OREGON DEPARTMENT OF AGRICULTURE

NATIVE PLANT CONSERVATION PROGRAM

Integrating California populations of *Fritillaria gentneri* into the 2003 USFWS Recovery Plan

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for
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Introduction

Fritillaria gentneri (Gentner's fritillary), a rare lily endemic to southwestern Oregon, occurs as a series of small, scattered populations, mainly in Jackson and Josephine Counties (Figure 1). Recently, two additional populations were discovered in northern California. Loss of habitat due to development, and degradation of existing habitat by exotic weed infestations threaten the continued existence of this species, prompting its listing as endangered by Oregon Department of Agriculture (ODA) in 1995, and by United States Fish and Wildlife Service (USFWS) in 1999. Additionally, over-collection by bulb enthusiasts, and habitat damage caused by timber harvest activities and livestock grazing may also affect populations negatively. To provide guidance for efforts to reduce the decline and increase the viability of *F. gentneri*, a Recovery Plan was issued by USFWS in 2003. As well as requiring the conservation of existing sites, this plan calls for the development of new protected populations of this rare species in order to prevent extinction and promote recovery. The Plan also identifies areas in which further research is needed, and establishes criteria for evaluating the progress of recovery.

Figure 1. A fine specimen of *Fritillaria gentneri* in flower. Photo by M. Carr.

At the time of the preparation of the Recovery Plan by ODA in 2001-2002, *F. gentneri* was thought to be endemic to Oregon, and four geographically based Recovery Units within Oregon were designated to guide conservation efforts (Figure 2). However, in 2003, a population of *F. gentneri* was discovered in northern California by Joseph Molter, a botanist with Bureau of Land Management's (BLM) Redding office. Subsequently, an additional population was located in the same general area. Although the ecology and morphology of these two populations had not been studied or evaluated, and they were not specifically discussed in the Recovery Plan, they were informally assigned to Recovery Unit 4 during the final stages of Recovery Plan development. This Unit includes the Cascade-Siskiyou National Monument, and supports numerous populations of *Fritillaria gentneri*.

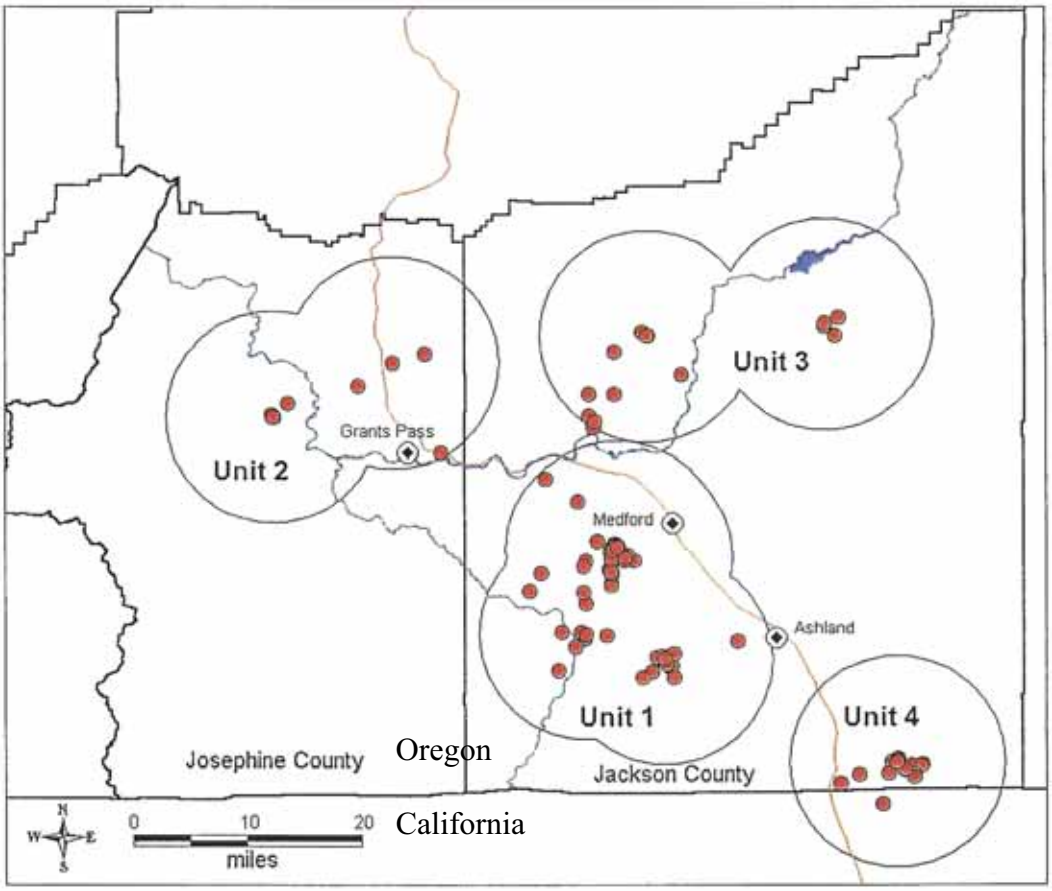

Figure 2. Four *Fritillaria gentneri* Recovery Units were designated in the Recovery Plan. The Brushy Creek site at Horseshoe Ranch can be seen just below the Oregon/California border in Recovery Unit 4 the bottom right.

Beginning in 1999, ODA, in cooperation with BLM, USFWS, and the City of Jacksonville, began studies focusing on a series of populations of *F. gentneri*, with the goal of providing information to be used in recovery planning. Reproductive biology and pollination ecology were researched (Amsberry and Meinke 2002), inter-population morphological variability and the likelihood of a hybrid origin for this species, were documented (Amsberry et al. 2006), and cytology and pollen viability were evaluated (Amsberry and Meinke 2007). ODA also developed cultivation protocols for producing transplants for population creation and augmentation projects, with mass production and outplanting of bulblets beginning in 2004 (Gisler and Meinke 2002, Amsberry and Meinke 2004, Maddux et al. 2005, Amsberry and Meinke 2005*a*, Amsberry and Meinke 2005*b*, Amsberry and Meinke 2008). Under the guidance of the Recovery Plan, we are currently continuing to collect, propagate and outplant bulblets with the goal of improving species viability and promoting recovery by creating new populations of *F. gentneri* in protected sites. Because the California populations of *F. gentneri* had not yet been discovered during the preparation and early implementation of the Recovery Plan, plants from these areas were not included in previous studies.

Objective

The goal of the current California Department of Fish and Game/ODA cooperative project is to incorporate plants from these newly discovered California *F. gentneri* populations into ODA's ongoing research, cultivation and outplanting efforts.

Site location documentation

Although the California populations of *F. gentneri* were discovered in 2003, the ownership of the sites where they occurred was originally in question. Fortunately, both were later determined to occur on BLM lands within Horseshoe Ranch, an area cooperatively managed for wildlife habitat by BLM and California Department of Fish and Game (CDFG). Subsequent to discovering these populations, Joe Molter (Redding BLM) retired, taking information concerning the location and access to these sites with him. Perceived access problems at Horseshoe Ranch exacerbated the difficulty in visiting this site. Fortunately, with the help of Nadine Kanim (USFWS) and directed by Joe Molter, we were finally able to

schedule a visit to the Brushy Creek site at Horseshoe Ranch, , in May 2007. Location information and access routes were well documented by ODA during this visit (see Appendices A and B). Because phenology of plants at the site was too advanced during this initial visit to allow for accurate identification, plants were not inventoried, marked for collection, or measured. However, robust plants, many with capsules, were observed throughout the study area.

Population inventory

The following year, the Brushy Creek site was re-visited in mid-April, and 94 identifiable flowering plants were counted by ODA and BLM botanists at that time. Additionally, an approximately equal number of flowering plants that could not be reliably identified due to phenological status or the effects of predation were observed. *F. recurva* and *F. gentneri* are difficult to distinguish in the absence of mature, non-damaged flowers, and the high levels of predation of developing flowers, (probably by insects) in 2008 made identification of many plants difficult (Figure 3). At least some, if not most, of these unidentified plants are probably *F. gentneri*, although *F. recurva* also occurs in this area. One hundred and one flowering plants were observed by BLM staff at this site in 2004.

Figure 3. In 2008, many flowers exhibited damage to stigmas, stamens and tepals. The identity of the predator causing this damage was not immediately apparent. Photo by R. Thomas.

Because only a few bulbs in any fritillary population produce flowers in a given year, population estimates based on flowering plants are very low. Although accurate estimates of the number of non-flowering vegetative plants associated with each reproductive plant have not

been developed, a 2000 monitoring study documented a mean of seven mature non-flowering plants of *Fritillaria* (probably *F. gentneri*, but potentially *F. recurva*) in plots associated with individual flowering plants of *F. gentneri* (USFWS 2003). In addition, each flowering plant has been estimated to support 44.9-54.2 barely emergent plants (with tiny grass-like leaves) or non-emergent attached "rice grain" bulblets (Gisler and Meinke 2002). Based on these rough estimates, the Brushy Creek site may support over 7,000 plants of *F. gentneri*, making it one of the largest populations of this species known (Figure 4). Although plants in this site suffered from high levels of predation in 2008, production of pods by surviving flowers was fairly common. Especially during our 2007 visit, when predation was not as prevalent, robust pods were frequently observed on plants of both *Fritillaria* species.

Figure 4. Pink flags (visible on right) mark locations of identifiable plants of *F. gentneri* at Brushy Creek. *F. gentneri* occurs in a wide variety of habitat types from riparian slopes to open grasslands. The oak and ceanothus dominated chaparral shown here is typical of habitat for this species in this site. Photo by R. Thomas.

Morphological evaluation

Introduction

The original description of *F. gentneri* (Gilkey 1951) stated that the newly described species "was remarkably constant in size and color", and that there was "no indication of intermediate forms" between *F. gentneri* and the sympatric congener *F. recurva*. However, Gilkey's statements were based solely on observations of populations occurring near Jacksonville. Since that time, the known range of *F. gentneri* has been greatly enlarged. The recently discovered populations located in northern California that are the subject of this report represent the southern end of the species range, with the Pickett Creek populations near Merlin representing the northern distribution limit. As populations increasingly disjunct from the Jacksonville sites were discovered and documented, they were (generally) readily recognizable as *F. gentneri* based on Gilkey's description, but encompassed increasing amounts of morphological variability. Differences in perianth form and color, plant size, and stigma lobing were reported, with plants tending to exhibit similar morphology within populations, but often displaying marked differences among populations. For example, the "somewhat spreading" tepal tips depicted in Gilkey's description are characteristic of the Jacksonville plants, but are not an accurate portrayal of the slightly flared to entirely unflared tepals of plants from most other populations, including those at the Brushy Creek site in California (Figure 5).

Methods

Our 2006 evaluation of inter-population variability (Amsberry et al. 2006) included nine populations encompassing the geographic and ecological range of the species (Figure 6; see Appendices C-G for specific site locations). As one of our objectives was to evaluate morphology in relation to Recovery Units, these populations were selected to represent three of the four Recovery Units (Figure 2). Because USFWS was considering integrating Units 1 and 3 at the time our initial study began (A. Robinson, 2002, personal communication), populations from Unit 3 were not included in our original study design. The Jacksonville Cemetery and Britt Grounds sites are less than ½ mile from each other, and were probably a contiguous population in the recent past. The Beekman Ridge population is within a mile of

these two sites, and Jacksonville Dump population is another ¾ mile to the east. These four populations, along with the Applegate site, represent Recovery Unit 1.

Jacksonville

Pickett Creek

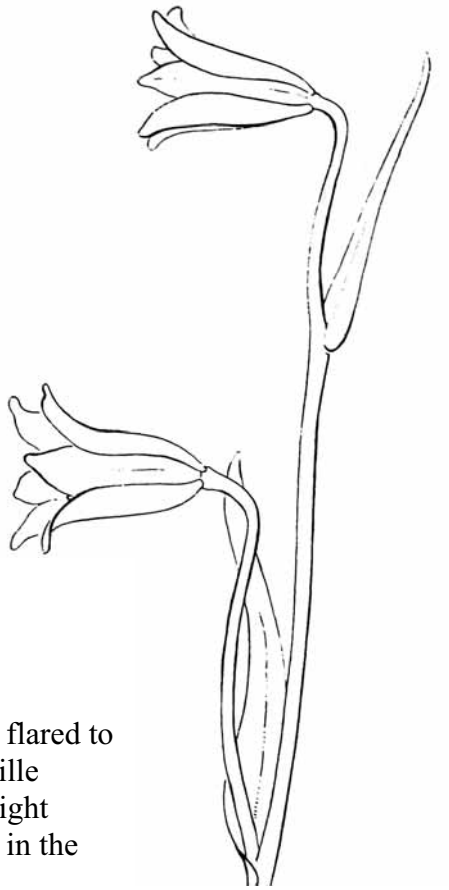

Figure 5. The drawing from Gilkey's description (right) depicts flared to recurved tepals, an accurate portrayal of the plant from Jacksonville shown on the left above, but not of the one from Pickett Creek (right above). These two plants were grown under identical conditions in the nursery yard at OSU. Photo by K. Amsberry.

Although the Grants Pass and Merlin populations are more widely separated, they both occur in Unit 2. The Siskiyou Pass and Pilot Rock sites are less than two miles apart, and represent Unit 4. The addition of the California Brushy Creek site brings the total represented in our study to ten, with this population hypothesized to be a component of Unit 4.

Because only one population each of *F. affinis* and *F. recurva* were included in the 2006 comparisons, data from 5-10 plants at five additional populations of *F. recurva* were collected in 2008, and are included in the current analyses. Flowers of *F. affinis* differ significantly from those of both *F. recurva* and *F. gentneri* in that they have highly lobed stigmas, large gland to tepal ratios and distinctive green mottling (Amsberry et al. 2006). Due to these distinctions, data from additional population of *F. affinis* were not deemed

necessary for the updated analyses presented in this report, and this species was dropped from the current between-species comparisons.

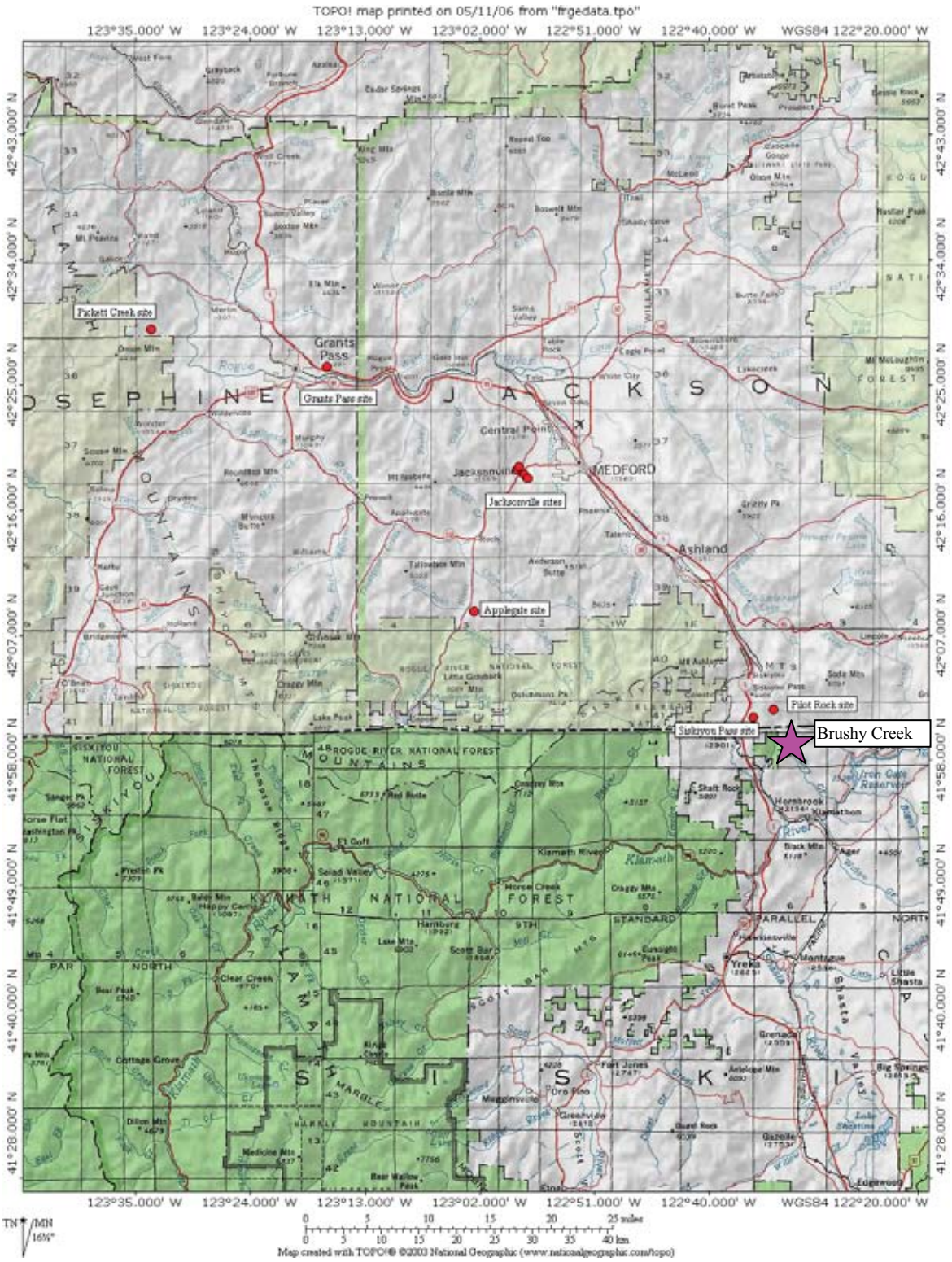

Figure 6. Location of the ten study sites, with the site at Brushy Creek indicated with a pink star. Jacksonville study sites include the Cemetery, Britt Grounds, Beekman Ridge and Jacksonville Dump sites. See Appendices for specific site locations.

In the Jacksonville Cemetery, Britt Grounds and Pickett Creek sites, plants were randomly selected by locating all flowering plants within each site, then flipping a coin to determine the inclusion or exclusion of each one from our study. However, this method was not needed for the Applegate, Pilot Rock, Siskiyou Pass and Jacksonville Dump sites, as locating ten flowering plants in these areas required diligent searching through thick brush and *Toxicodendron diversilobum* (poison oak), and the first ten plants discovered were used. Populations in these latter sites are all fairly small, with only a small portion of the total number of individuals present producing flowers each year (a common situation for *F. gentneri*), allowing us to feel confident that this unbiased method of study plant selection provided adequate representation of these smaller populations. A minimum of 94 reproductive plants of *F. gentneri* occur in the Brushy Creek site; however, predation of flowers was severe during our 2008 visit, and diligent searching was required to find ten plants with undamaged flowers.

Data on a series of parameters were collected from each of the ten plants selected per site for the 2006 study; 18 of these same characters were used for the current analysis (Table 1). Both vegetative and reproductive features were represented, with information on all taxonomically important characters included. Outer tepal length, degree of style branching, perianth shape (degree of tepal recurving), tepal color, and gland length were important traits used for defining *F. gentneri* in the original description of this taxon (Gilkey 1951), and were included in our evaluation. Similar characters (except tepal color) were also used to differentiate a series of related taxa in Iran (Rix 1997). Perianth color was very variable, and not useful in differentiating taxa in the Iranian study. However, we included both tepal background color and checkering color in our analyses, as color was the outstanding character that allowed this unique species to be recognized, and was a focus of Gilkey's description.

In 2008, color traits (of perianth and stigma) were categorized using an expanded color wheel that was created using paint samples. To ensure compatibility of the complete dataset for analysis, previously collected data were re-evaluated and standardized to match the 2008 data. Fritillary flowers without stigmas occur occasionally – these flowers were not

included in the analysis. All measurements were taken in cm in the field, and flowers were also photographed as vouchers for future reference (Figure 7).

Table 1. Morphological traits measured with descriptions of procedures used for their determination. All linear measurements are in centimeters.

Flower part characters	
Gland length	Distance from base to tip of nectary gland
Gland width	Distance across gland at the widest point
Gland length to tepal length ratio	Gland length divided by tepal length
Tepal length	Length of tepal (outer whorl)
Tepal width	Width of tepal (outer whorl)
Style height	Length of style from attachment point above ovary to stigma tip
Style branching percentage	Ratio of style length that is branched into lobes
Style branching form	Style branching form was categorized as not spreading (0; as in *F. recurva*), slightly spreading (1), spreading (2), or widely spreading (3)
Style color	Style coloration was categorized as green (0), yellow (1), red and yellow mixed (2), or all red (3)
Ovary length	Length of ovary from lower attachment point to base of style
Flower general characters	
Tepal background color	Color of petal background was evaluated using a color wheel created from paint samples and assigned values from 1 to 8
Tepal checkering color	Color of petal checkering was evaluated using a color wheel created from paint samples and assigned values from 1 to 5
Number of flowers	Number of flowers on stalk
Flower orientation	Angle of flower in relation to stem (0-45°=1, 46-90°=2, 91-135°=3, and 136-180°=4)
Degree of tepal recurving	Degree of recurving was categorized as 0 (campanulate), 1 (1-25%), 2 (26-50%), 3 (51-75%), 4 (76-100% [tepals completely recurved])
Vegetative characters	
Flower stalk leaf length	Length of longest leaf on flowering stalk
Flower stalk leaf width	Width of longest leaf on flowering stalk
Plant height	Height of plant from ground to tip of top flower

Figure 7. Morphological measurements of *Fritillaria* plants were largely completed in the field. These robust plants of *F. recurva* occur near Jacksonville. Photo by R. Thomas.

In the current study, morphological data were analyzed using principal components analysis (PCA) in order to compare groups of specimens from the ten populations of *F. gentneri*, and to evaluate differences between *F. gentneri* and *F. recurva.* PCA is a method for selecting several linear combinations of variables that capture most of the variation of a set of multi-variate responses (Ramsey and Schafer 2002). When relatively few linear combinations capture most of the variability, and linear combinations lend themselves to useful interpretation, this type of analysis provides valuable insights into data groupings. The eigenvalue represents the relative value, expressed as a percentage, of each component, and factor values indicate the importance and correlation of each morphological character to each component. PCA has been used successfully in morphometric studies, as it provides a concise method for evaluating and presenting plant groupings (Kaye 2001).

Additionally, two character traits considered important for distinguishing *F. gentneri* from *F. recurva* (USFWS 2003) were compared using a Student's t-test. Measurements of both the percent of the style that is lobed and the ratio of the length of the nectary gland to the length of the tepal were reported to differ between these two species, and were recommended as useful criteria for their reliable identification.

Disriminant function analysis (DFA) was used to test the validity of the Recovery Unit assignment of each specimen. The object of DFA is to develop a rule involving multi-variate responses that best discriminates among individuals that have been previously assigned to various groups (Ramsey and Schafer 2002). Individuals that are incorrectly assigned to groups are identified, and functions that can help predict the correct group to which future observations should belong are developed (Manguistics 2000). This analysis was useful in evaluating the ability of Recovery Unit designations to assign individuals to their "correct" groupings, based in morphological similarity.

Results

Population differentiation. Trait means differed among the ten populations of *F. gentneri* studied (Table 2), and in PCA, the first three components accounted for over 57.5% of the variation in the morphological data (Table 3). The remaining components did not explain interpretable variation and were discarded. Tepal length, plant height and flower stalk leaf length were heavily weighted on the first component, and the second was strongly loaded by gland to tepal length ratio, style branching percentage, and flower stalk leaf width, and the third represented tepal checkering color, tepal recurving and tepal width. The plot of the first two components separates most populations well, with the exception of separating Siskiyou Pass (Figure 8). Plants from this population are interspersed within those from the Applegate site; however, a plot of components one and three results in good separation of this population (Figure 9). Recovery Unit groupings are also evident in this analysis, with populations assigned to Recovery Units, 1, 2 and 4 grouping together in three-dimensional space.

Table 2. Trait means for ten populations of *F. gentneri.* AP = Applegate, BR = Beekman Ridge, BG = Britt Grounds, JC = Jacksonville Cemetery, JD = Jacksonville Dump, PC = Pickett Creek, PR = Pilot Rock, SP = Siskiyou Pass, BC = Brushy Creek. Trait means for *F. recurva* are included in the far right column. Traits that differ significantly between F. *gentneri* and *F. recurva* are represented by bold text ($p < 0.01$ from ANOVA).

	AP	**BR**	**BG**	**GP**	**JC**	**JD**	**PC**	**PR**	**SP**	**BC**	***Total FRGE***	***Total FRRE***
n =	9	9	9	10	10	10	10	10	10	5	92	46
Flower part traits												
Gland length	1.17	1.31	1.57	1.01	1.54	1.59	0.92	1.28	1.22	1.24	**1.29**	**0.76**
Gland width	0.26	0.23	0.45	0.32	0.30	0.24	0.29	0.15	0.22	0.22	**0.27**	**0.17**
Gland to tepal ratio	0.33	0.35	0.39	0.29	0.37	0.37	0.27	0.41	0.37	0.40	**0.35**	**0.22**
Tepal length	3.47	3.76	4.02	3.47	4.21	4.35	3.44	3.11	3.27	3.10	3.64	4.21
Tepal width	1.16	1.24	1.26	1.44	1.33	1.13	1.28	1.18	1.07	0.91	**1.22**	**0.80**
Style height	1.99	2.12	2.11	1.97	2.13	2.12	1.97	2.0	1.71	1.62	**1.99**	**1.65**
Style branching percentage	50.0	46.1	50.6	40.0	50.0	49.5	30.0	49.0	37.0	43.0	**44.5**	**17.8**
Style branching form	1.6	2.2	1.7	1.3	1.5	2.1	1.8	1.9	1.8	1.2	**1.8**	**0.39**
Style color	2.7	2.5	2.2	1.9	2.2	2.4	1.5	2.0	3.0	1.0	2.2	2.1
Ovary length	0.90	0.95	0.96	0.89	0.99	0.98	0.88	0.78	0.91	0.67	**0.90**	**0.77**
Flower general traits												
Tepal background	6.8	6.8	4.9	6.3	5.0	5.5	6.3	7.4	5.9	5.8	**6.1**	**3.1**
Tepal checkering	3.1	2.4	3.3	2.8	3.2	3.5	3.3	1.2	5.0	2.6	**3.1**	**3.3**
Number of flowers	2.2	4.0	3.1	3.9	4.8	2.5	2.5	2.0	2.1	2.4	3.0	2.9
Flower orientation	3.0	2.7	3.3	3.2	3.3	3.7	2.3	2.6	3.0	2.8	**3.0**	**2.7**
Tepal recurving	1.0	1.6	1.2	0.1	2.6	3.0	0.9	0.2	2.2	1.0	**1.4**	**3.4**
General plant traits												
Stalk leaf length	8.1	12.7	10.5	10.1	13.4	10.9	7.3	8.2	8.7	8.5	9.9	8.9
Stalk leaf width	1.40	1.64	1.27	1.12	1.52	1.33	1.04	2.61	1.54	1.16	**1.48**	**0.9**
Plant height	68.8	88.0	76.3	55.6	93.2	69.2	39.5	50.4	50.7	43.6	64.2	55.3

Table 3. Factor loadings and eigenvalues for the first three principal components for analysis of ten populations of *F. gentneri*, and the first two principal components for the evaluation of *F. gentneri* and *F. recurva*. Factor loading values in bold represent morphological characters with the greatest importance to each component.

	10 populations of *F. gentneri*			*F. gentneri*/*F. recurva* compared	
Traits	Component 1	Component 2	Component 3	Component 1	Component 2
Flower orientation	0.575	0.019	0.169	0.434	0.397
Flower stalk leaf length	**0.766**	-0.148	0.231	0.468	-0.291
Flower stalk leaf width	-0.004	**0.543**	-0.347	0.508	**0.664**
Gland length	0.762	0.530	0.147	**0.872**	0.000
Gland to tepal length ratio	0.187	**0.867**	0.102	0.748	-0.378
Gland width	0.357	-0.251	0.045	0.395	0.277
Number of flowers	0.538	-0.301	-0.413	0.282	0.587
Ovary length	0.706	-0.303	0.079	0.396	0.210
Plant height	**0.843**	0.032	-0.265	0.582	**0.640**
Style branching percentage	0.568	**0.593**	-0.246	**0.871**	-0.195
Style branching form	0.123	0.131	-0.013	**0.762**	-0.272
Style color	0.262	0.226	0.377	0.083	0.062
Style height	0.658	-0.063	0.442	0.663	0.073
Tepal checkering color	0.211	-0.357	**0.801**	-0.191	0.397
Tepal color	-0.501	0.176	-0.486	0.676	-0.575
Tepal length	**0.870**	-0.163	0.066	0.518	**0.649**
Tepal recurving	0.607	0.104	**0.541**	-0.504	**0.626**
Tepal width	0.287	-0.533	**-0.499**	**0.808**	-0.068
Eigenvalue	5.502	2.500	2.304	6.171	3.129
Percent variance	30.567	13.889	12.803	34.283	17.382
Cumulative percent	30.567	44.456	57.258	34.283	51.665

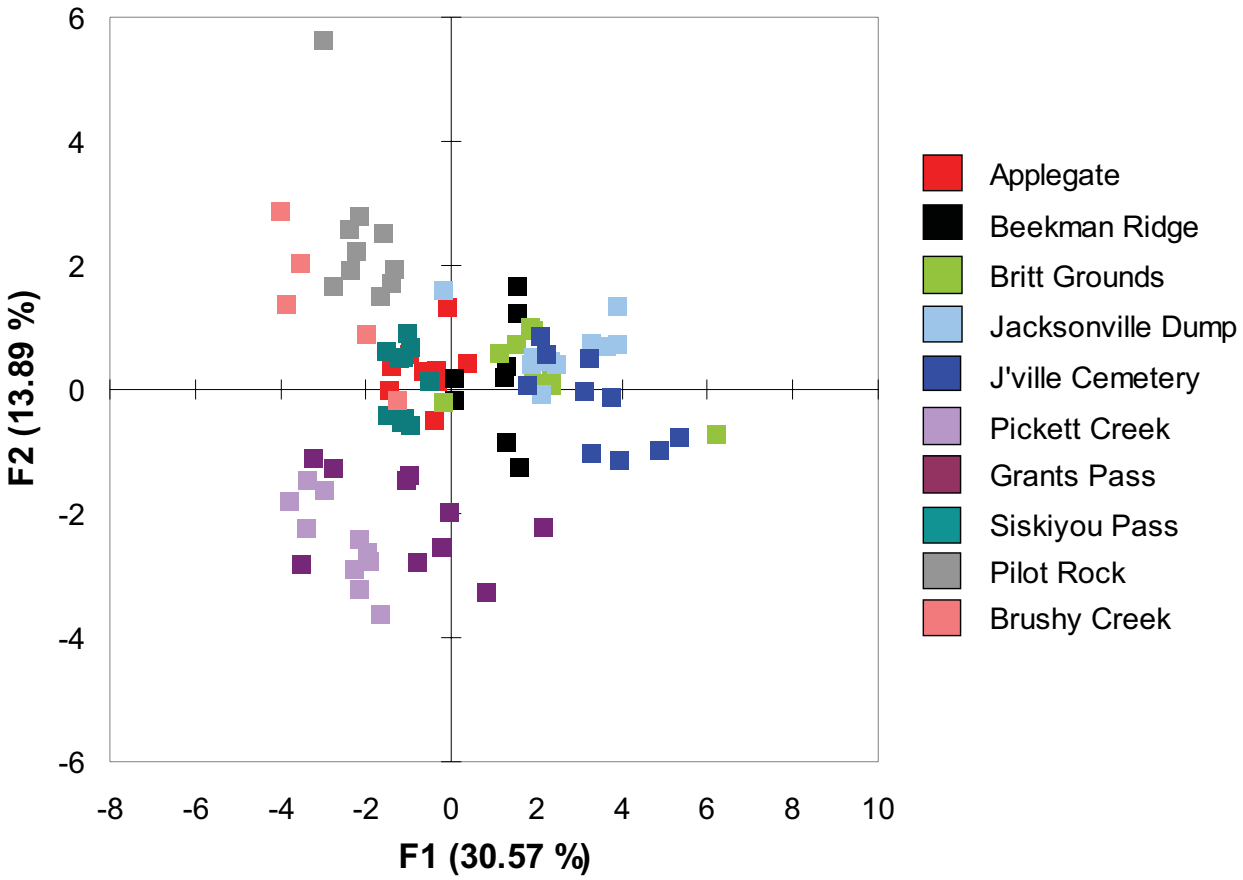

Figure 8. Phenotypic relationships among 10 Oregon and California populations of *F. gentneri* (components 1 and 2). Recovery Unit 1 consists of the Applegate, Beekman Ridge, Britt Grounds, Jacksonville Dump, and Jacksonville Cemetery sites; Recovery Unit 2 consists of the Pickett Creek and Grants Pass sites, and Recovery Unit 4 consists of the Pilot Rock, Siskiyou Pass and Brushy Creek sites.

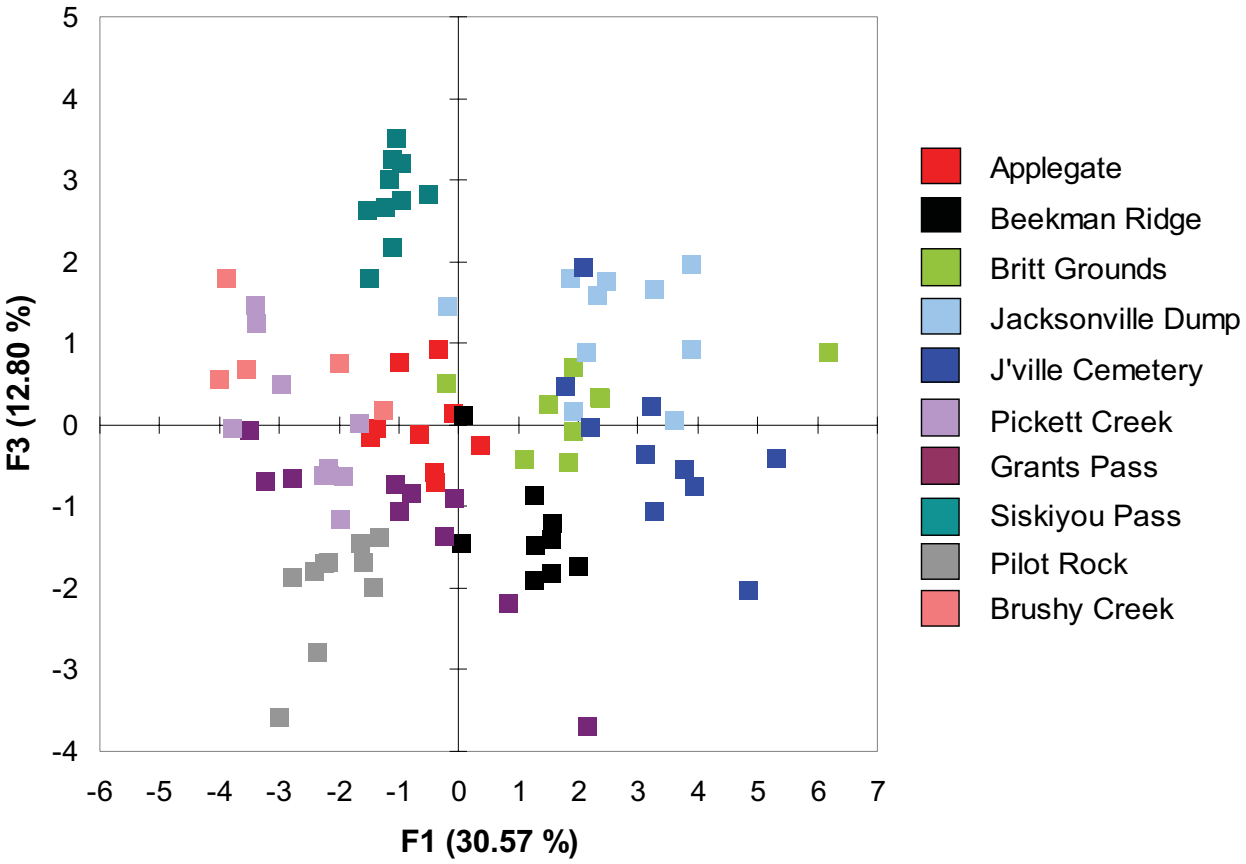

Figure 9. Phenotypic relationships among 10 Oregon and California populations of *F. gentneri* (components 1 and 3). Recovery Unit 1 consists of the Applegate, Beekman Ridge, Britt Grounds, Jacksonville Dump, and Jacksonville Cemetery sites; Recovery Unit 2 consists of the Pickett Creek and Grants Pass sites, and Recovery Unit 4 consists of the Pilot Rock, Siskiyou Pass and Brushy Creek sites.

Differentiation of F. gentneri from F. recurva. *Fritillaria gentneri* has been hypothesized to be a subspecific element of *F. recurva* "in the earliest stages of speciation" (Carey and Jessup 2004), and has been considered a "form" of *F. recurva* by horticulturists (Pratt and Jefferson-Brown 1997). Although our 2006 review suggested that the two are morphologically distinct taxa, the inclusion of only a single population of *F. recurva* in the analysis did not allow for definitive conclusions on species differentiation. The current evaluation, made up of 10 populations of *F. gentneri* and six of *F. recurva*, is much more robust, with significant differences between means for most traits (Table 2).

Principal component analyses also separates these two species separate well, with 51.7% of the variation explained by the first two components (Table 3, Figure 10). Gland length, style branching percentage, tepal width and style branching form were heavily weighted on the first axis, and flower stalk leaf width, tepal length, plant height and tepal recurving were represented by the second Component. Components beyond the first two did not explain additional variation, and are not presented.

In our study, nectary glands extended slightly more than 1/3 the length of tepals of *F. gentneri* (35.3%), and slightly less than ¼ the length of tepals of *F. recurva* (21.8%). Styles of flowers of *F. gentneri* were divided 44.5% of their length, and those of *F. recurva* 17.8% of their length (Table 2). Comparison of these traits (along with flower color and shape, and degree of stamen exertion), is recommended as a method for differentiating sympatric *Fritillaria* species in the Recovery Plan, and was validated in our 2006 study using a fairly small sample of fresh and herbarium specimens (only 16 individuals of *F. recurva* were used). Analysis of the much larger dataset used in the current study (n=92 for *F. recurva* and n=46 for *R. recurva*) reveals significant differences between the two species in both of these traits and corroborates their use for identification purposes (two-tailed $p<0.01$ for both traits from a Student's t-test; Figure 11).

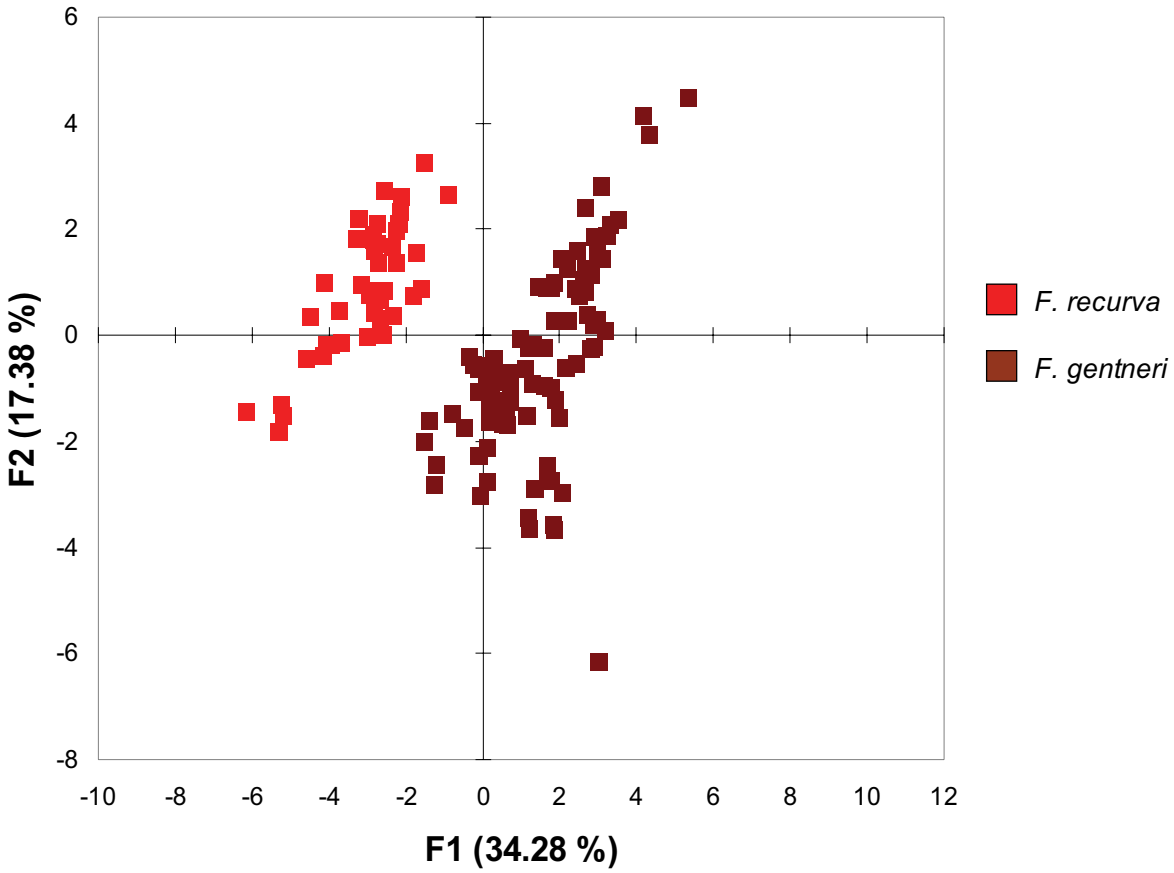

Figure 10. Phenotypic relationship between *Fritillaria recurva* and *F. gentneri.*

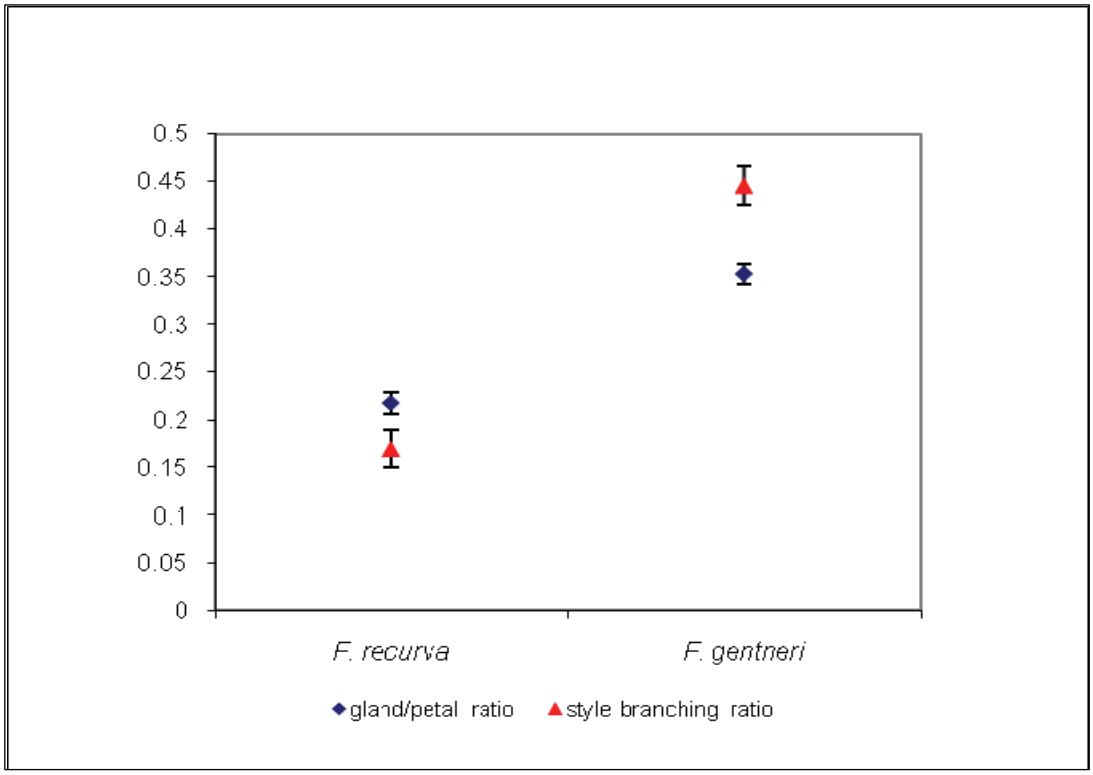

Figure 11. The mean length of the nectar gland relative to the tepal length, and the mean degree to which the style is branched (here both expressed as ratios) differ significantly between the two *Fritillaria* species. Error bars represent 95% confidence intervals; n=46 for *F. recurva*, and 92 for *F. gentneri*.

Recovery Unit evaluation. Recovery Unit groupings were easily distinguishable by DFA ($p < 0.001$). Individuals in each Recovery Unit grouped together, and groupings separated from one another. Unit 1 was separated from the southern Unit 2 along function 1, and the southern group (Unit 4 including the Brushy Creek site) separated from the others along function 2 (Figure 12). The most important traits for the first discriminant function were plant height, number of flowers and style branching percentage. Gland to tepal length ratio, gland length and tepal length (obviously correlated variables) were highly ranked in the second disriminant function (Table 4). All but one of the 92 plants analyzed were assigned to the correct Recovery Unit, validating the use of these designated units as groupings to identify morphologically similar individuals and guide management of this species.

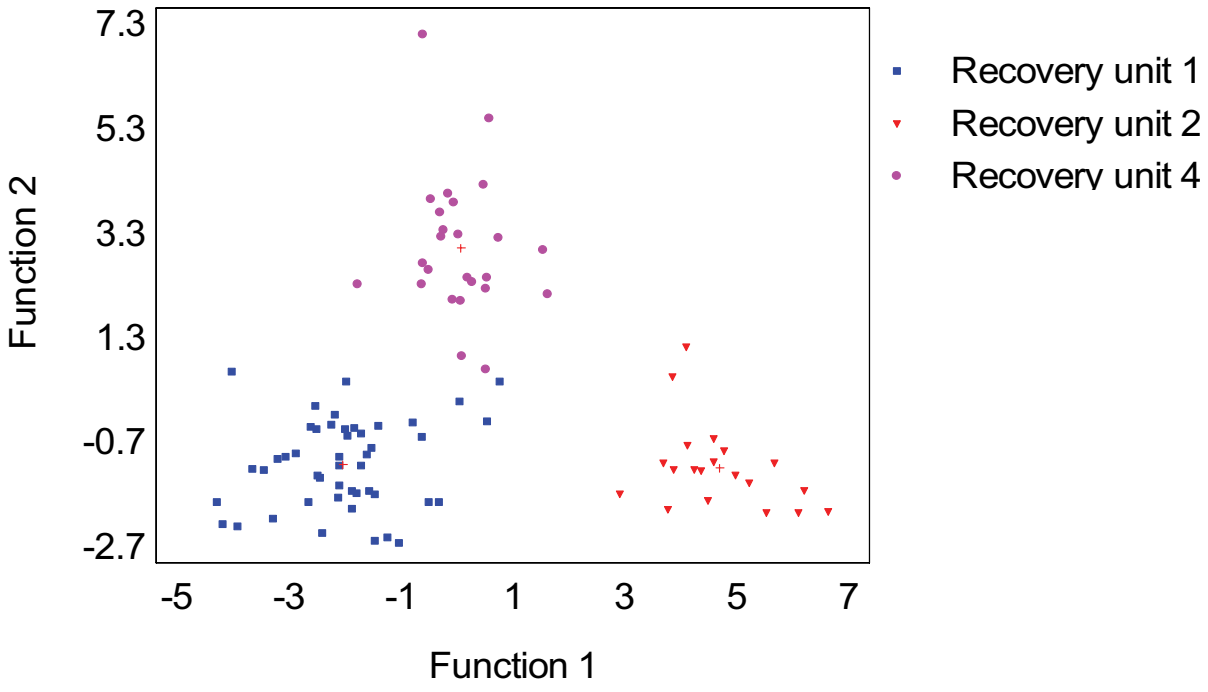

Figure 11. Discriminant function analysis of 92 samples of *F. gentneri*. Morphological data support the designation of Recovery Units as designated in the 2003 Recovery Plan. Recovery Unit 1 consists of the Applegate, Beekman Ridge, Britt Grounds, Jacksonville Dump, and Jacksonville Cemetery sites; Recovery Unit 2 consists of the Pickett Creek and Grants Pass sites, and Recovery Unit 4 consists of the Pilot Rock, Siskiyou Pass and Brushy Creek sites.

Table 4. **Discriminant function coefficients (standardized) for each morphological trait from discriminmant analyses of *F. gentneri* in three Recovery Units**. Only the first two functions are shown; large coefficients are in bold type.

Traits	Function 1	Function 2
Flower orientation	0.22416	-0.10870
Flower stalk leaf length	0.11301	0.47330
Flower stalk leaf width	-0.16805	0.62133
Gland length	0.15109	**-1.68669**
Gland to tepal length ratio	-0.47188	**1.96724**
Gland width	0.11569	-0.21350
Number of flowers	**0.91343**	-0.01990
Ovary length	-0.26536	-0.10738
Plant height	**-1.07959**	-0.51929
Style branching percentage	**0.74982**	-0.11442
Style branching form	0.030724	0.29815
Style color	-0.31184	0.11722
Style height	0.14009	0.50199
Tepal checkering color	0.01900	0.33525
Tepal color	-0.04669	-0.06172
Tepal length	-0.44319	**0.89011**
Tepal recurving	-0.24855	-0.22012
Tepal width	0.62211	0.12239
Eigenvalue	7.1	3.5
df	36	17
Chi square	290.1	121.7
p	<0.001	<0.001

Discussion

Population differentiation. In most populations of *F. gentneri* in which reproductive rates have been evaluated, the production of seed in the field is a rare event (Amsberry and Meinke 2002). Plants of this species procreate largely through asexual means, prolifically producing rice grain bulblets on the surface of mature bulbs (Amsberry and Meinke 2004). The Brushy Creek site is an exception with pod production relatively common, at least in some years. Because most plants rarely reproduce sexually, genetic mixing within populations of this species is minimal, and gene flow among populations is presumed to occur at even lower rates. Our recent studies indicate that morphologically distinct populations (or population groupings) of this species probably developed from individual hybridization events between the occasionally sympatric congeners, *F. recurva* and *F. affinis* (Amsberry et al. 2006). Viable hybrid progeny survived in sites in which they were adapted, persisting indefinitely through prolific vegetative reproduction.

Our morphological analyses demonstrate that plants within a population of *F. gentneri* are quite similar, as would be expected of very closely related individuals. The low level of within-population variation we do observe may be due to somatic mutation in some individuals subsequent to the originating hybridization event, or to occasional episodes of sexual reproduction. Additionally, populations may have developed from sibling seedlings produced by the original hybridization, producing a population of closely related, but not identical, individuals.

Differentiation among populations is more pronounced than variation among individuals within a population. Various populations exhibit unusual tepal coloring, entirely unrecurved tepals, a larger overall plant size and other unique traits. Some populations are also phenologically and ecologically distinct, exhibiting earlier bloom times and greater cold requirements for emergence than the species as a whole (Gisler and Meinke 2002). However, the ten populations we evaluated are generally similar in most traits, and this inclusive group is distinctly different from its two common congeners. Although *F. gentneri* probably developed through a series of independent hybridization events, the recognizable character combinations and persistence (through vegetative proliferation and occasional

sexual reproduction) of this species indicate that it should continue to be recognized as a taxonomic species (Grant 1981).

Recovery Unit evaluation. Recognizable taxonomic entities are deserving of conservation status - this status, for taxa that "have developed outside of confinement, (and) are self-sustaining, naturally-occurring taxonomic species" is provided by the Endangered Species Act (USFWS 1996). Although the intercross policy formulated by USFWS in 1996 to insure this protection has never formally been adopted, it has never been withdrawn. Taxa of hybrid origin such as *Helianthus paradoxus* (puzzle sunflower), a "stable and self-sustaining, biological unit" of documented hybrid origin (Riesberg et al. 1990) continues to receive protection as a Threatened species under the Act. Other land management agencies also recognize the need to conserve hybrid taxa. *Fritillaria eastwoodiae* (Eastwood's fritillary) is managed as a Sensitive Species by the Bureau of Land Management (BLM Redding Field Office 2002), and is listed as "rare, threatened or endangered in California and elsewhere" (1B) by the California Native Plant Society (CNPS 2007), despite its purported hybrid origin (Macfarlane 1978).

As well as corroborating the scientific community's recognition of *F. gentneri* as a valid taxonomic entity, our morphological work lends support to the use of the Recovery Units as designated in the Recovery Plan. Morphologically similar populations probably developed from the same hybridization event, (or possibly from more than one event involving the same parental groups). As well being similar in appearance, these population groupings presumably share similar adaptations and habitat preferences. The geographically-based delineation of these Units accurately captures the observed morphological variation, making the designated Units valuable in managing for this species.

Although the U.S. Endangered Species Act protects "distinct population segments" of vertebrate fish or wildlife, but not of *plants* (Waples 1995), the model of conserving identifiable taxonomic or 'Evolutionarily Significant' Units (ESUs), as it has been applied to fish and other vertebrates, is also applicable to rare members of the floral community. As the purpose of defining these subspecific groups of populations is to ensure that evolutionary

heritage is recognized and conserved (Moritz 1994), identification of these units (as ESUs, Recovery Units, or other designated management units) provides a framework for conservation planning for all taxa, whether animal or vegetable. As expected, Brushy Creek plants fall into Recovery Unit 4, and should be managed in conjunction with other populations within this Unit.

Differentiation of F. gentneri and F. recurva. As mentioned above, gland to tepal length ratio and style branching ratio are valid criteria for distinguishing *F. gentneri* from *F. recurva*. Although these traits vary somewhat from individual to individual, evaluation of these two characters, especially in conjunction with tepal color and the degree of tepal recurving, allows for positive identification of most specimens. The addition of five populations of *F. recurva* and one of *F. gentneri* to the current analysis provided a more robust dataset for analysis and further validated the use of these traits to distinguish these two species.

Cytology

Introduction

Cytological research can be useful in identifying naturally occurring plant hybrids, especially in those genera for which experimental crossing studies are time consuming and difficult (Motley and Carr 1998). Because abnormal meiosis due to poor chromosome pairing occurs in many hybrid taxa, observations of chromosomes during the meiotic process can provide evidence of hybridity. Cytological studies also document the number of chromosomes present in cells undergoing meiotic or mitotic division, providing evidence of polyploidy. Polyploidy can occur due to autopolyploid increases in chromosomes resulting from non-reduction of gametes during intraspecific sexual reproduction, or due to allopolyploidy resulting from hybrid crosses between taxa with different chromosome numbers (Lewis 1979). Because fritillaries take years to bloom from seed, evaluation of the progeny of crossing studies (Amsberry and Meinke 2002) has not yet been completed for artificially created hybrids of *F. recurva* and *F. affini*s (putative *F. gentneri*). Cytological studies provided an opportunity to collect additional information regarding the possibility of a hybrid

origin for this species, as well as providing insight into the causes for the varying levels of fertility observed among populations. Chromosome numbers were available in the literature for *F. affinis* and *F. recurva,* (Table 5) and we wanted to verify the chromosome number of these "parental" taxa in our study sites. We also wanted to document the chromosome number for *F. gentneri*, as this information was not available prior to our 2006 study.

Table 5. Chromosome numbers in *Fritillaria affinis* and *F. recurva*

Fritillaria affinis	
2*n* = 24+B, 36	Darlington, C. D. 1936. The external mechanisms of the chromosomes. Proc. Roy. Soc., Ser. B, Biol. Sci. 121, 823:264-319.
2*n* = 24, 24+1-8B, 36, 48	Beetle, D.E. 1944. A monograph of the North American species of *Fritillaria*. Madrono 7:133-159. La Cour, L. F. 1951. Heterochromatin and the organization of nucleoli in plants. Heredity 5, 1:37-50
n = 12, 12+f	Cave, M. S. 1970. Chromosomes of California Liliaceae. Univ. Calif. Publ. Bot. 57:1-58.
2*n* = 24	Schweizer, D. 1973. Differential staining of plant chromosomes with giemsa. Chromosoma (Berl.) 40:307-320.
n = 12+0-3B	Taylor, R. L. & S. Taylor. 1977. Chromosome numbers of vascular plants of British Columbia. Syesis 10:125-138.
n = 12	La Cour, L. F. 1978. The constitutive heterochromatin of chromosomes of *Fritillaria* sp., as revealed by giemsa banding. Phil Trans. Roy. Soc. London, Ser. B, 285:61. La Cour, L. F. 1978. Two types of constitutive heterochromatin in the chromosomes of some *Fritillaria* species. Chromosoma (Berl.) 67:67-75.
2*n* = 24, 36	Marchant, C. J. & R. M. Mcfarlane. 1980. Chromosome polymorphism in triploid populations of *Fritillaria lanceolata* Pursh (Liliaceae) in Calif. Bot. J. Linn. Soc. 81:135-154.
Fritillaria recurva	
2*n* = 24+f, 36	Darlington, C. D. 1936. The internal mechanics of the chromosomes, V. Relational coiling of chromatids at mitosis. Cytologia 7, 1-2:248-255.
2*n* = 36	Frankel, O. H. 1937. Inversions in *Fritillaria*. Jour. Genetics 34, 3:447-462.
n = 12+1f	Tischler, G. 1938. Pflanzliche Chromosomenzahlen. Tab. Biol. 16 (3):162-218.
2*n* = 24	Beetle, D.E. 1944. A monograph of the North American species of *Fritillaria*. Madrono 7:133-158.
2*n* = 24+B, 36	La Cour, L. F. 1951 Heterochromatin and the organization of nucleoli in plants. Heredity 5, 1:37-50
2*n* = 24	Beck, C. 1953. A gardener's introduction to the genus *Fritillaria*. London, Faber & Faber Ltd. 1-96.
n = 12, 12+f, 18+f [var. *coccinea*]	Cave, M. S. 1970. Chromosomes of California Liliaceae. Univ. Calif. Publ. Bot. 57:1-58.
2*n* = 24 [var. *recurva*]	Cave, M. S. 1970. Chromosomes of California Liliaceae. Univ. Calif. Publ. Bot. 57:1-58.
n = 12	La Cour, L. F. 1978. The constitutive heterochromatin of chromosomes of *Fritillaria* sp., as revealed by giemsa banding. Phil Trans. Roy. Soc. London, Ser. B, 285:61.

Methods

Using traditional methods of chromosome counting, 17 individuals of *F. gentneri*, along with four individuals of *F. affinis* and six of *F. recurva* were included in our 2006 study. Counts were completed by Dr. Gerald Carr at Oregon State University (OSU) using bud or root material, depending on availability.

In order to incorporate the Brushy Creek site into our cytology work, we collected fresh leaf tissue from five plants of *F. gentneri* and five of *F. recurva* during our 2008 visit. These specimens were transferred as quickly as possible to the OSU Seed Lab, where they were evaluated using flow cytometry by Dr. Sabry Elias. Flow cytometry provides an inexpensive and expedient way to determine ploidy levels by comparing the weight of chromosome material from the target specimens with that of controls with known ploidy levels. In addition to collections made at Brushy Creek, specimens were also collected and evaluated from four plants of *F. recurva* in the Jacksonville area, and from three plants each of *F. affinis* and *F. recurva* at a study site near Siskiyou Pass.

Results

Our 2006 chromosome counts for *F. affinis*, *F. recurva* and *F. gentneri* indicate that both diploid and triploid individuals of all three of these species occur (Table 6). Our counts of *F. recurva* and *F. affinis* corroborate previously published reports of dipoidy and triploidy in these species (Table 5), and our documentation of both ploidy levels within the species *F. gentneri* is valuable in assessing the potential fertility and origin of this taxon.

Interestingly, although most of the plants of *F. gentneri* evaluated in our 2006 study were triploid, all plants of both species collected at Brushy Creek were diploid. Additional collections of *F. affinis* and *F. recurva* from Siskiyou Pass were also diploid, as were collections of *F. recurva* made in 2008 in Jacksonville.

Table 6. Chromosome counts for three fritillary species at six study sites. 2008 collections are shown in bold type; chromosome numbers for these specimens were determined using flow cytometry.

site	*F. affinis*	*F. gentneri*	*F. recurva*
Brushy Creek		**2n=24, 2n=24, 2n=24, 2n=24, 2n=24**	**2n=24, 2n=24, 2n=24, 2n=24, 2n=24**
Jacksonville	2n=36, 2n=36	2n=36, 2n=36, 2n=36, 2n=36, 2n=36, 2n=36	**2n=24, 2n=24, 2n=24, 2n=24**
Siskiyou Pass	2n=24, 2n=24, **2n=24, 2n=24, 2n=24**	2n=36, 2n=36	2n=24, 2n=24, 2n=36, 2n=36, 2n=24, **2n=24, 2n=24, 2n=24**
Pickett Creek		2n=36, 2n=36, 2n=36, 2n=36, 2n=36, 2n=36	
Pilot Rock		2n=36, 2n=24	
Grants Pass		2n=36	

Discussion

Unlike the situation in most plant species, the sister chromatids of chromosomes in anaphase I of meiosis in *Fritillaria* are clearly visible as distinct entities held together only at the centromere. The basic karyotype of diploid species in this genus with n=12, found in North American as well as Mediterranean species, consists of two nearly metacentric, and ten nearly telocentric, chromosomes. (See Amsberry and Meinke 2007 for further cytological discussion of *Fritillaria.*)

Based on the pollen stainabilities and chromosome observations available, the populations of *F. recurva* sampled in both the 2006 and current studies consist mostly of individuals with normal or near normal meiosis. In contrast, all pollen samples of *F. gentneri* evaluated in 2006 exhibited high frequencies of abortive pollen consistent with abnormal meiosis

(Amsberry and Meinke 2007). Additionally, most individuals of this species evaluated prior to the current inclusion of the Brushy Creek site have been demonstrated to be triploid (2n = 36); these combined observations provide a potential explanation of the very low levels of capsule production observed in most sites, especially in the Jacksonville area (Knight 1991, Knapp 1999, Amsberry and Meinke 2002).

The documentation of diploidy in five randomly selected plants of *F. gentneri* at the Brushy Creek site is valuable new evidence that ploidy levels vary among populations. Because these diploid plants probably do not experience the chromosomal abnormalities associated with triploidy, functional pollen may routinely be produced by plants in this population. Diploidy and functional pollen, combined with the relatively large size of the population in this site, may contribute to the higher levels of capsule production observed at Brushy Creek, and make active conservation of this population especially important to species viability. However, as capsules are more likely to be produced from inter-specific pollinations of *F. gentneri* with pollen from *F. recurva* than from conspecific pollinations (Amsberry and Meinke 2002), the higher apparent fertility in this site could alternatively be explained as the result frequent interspecific fertilization events. Additional controlled pollination studies are needed to provide information on the fertility of the *F. gentneri* plants at the Brushy Creek site.

Molecular evaluation – a hybrid origin?

Prior to our 2006 study, a molecular study of *F. gentneri*, using a popular molecular technique known as RAPD (Random Amplification of Polymorphic DNA), was conducted at Southern Oregon University (Carey and Jessup 2004). The goal of this study was to determine the evolutionary history of *F. gentneri*, and evaluate the hypothesis that this taxon originated as a hybrid between *F. affinis* and *F. recurva*. Although the results of this study were inconclusive, the authors stated that “the results, as detailed in the report, do not support that hypothesis” (hybrid origin), and "...that *F. gentneri* is most likely a subspecific element of the *F. recurva* lineage." However, the use of RAPDs in plant systematic and population studies has come under great scrutiny by many molecular plant researchers (Penner et al. 1993, Williams et al. 1993, Ayliffe et al. 1994, Säll and Nilsson 1994, Skroch and Nienhuis

1995, Halldén et al. 1996, Novy and Vorsa 1996, Hansen et al. 1998). The drawbacks of the RAPD technique include poor reproducibility, heteroduplex formation of homologous sequences and competition between different DNA fragments for amplification. In light of these problems, we believe that RAPDs are not the best method for determining the evolutionary history of *F. gentneri* – use of this technique probably contributed to the equivocal results reported in the Carey and Jessup study.

Instead, our 2006 molecular research focused on unambiguous sequence data. We analyzed nuclear sequences and SNAPs (Superimposed Nucleotide Additivity Patterns) of leaf tissure collections of each of the three *Fritillaria* species from two populations. Nuclear sequences and SNAPs have been used by past authors to determine the hybrid origin of several plant species (Kim and Jansen 1994, Campbell et al. 1997, Fuertes-Aguilar et al. 1999, Sang and Zhang 1999, Whittall et al. 2000). Using this technique, our initial results indicate that *F. gentneri* is a hybrid of *F. affinis* and *F. recurva*. Additionally, our results suggest that separate hybridization events, leading to the speciation of local *F. gentneri* populations, have occurred on multiple occasions across the range *F. gentneri*. This research is currently ongoing, with results scheduled to be published in the near future; we hope to include our collections from Brushy Creek in the final portion of this study.

Data from our pollen viability, breeding system and cytology studies corroborate the results of the initial molecular work. Triploidy, high levels of sterile pollen and the inability to reproduce more than occasionally are all characteristics of hybrid taxa. Small populations of *F. gentneri* are probably created from seed produced by a single hybridization event, with asexual propagation of these initial seedlings eventually producing multiple flowering adults.

Although *F. gentneri* is of hybrid origin, we do not feel this should affect the species taxonomic status. The role of hybrid speciation in the creation of new plant species is a well documented phenomenon (Coyne and Orr 2004). *F. gentneri* populations persist through bulblet production, and reproduce occasionally as evidenced by our observations of capsules in the Brushy Creek site. *F. gentneri* simply represents another example of a common speciation event in the plant kingdom.

Bulblet harvest and cultivation

Several dozen *F. gentneri* plants were flagged for future bulblet collection at the Brushy Creek site during our April 2008 visit. In early August, we returned to the site and harvested approximately 550 bulblets from six flagged plants. (Many flags disappeared during the interim between our two visits, and only six bulbs were locatable.) Each "mother' bulb was carefully dug from the soil, bulblets were removed, and donor bulbs were carefully replanted (Figure 12). Bulblets were placed in plastic bags, labeled and returned to OSU in coolers to prevent desiccation.

Figure 12. These large mother bulbs were dug from the Brushy Creek site. Bulblets have been removed, and the mother bulbs are ready to be replanted. A few loose bulblets can be seen on the lower left. Photo by R. Thomas.

Figure 13. One hundred small bulblets (inset) were planted in each flat. Photos by M. Carr.

Upon arrival at OSU, collected bulblets were removed from their bags and prepared for planting in deep flats in the nursery yard. Subsequent to lining with screen to prevent soil loss, each flat was filled to a height of three cm with Perlite® to enhance drainage and prevent bulblet rotting. Finally, each flat was filled with standard potting mix (SB-40 Grower's Mix), and an array of 100 holes (each 1 cm deep) were dug in each flat (Figure 13). Bulblets were carefully placed in the holes and covered with soil, and each flat was labeled with the population of origin. Bulblets were watered thoroughly at the completion of planting, and flats were placed in the OSU nursery yard (Figure 14).

Plants were kept moist throughout the winter, and fertilized with Osmocote® 14-14-14 when leaves emerged in spring. Flats were watered daily or as needed throughout the growing

period, and treated with Sluggo® bait to prevent herbivory damage by slugs. As plants senesced in mid-summer, watering was reduced to once per week. Bulbs are currently dormant, and will emerge again in February - March of 2010.

Figure 14. Our nursery yard at OSU now contains more than 10,000 bulbs of *F. gentneri*. Photo by R. Thomas.

In 2009, ODA submitted a cost-share proposal to the BLM Redding Office to fund a cooperative outplanting project on BLM land. If this proposal is funded, the 550 bulbs collected from the Brushy Creek site will be planted on a site selected by BLM, either to augment an existing population, or create a new one as specified in the Recovery Plan.

Summary

- The Brushy Creek site supports a relatively large population of *F. gentneri*.
- Predation of flowers, presumably by insects, was very common at Brushy Creek in 2008.
- Plants in this site are morphologically similar to each other, and are readily identifiable as *F. gentneri*.
- Recovery Unit 4 is the appropriate designation for the Brushy Creek population.
- Sampled plants at Brushy Creek were diploid (rather than triploid as expected); diploidy may provide an explanation of higher levels of capsule production at this site.
- 550 bulblets harvested from donor bulbs at Brushy Creek are currently being cultivated in the OSU nursery yard for use in future outplanting projects.

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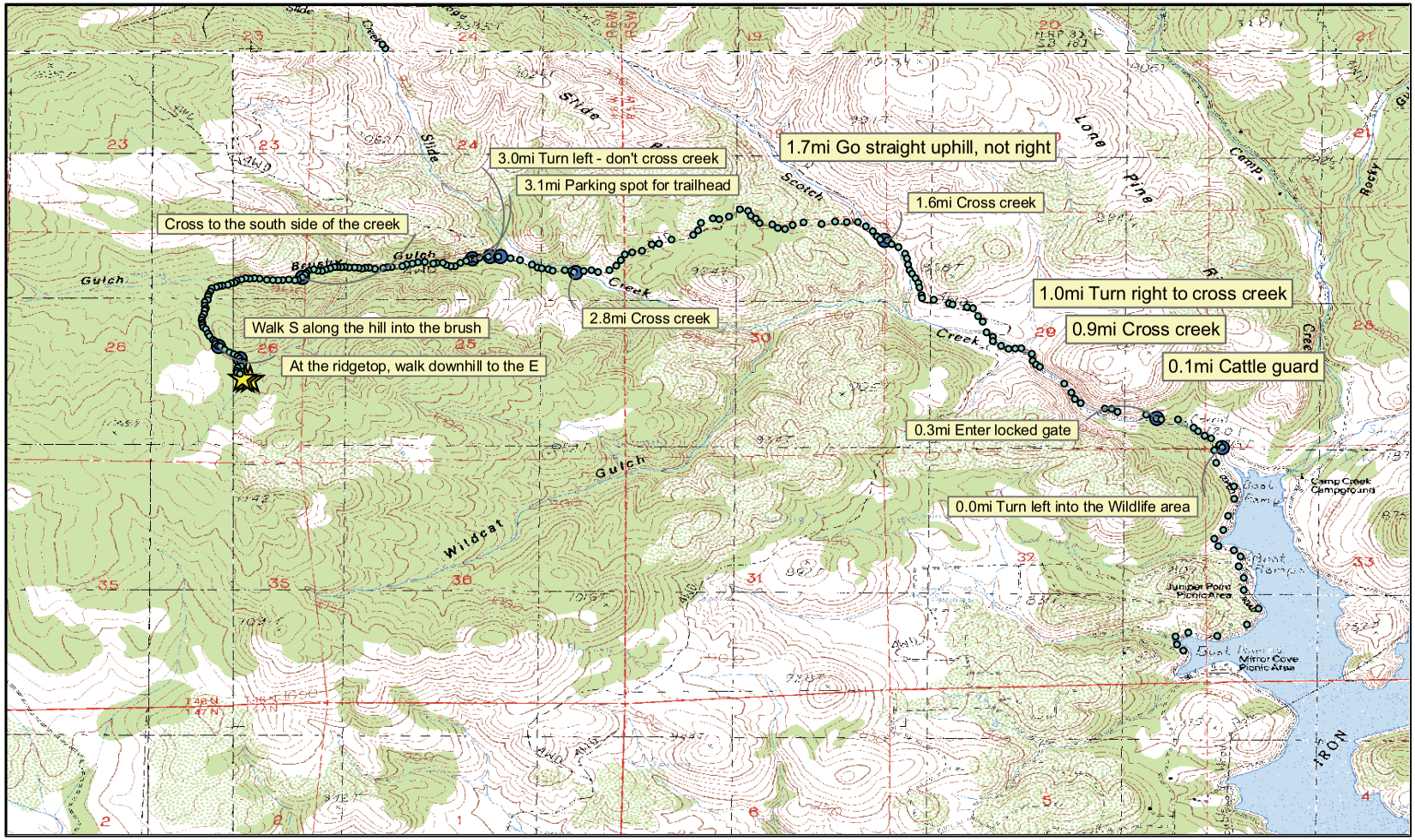
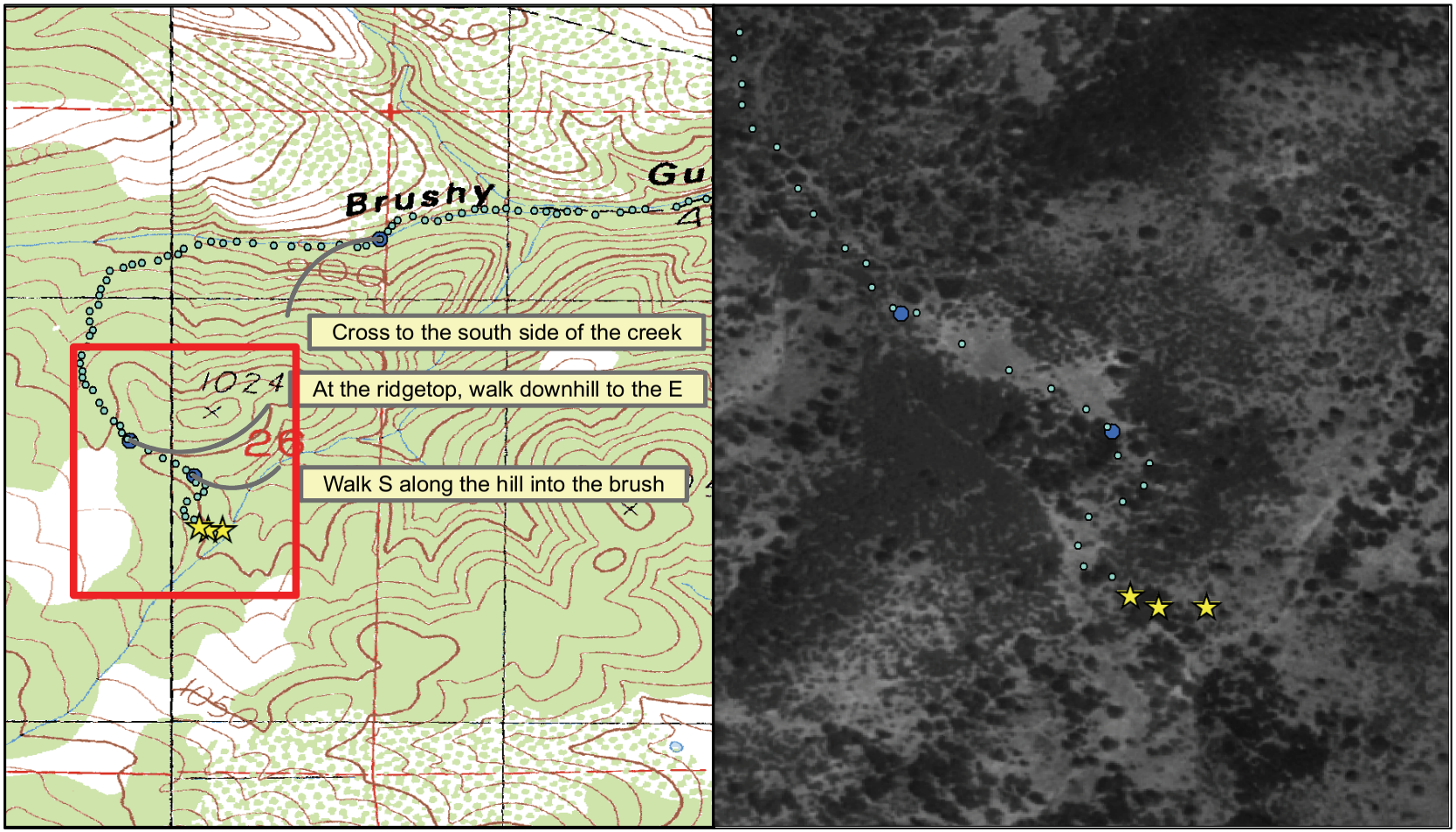
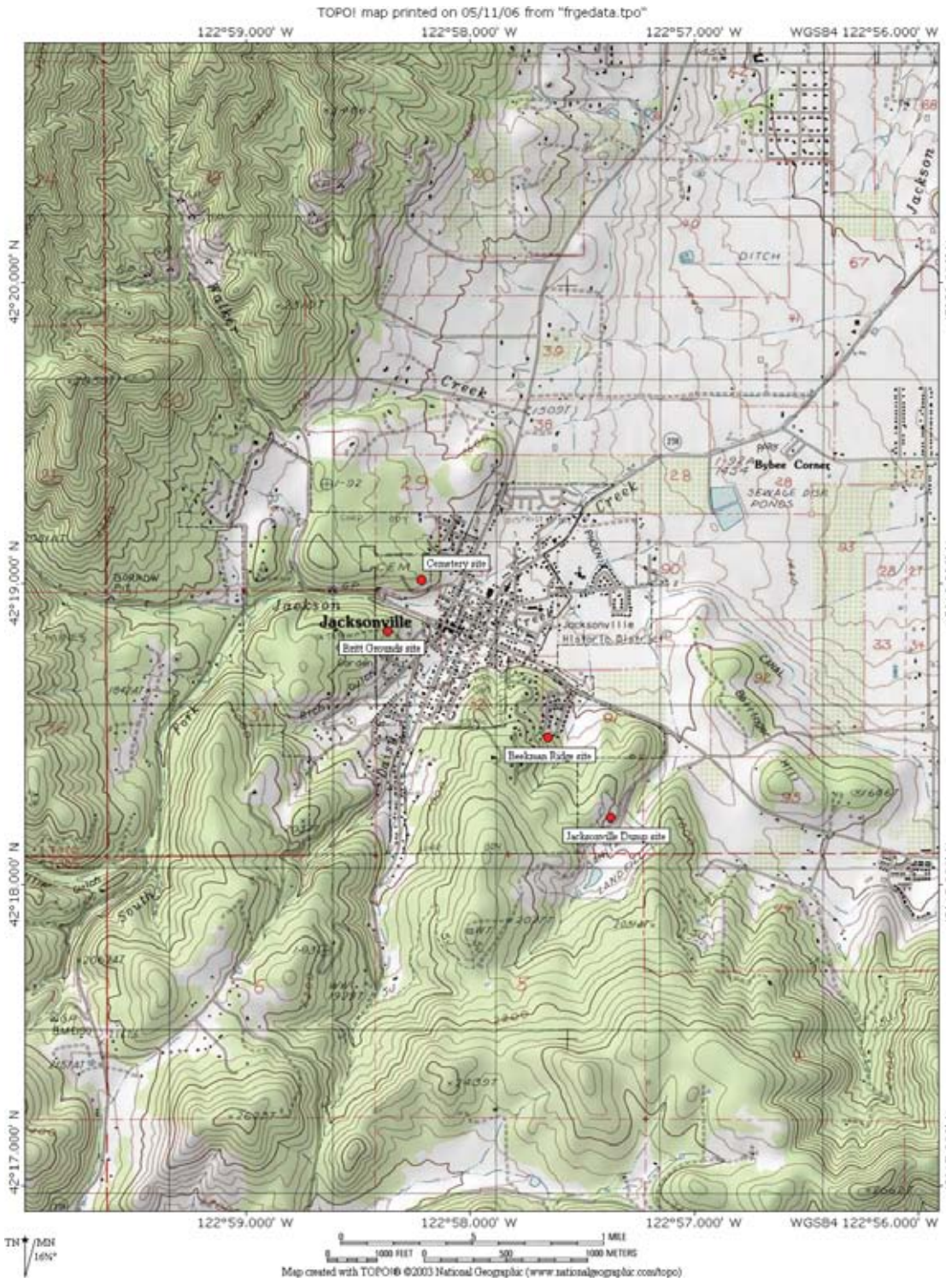
Appendices



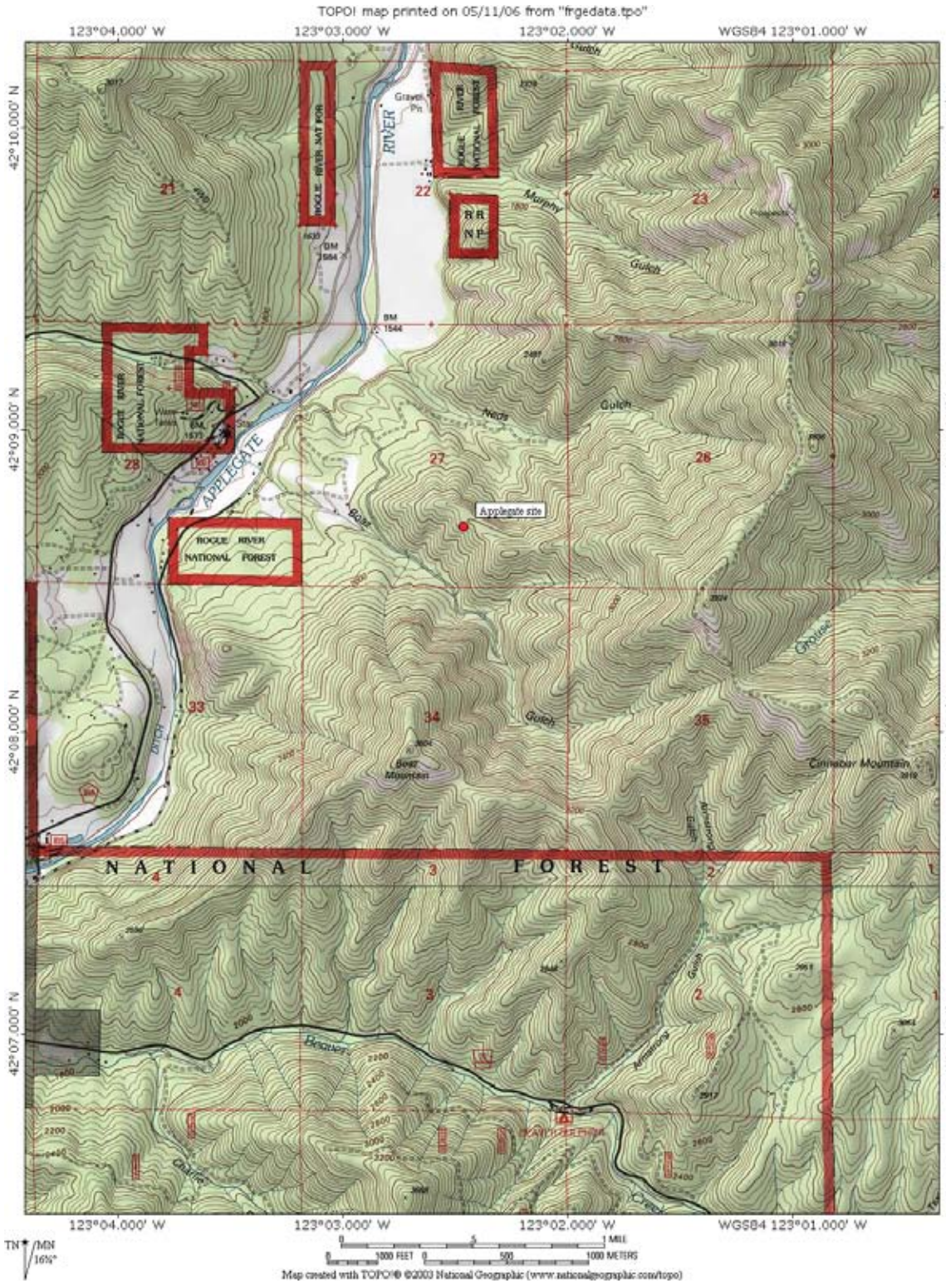
Appendix A. Location and directions to the Brushy Creek site. This site is in Recovery Unit 4.



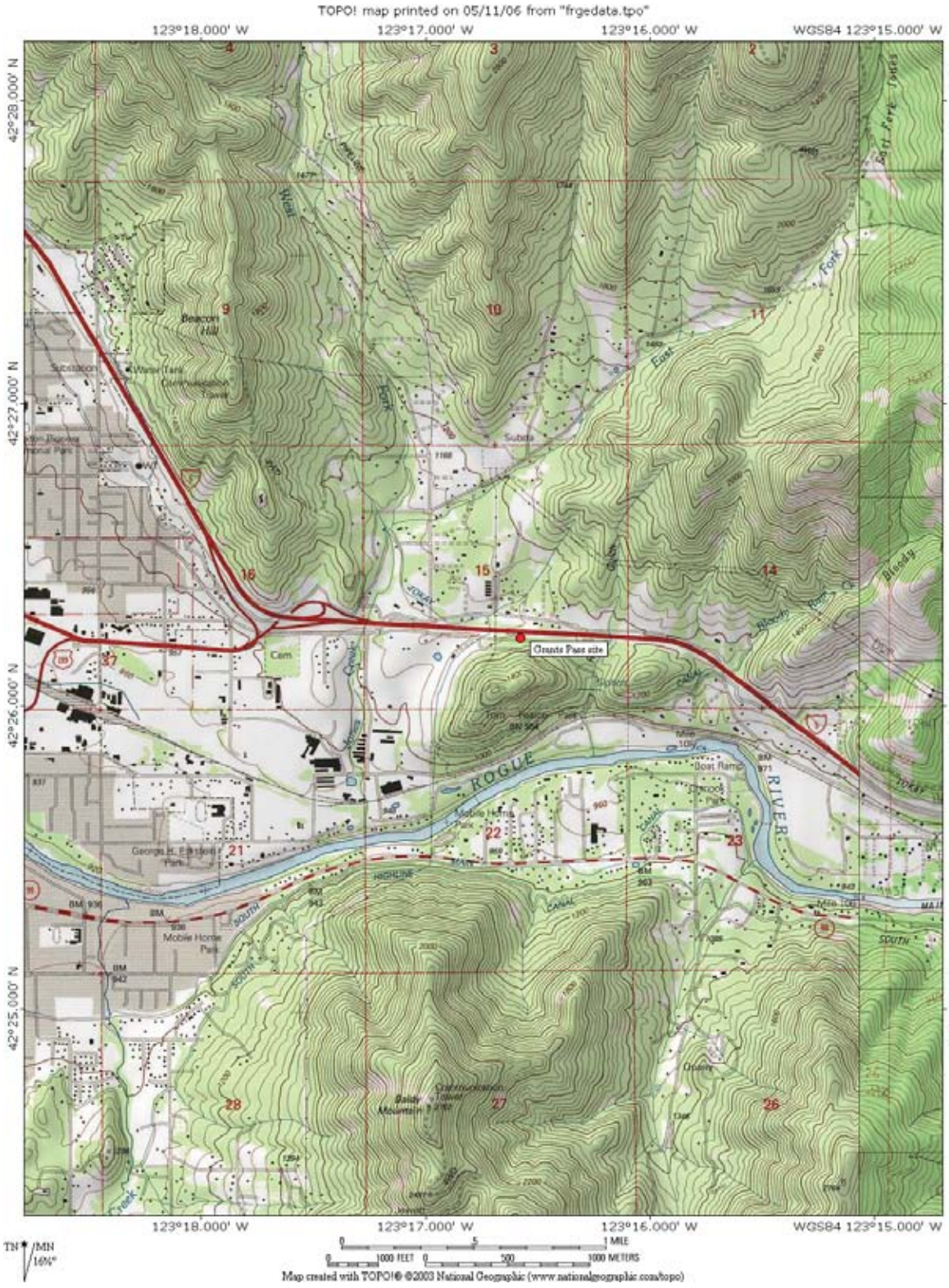
Appendix B. Closeup map and aerial photo of the Brushy Creek site.



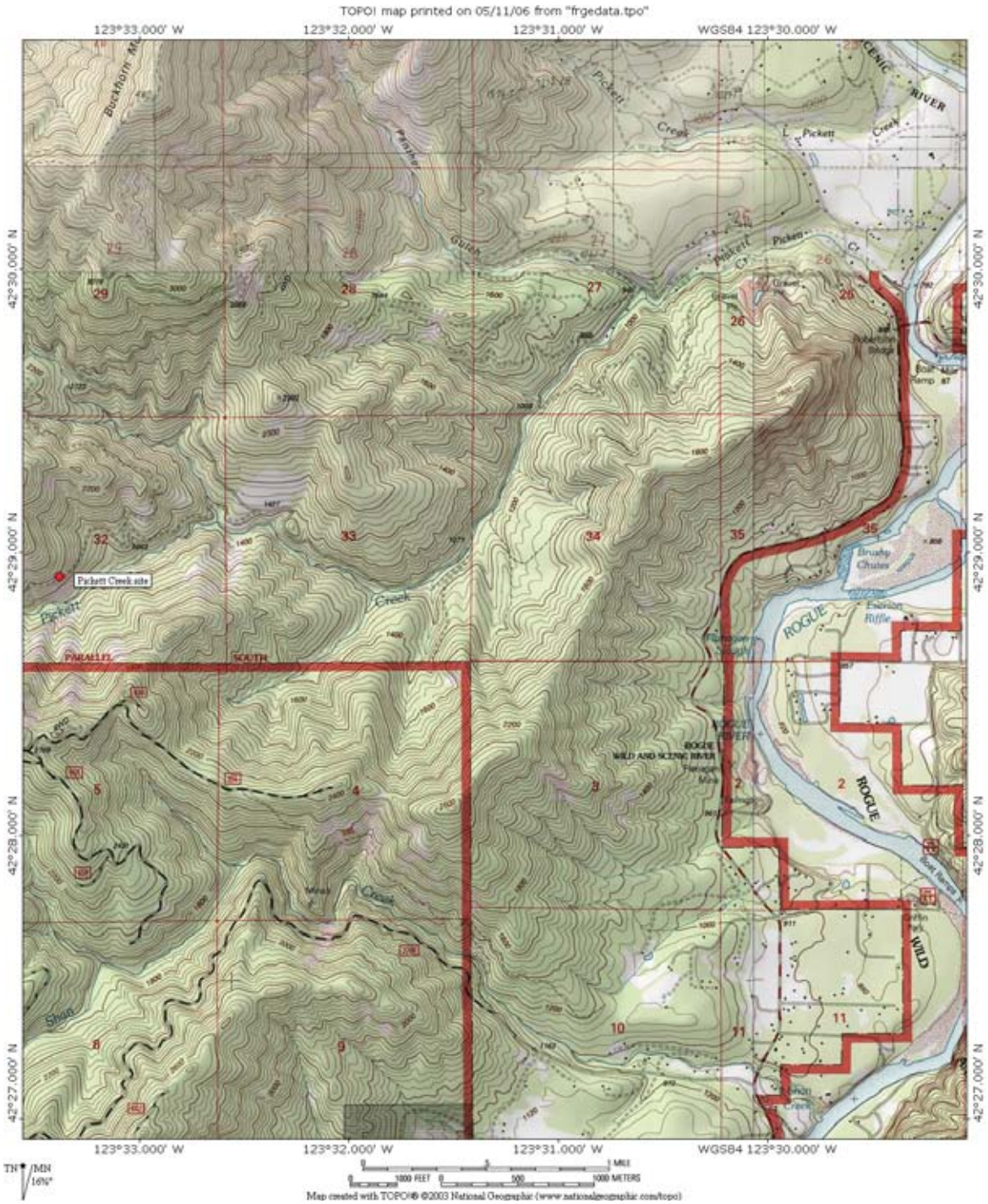
Appendix C. Location of the Jacksonville study sites in Recovery Unit 1.



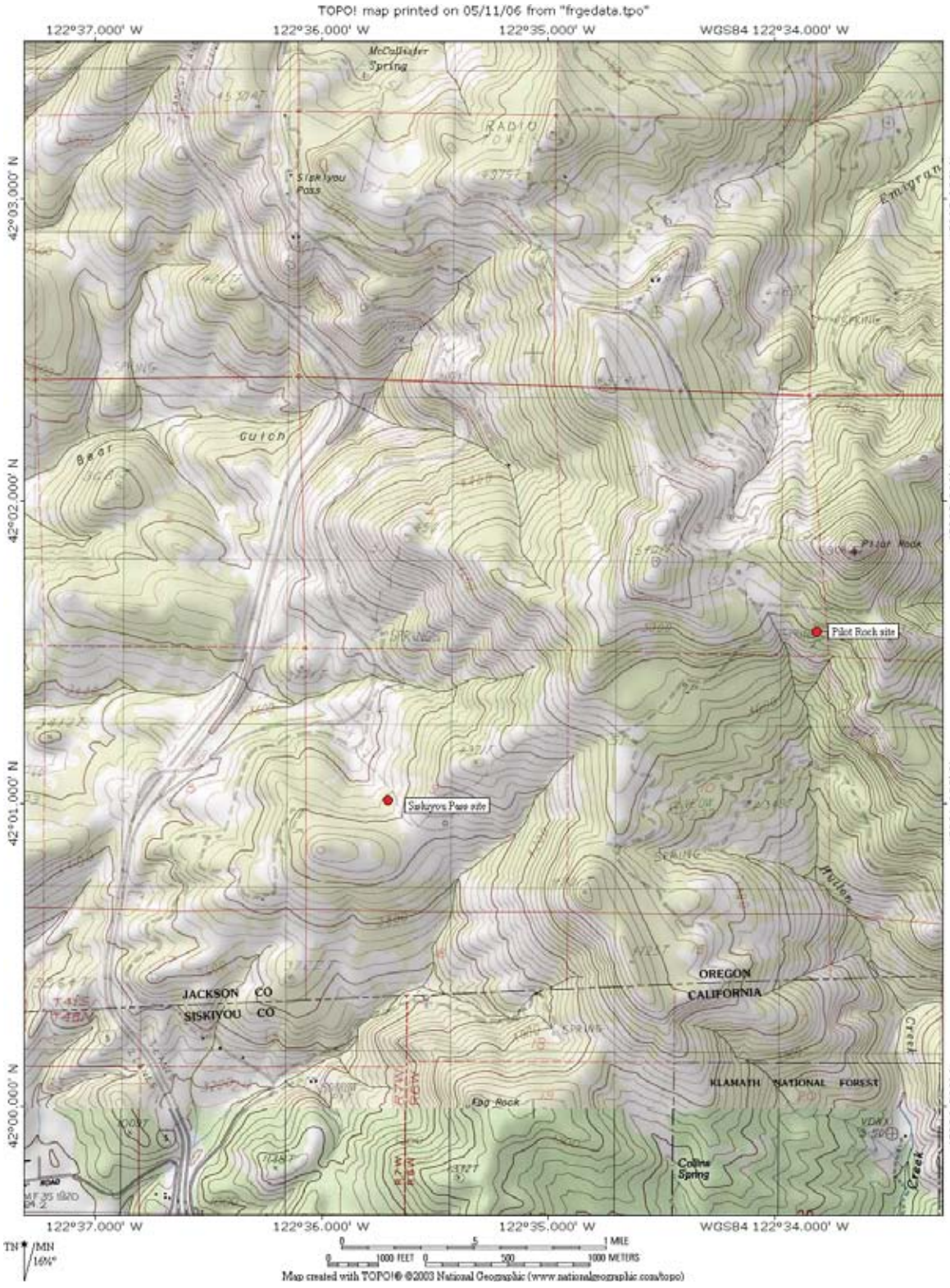
Appendix D. Location of the Applegate site in Recovery Unit 1.



Appendix E. Location of the Grants Pass site in Recovery Unit 2.



Appendix F. Location of the Pickett Creek site in Recovery Unit 2.



Appendix G. Location of the Pilot Rock and Siskiyou pass sites in Recovery Unit 4.