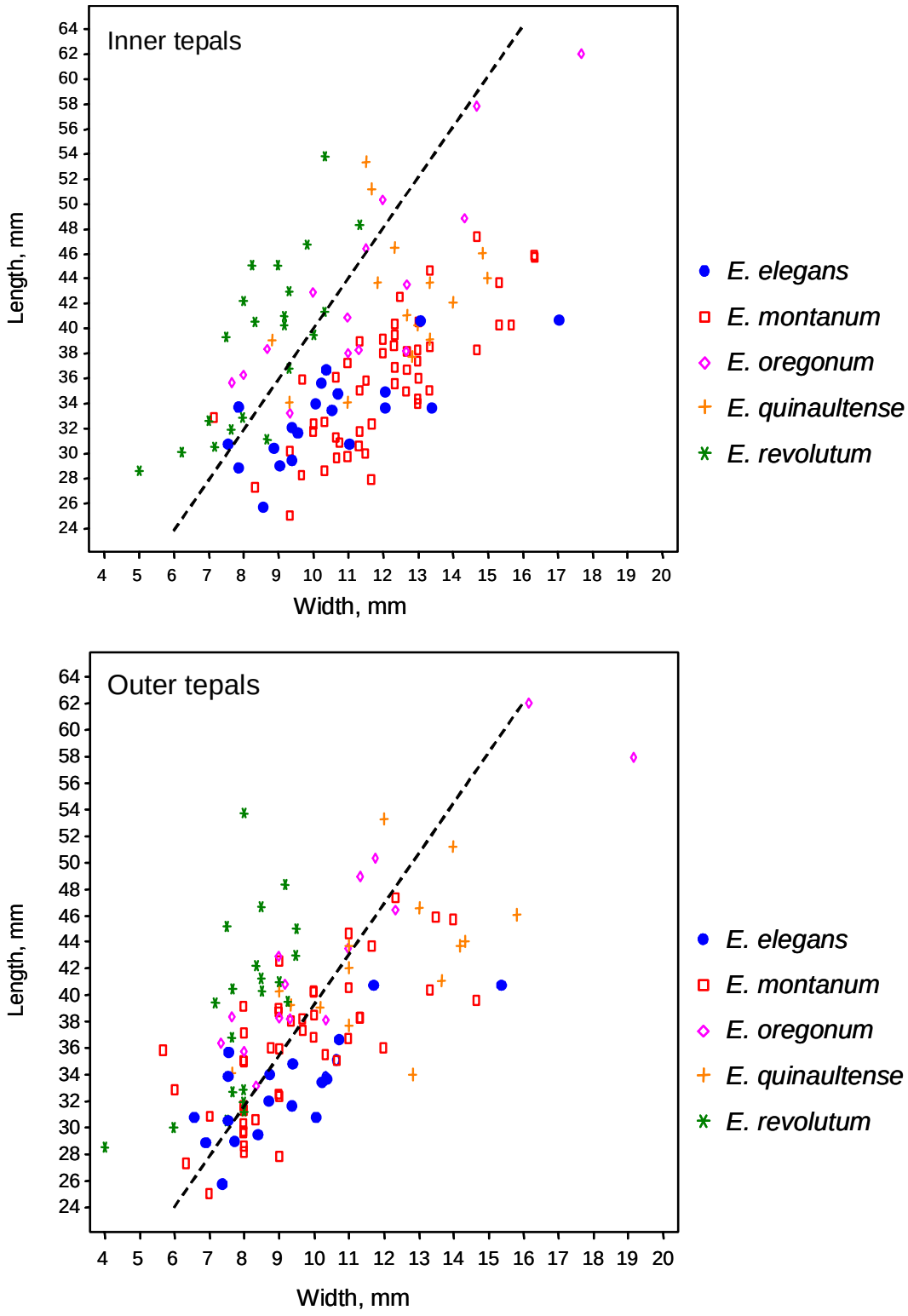


Fig.2. Tepal length vs. width in *Erythronium elegans* and four related species.



Principal component analysis plots of *E. elegans* with each of its candidate parent species (*E. montanum, E. oregonum* and *E. revolutum*) are shown in Fig. 3. These summarize flower size and shape differences overall, showing degree of overlap among the species pairs.

Erythronium elegans flowers show the greatest overlap with those of *E. montanum*, differing in their wider anther filaments and slightly smaller size. They show less overlap with *E. oregonum* or *E. revolutum*. Both of the latter species (but especially *E. revolutum*) differ from *E. elegans* in their wider anther filaments and longer, narrower tepals. These two species are also distinctly different from *E. elegans* in tepal color and leaf markings, which were not included in this analysis.

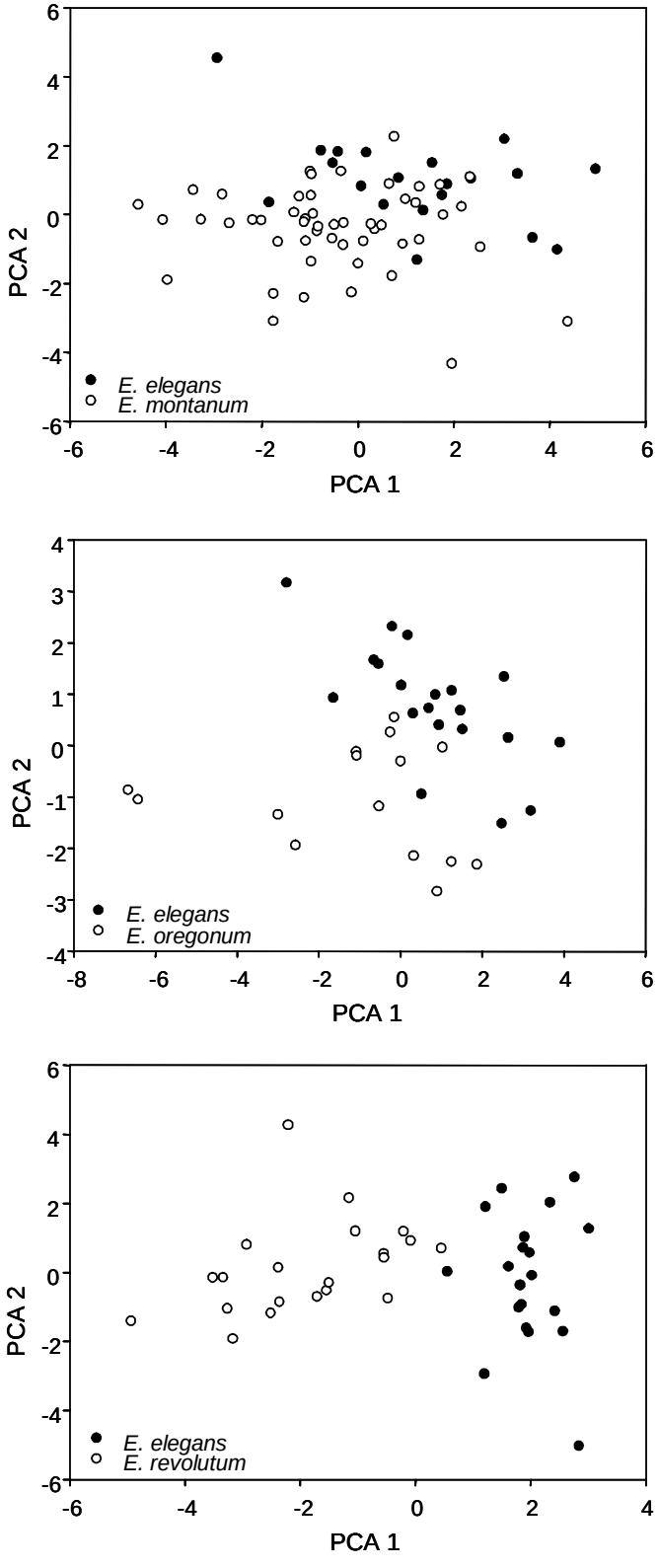


Fig. 3.
Principal components analyses of *Erythronium elegans* and three related species.
Top: *E. elegans* and *E. montanum*
*M*iddle: *E. elegans* and *E. oregonum*
Bottom: *E. elegans* and *E. revolutum*

Results of Molecular Studies

Internal transcribed spacer (ITS)

The ITS analysis presented here includes DNA data from all Pacific Northwest *Erythronium* species, and nearly all of the species indigenous to western North America. On the basis of ITS sequences, the western North American fawn-lilies fall into three distinct lineages or groups (Fig.4). The first group (indicated by A in Fig. 4) includes the southern Oregon species (*E. citrinum, E. hendersonii* and *E. klamathense*), as well as all of the California species, both low elevation and montane (*E. californicum, E. helenae, E. multiscapideum, E. purpurascens, E. taylori* and *E. tuolumnense*). Within group A, *E. klamathense* is the earliest-diverging branch.The second group (B in Fig. 4) includes the high-elevation species *E. montanum* and *E. grandiflorum*. The third group (C in Fig. 4) includes *E. elegans* and the closely related species *E. oregonum, E. quinaultense,* and *E. revolutum*.

Within group C, the four species (particularly *E. elegans* and *E. quinaultense*) do not form distinct clusters. In particular, some populations of *E. elegans* are more closely associated with populations of *E. oregonum* than with other *E. elegans* populations. This pattern is consistent with the proposed hybrid origin of *E. elegans*.

Chloroplast spacer regions (psbA-trnH, trnS-G and accD-psaI)

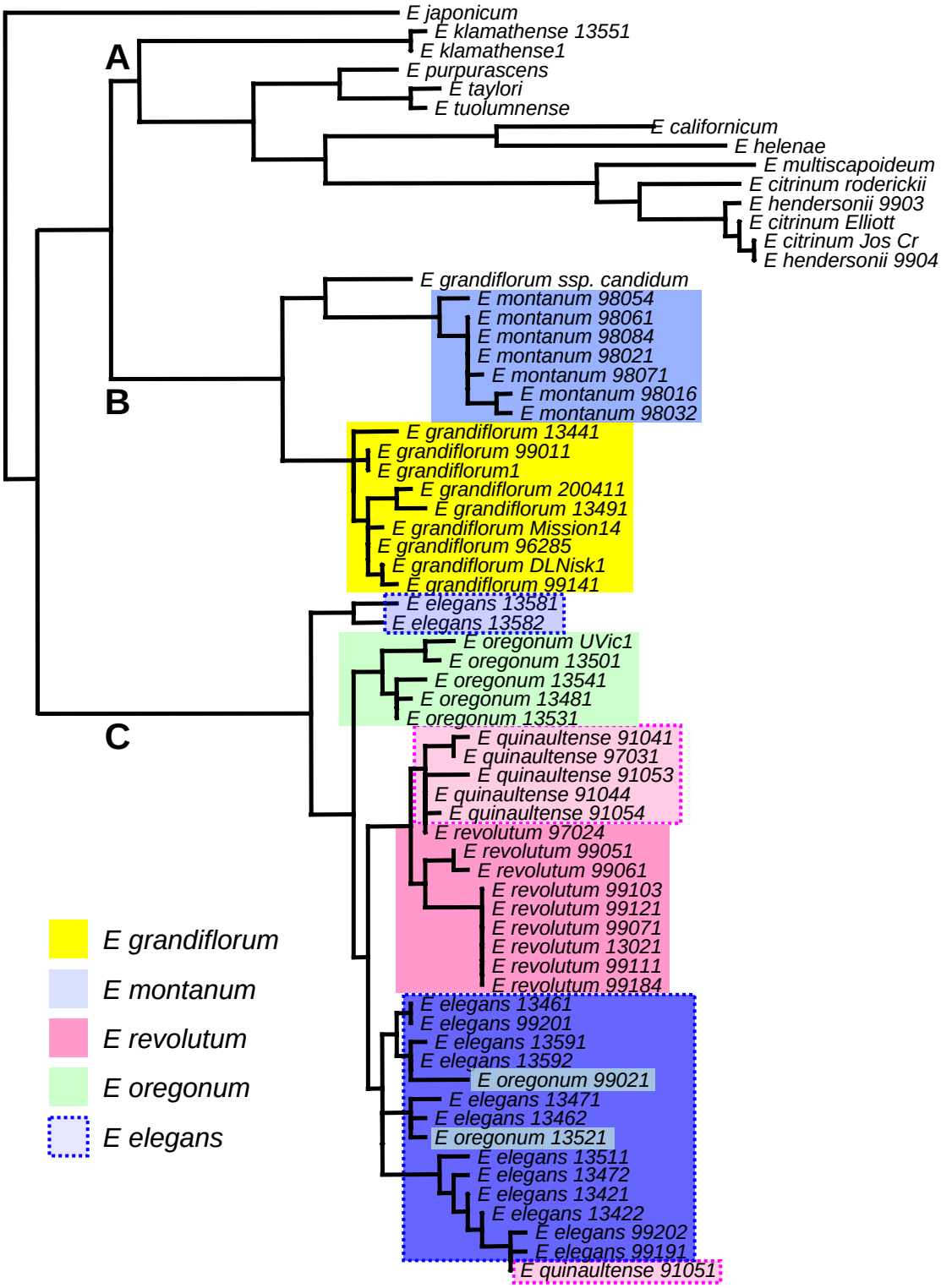
Analysis of the combined chloroplast DNA sequences of 15 Erythronium species yielded 38 distinct sequence variants or haplotypes. These fall into five groups, as follows: (1) *E. klamathense*; (2) *E. grandiflorum*; (3) *E. montanum*; (4) seven California species (*E. californicum, E. helenae, E. pluriflorum, E. purpurascens, E. pusaterii, E. taylori,* and *E. tuolumnense*); and five Pacific Northwest/Oregon species (*E. elegans, E. citrinum, E. hendersonii, E. oregonum, E. quinaultense,* and *E. revolutum*). *Erythronium klamathense* sequences are highly distinct (distinguished from all other groups by at least 22 molecular differences), as are *E. grandiflorum* (distinguished by 7 differences) and *E. montanum* (distinguished by 6). The California group is closer to the *E. elegans* group, but still distinguished from it by at least 4 differences.

Within the group containing *E. elegans*, 21 chloroplast DNA haplotypes were identified (Fig. 5; Table 3).Nine sequence haplotypes were found in *E. elegans*, forming two distinct clusters of sequences (Fig. 5). One cluster of five *E. elegans* haplotypes (A, C, E, F, G) is most closely associated with *E. revolutum* (haplotype C is common to both species, Table 3). The other cluster of four haplotypes (O, P, Q, R) is most closely associated with *E. oregonum*.

This pattern is echoed in *E. quinaultense*, an endemic from the Olympic Peninsula, Washington State. One population of *E. quinaultense* has a haplotype (A) also found in E. revolutum. The other two populations have two haplotypes (M, N) that are much more similar to haplotype I from *E. oregonum*.

The two clusters of *E. elegans* haplotypes show no obvious geographic pattern within the range of the species. The northernmost (Triangulation Point) and southernmost (Fanno Ridge) populations both have *E. oregonum*-type chloroplast DNA. The largest known population of *E. elegans* (Mt. Hebo), a northerly population, has *E. revolutum*-type cpDNA. Further south, three *E. elegans* populations occur close together within a small geographic area. Of these, two (Lost Prairie and Rocky Point) have *E. revolutum*-type cpDNA, and one (Saddle Bag Mt.) has *E. oregonum*-type cpDNA.

Fig. 4. Neighbor-joining phylogenetic tree of western North American *Erythronium* based on ITS nuclear DNA sequences.



Populations of *E. elegans* show substantial variation at the molecular level in both nuclear (ITS) and in chloroplast DNA sequences. A total of 12 different ITS sequence variants were found. Although no populations were shown to have both *E. oregonum*-type and *E. revolutum*-type chloroplast DNA, each population has a different mix of haplotypes. Of the six localities sampled, five have multiple haplotypes; each of the six localities has chloroplast haplotypes not found in any other population (Table 3).

Fig. 5. Chloroplast DNA haplotype network for six western North American *Erythronium* species, based on sequence data from three chloroplast spacer regions. Each letter corresponds to a distinct haplotype (sequence variant); line segments connecting them indicate the numbers of differences between haplotypes.

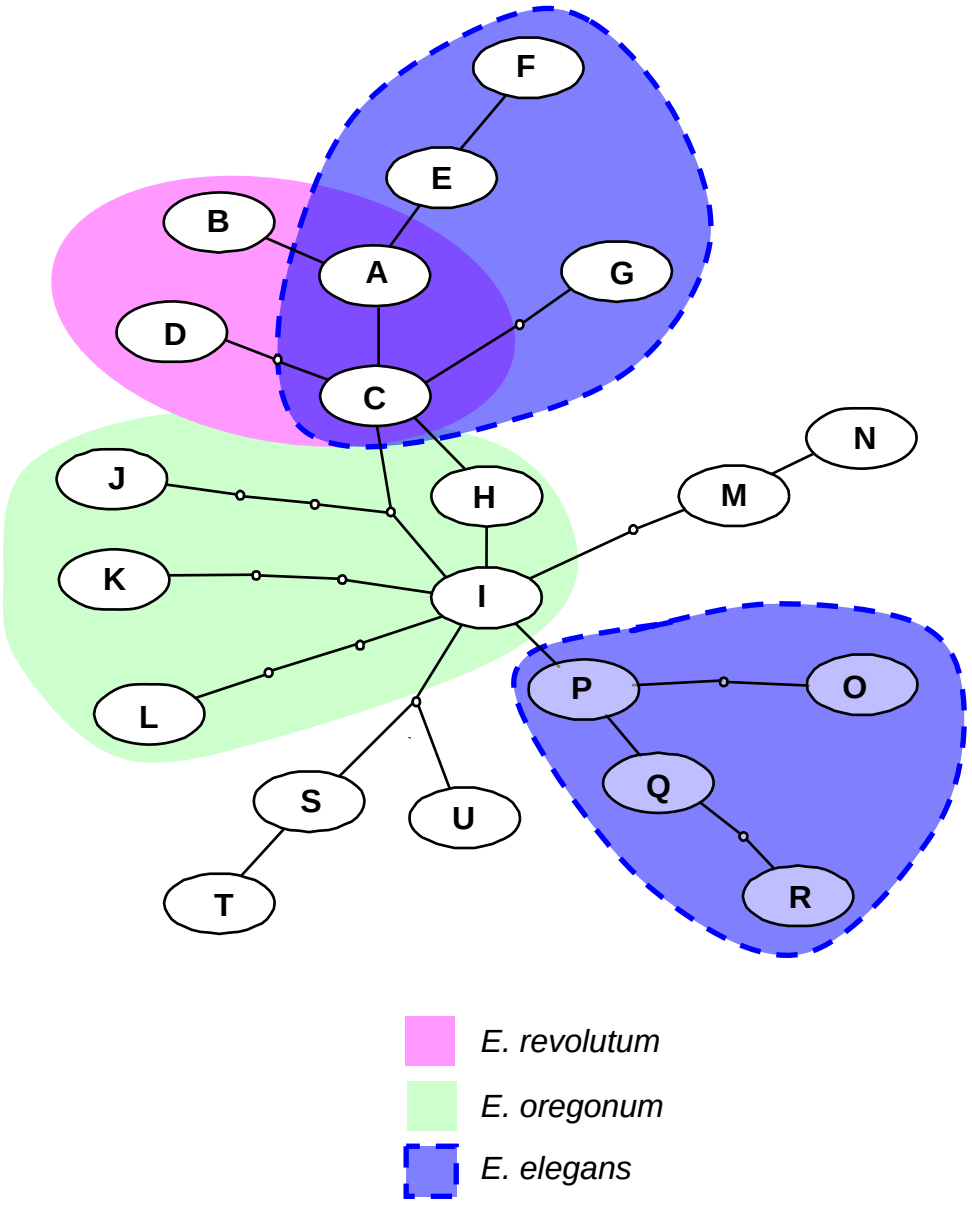


Table 3. Chloroplast DNA sequence haplotypes found in *Erythronium elegans* and five related species. Letters used for the different haplotypes correspond to those in Fig. 5.

	Chloroplast DNA sequence haplotype																				
Population	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**
E. elegans																					
1342 (Mt Hebo W)	...	...	...	...	X	…	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9919 (Mt Hebo E)	...	...	X	...	…	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1346 (Lost Prairie)	X	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1359 (Rocky Point)	...	...	X	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1347 (Saddle Bag Mt)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	...	X	...	...	...
1351 (Fanno Ridge Bog)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...
9920 (Fanno Ridge Bog)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	X	...	...	...	...
1358 (Triangulation Point)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...
E. oregonum ssp. leucandrum																					
1348 (Corvallis)	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...
1350 (Mary's Peak)	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...
1352 (S Fk Alsea River)	...	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...	...
1353 (Mary's Peak)	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...
1354 (Condenser Peak)	...	...	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...
9902 (Wolf Creek)	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...
E. oregonum ssp. oregonum																					
UVic01 (Victoria)	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. revolutum																					
1302 (WA, Beaver Cr)	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9702 (BC, Harris Cr)	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9905 (Kentucky Creek)	...	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9906 (Sweet Creek)	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9907 (Mt Gauldy)	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9910 (Alder Creek)	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9911 (Neahkanie Mt)	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Population	Chloroplast DNA sequence haplotype																				
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**
E. revolutum (cont'd)																					
9912 (WA, Naselle River)	...	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9918 (Saddle Mt)	...	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. quinaultense																					
9104 (Higley Peak)	...	...	...	...	...	...	...	...	...	...	...	...	X	...	...	...	...	...	...	...	...
9105 (Matheny Ridge)	...	...	...	...	...	...	...	...	...	...	...	...	X	X	...	...	...	...	...	...	...
9703 (Manor Ridge)	X	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. citrinum																					
Elliott Creek	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	...	...
Josephine Creek	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X	...
E. hendersonii																					
9904 (Fielder Creek)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	X

CONCLUSIONS AND RECOMMENDATIONS

Taxonomic affinities of *Erythronium elegans*

Morphological evidence suggests a close relationship between *E. elegans* and the avalanche lily *E. montanum.* The two species are very similar morphologically, although their habitats and elevation preferences are different and their geographic distributions do not overlap. *Erythronium elegans* is tetraploid and probably of hybrid origin (Hammond and Chambers 1985; Allen 2001) and it seems likely that *E. montanum* is one of its parents.

Molecular evidence, on the other hand, suggests that *E. elegans* is closely related to the two lowland species *E. oregonum* and *E. revolutum* (although it differs from each of these in several morphological features). This does not rule out a hybrid origin, as molecular traits of hybrids are often more similar to one parent, especially in the case of the chloroplast DNA which is inherited from the maternal parent (Allen et al. 2003). Populations of *E. elegans* fall into two groups each resembling one of the above species in molecular characters, suggesting that the two population groups may have arisen separately. Since hybrid species often arise through multiple hybridization events, this is a likely scenario.

The combined morphological and molecular data thus strongly support a hybrid origin of *E. elegans* from *E. montanum* and either *E. oregonum* or *E. revolutum*. The lowland ancestor of *E. elegans* could even have been a hybrid between these two species. (The Washington endemic species *E. quinaultense* shows essentially the same pattern, and likely originated in a very similar fashion, probably from the same parent species.) *Erythronium elegans* does not appear to be closely related to *E. klamathense*, to any other southern Oregon or California species, or to the widespread alpine species *E. grandiflorum*.

An updated key to *Erythronium* of the Pacific Northwest is included at the end of this report.

Genetic variation in *Erythronium elegans* populations

Levels of genetic variation detected in *E. elegans* are fairly high for such a rare and localized taxon. Populations at three localities (Mt. Hebo, Lost Prairie and Rocky Point) have chloroplast DNA features similar to *E. revolutum*, and those at three others (Fanno Ridge, Saddle Bag Mountain and Triangulation Point) have chloroplast features of *E. oregonum*, although no morphological differences are apparent between these two groups of populations. Additionally, within these two groups, unique genetic variants were detected in each population. Therefore, as far as possible, individual populations should be preserved as distinct entities.

Management plans and conservation efforts for *E. elegans* should take into account the need to maintain its genetic diversity as far as possible. This is best done by preserving each population in situ, with special emphasis on maintaining plant numbers in the smallest populations. However, *E. elegans* is amenable to cultivation (Allen, personal observation) and it might also be possible to collect seed and grow plants for later return to their locality of origin. If it became necessary to re-establish populations using plants from other localities, both geographic distance and molecular differences should be taken into consideration in selecting such plants, although such a step would be likely to result in some loss of genetic diversity.

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Key to Pacific Northwest Species of *Erythronium* (updated 2009)

1a. Leaves strongly mottled with white to brown markings; stamen filaments >1.5 mm wide; lf margin often planar

2a. Tepals uniformly violet-pink, 4-12 mm wide; stamen filaments 1.5 to 3.0 mm wide at midpoint; anthers yellow; leaves mottled with white and usually with brown; plants often riparian, usually within 100 km of coast (British Columbia to northern California) ..... *E. revolutum* Smith

2b. Tepals white to cream colored, 7-15(-18) mm wide, often becoming pinkish in age; stamen filaments 1.5 to 2.5 mm wide at midpoint; anthers yellow to cream-colored; leaves distinctly mottled with purple-brown and white; plants in well-drained lowland to montane habitats, mostly east of the Coast Ranges (British Columbia to southern Oregon) ..... *E. oregonum* Applegate

3a. Tepals cream-colored; anthers cream-colored; stamen filaments <2.0 mm wide ..... *E. oregonum* ssp. *leucandrum* (Applegate) Applegate

3b. Tepals white, yellow at base, sometimes with reddish bands; anthers yellow; stamen filaments 1.5-2.5 mm wide ..... *E. oregonum* ssp. *oregonum*

1b. Leaves green and unmottled, or faintly white- or brown-mottled; stamen filaments < 1.8 mm wide; lf margin often sinuose

4a. Style <10 mm long; stigma capitate or with very short lobes ..... *E. klamathense* Applegate
(other Californian Sierra Nevada species also key here)

4b. Style usually 10-18 mm long; stigma lobes elongate, 1-3 mm long, ± recurved

5a. Tepals yellow; stamen filaments linear, 0.5-1.0 mm wide; leaves green, glaucous, uniformly colored; plants mostly at higher elevations in the Cascade Mts and eastward, occasionally at the highest elevations on Coast Range peaks (British Columbia to California, east to the Rocky Mountains) ..... *E. grandiflorum* Pursh

5b. Tepals white, yellow at base, sometimes tinged with pink; stamen filaments linear to slightly widened, 0.5-1.8 mm wide; leaves green, sometimes with faint white markings, not glaucous; distribution various

6a. Tepals white; filaments linear, 0.5-1.0 mm wide; plants of the Cascade Mtns, usually at elevations ≥ 3,000 feet (British Columbia to central Oregon Cascades) ..... *E. montanum* S. Wats.

6b. Tepals white to pink-tinged; filaments slightly widened, ≥ 0.8 mm wide; endemic plants of the coastal mountains in northwest Oregon and western Washington

7a. Tepals ± white to slightly pinkish, the outer ones often pinkish on the outer surface near the base; stamen filaments 0.8–1.4(-1.8) mm wide, white; known only from the Coast Range in northwestern Oregon ..... *E. elegans* P. C. Hammond & K. L. Chambers

7b. Tepals ± white near base, often shading to pink at margins and tips; stamen filaments 1.0–1.8 mm wide, often pink-tinged; known only from the Olympic Mountains in western Washington ..... *E. quinaultense* G. A. Allen

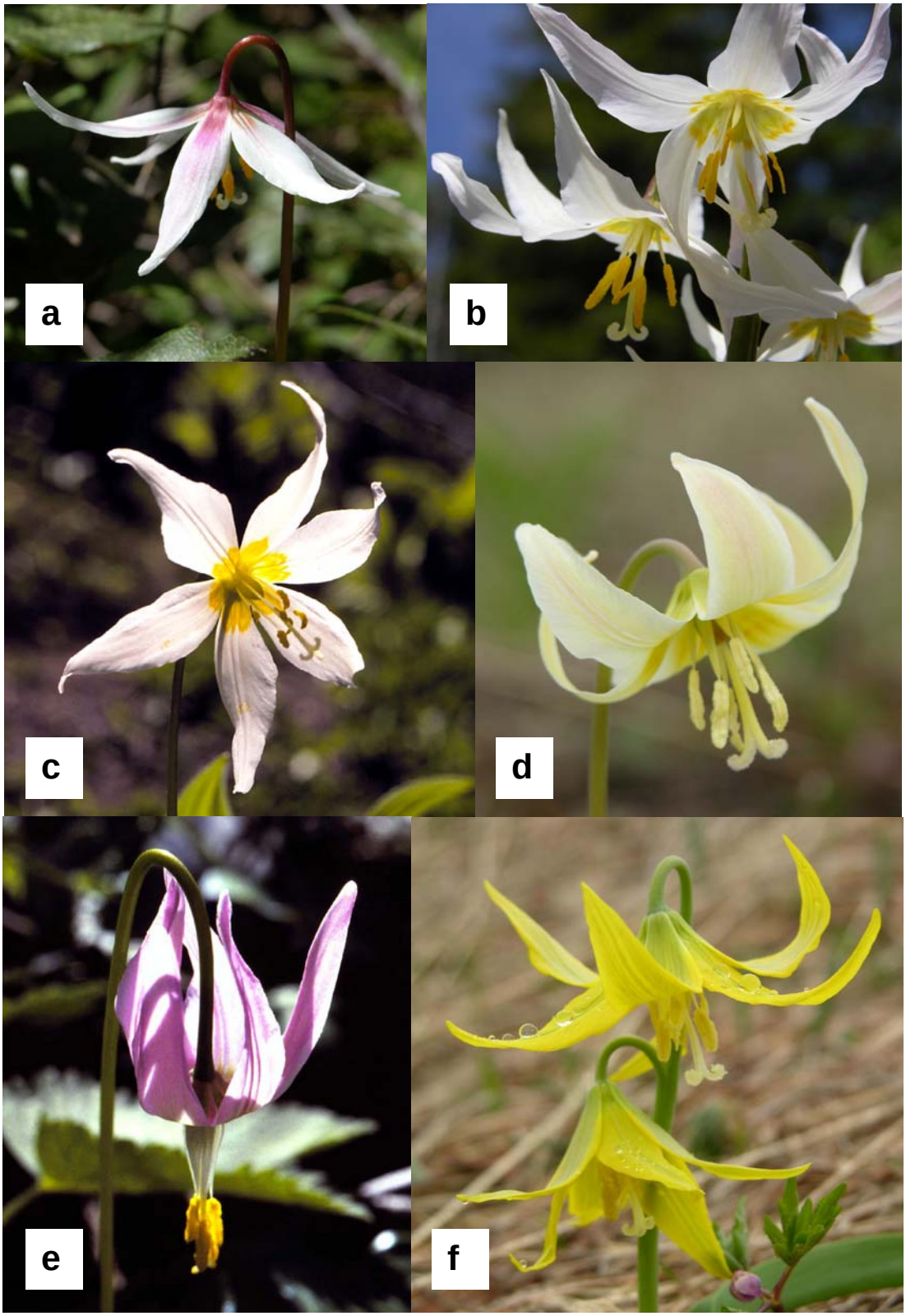

Fig. 6. *Erythronium elegans* with other Pacific Northwest species. (a, b) *E. elegans,* (c) *E. montanum*, (d) *E. oregonum* ssp. *leucandrum*, (e) *E. revolutum*, (f) *E. grandiflorum*.