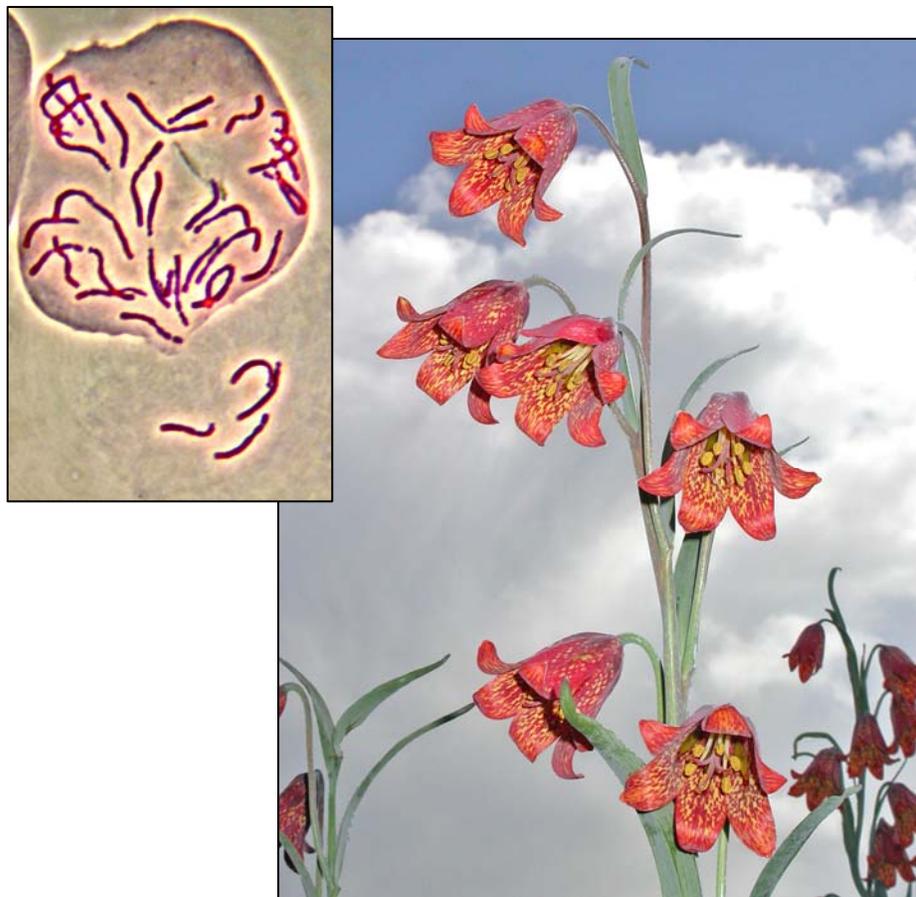


**Continuing investigations of
hybridization and fertility of *Fritillaria
gentneri* through cytological evaluations
and pollen viability analysis**



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for
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Introduction

Conservation status

Fritillaria gentneri Gilkey (Gentner's fritillary) was originally described from a collection made near Jacksonville, Oregon, in the 1940s (Gilkey 1951). Due to habitat losses associated with rapid development in southern Oregon, and competition from exotic weeds, *F. gentneri* is listed as endangered by the Oregon Department of Agriculture (ODA), and the U.S. Fish and Wildlife Service (USFWS). Gentner's fritillary is on the Oregon Natural Heritage Information Center (ORNHIC) List 1 (endangered or threatened throughout its range), and has a Natural Heritage Network Rank of G1/S1 (critically imperiled throughout its range/critically imperiled in Oregon; ORNHIC 2007). A Recovery Plan, including recommendations for the augmentation of existing populations and the creation of new ones, was issued by USFWS in 2003.

Description of species

Fritillaria gentneri is a member of the lily family (Liliaceae) with showy flowers ranging from deep red to maroon (Figure 1). Both flowering and non-flowering individuals occur in most natural populations, with vegetative individuals vastly outnumbering reproductive plants. The single, ovate, entire, basal leaves of non-reproductive *F. gentneri* are virtually identical with co-occurring *Fritillaria* species (*F. recurva* and *F. affinis*), making specific identification of vegetative plants difficult. Basal leaves of these three *Fritillaria* species vary considerably in



Figure 1. *F. gentneri*'s showy flowers make this species a popular wildflower. Photo by M. Carr.

size, from less than one centimeter in length for leaves produced by small bulblets, to 20 centimeters or more for leaves emerging from large vegetative bulbs (Gisler and Meinke 2002).

Flowering individuals produce single erect flowering stems 50 to 70 centimeters tall (and no basal leaves), with groups of narrow leaves arranged in several whorls around the stems.

Like leaves produced by vegetative bulbs, the leaves and stems of flowering plants are glaucous. Flowers vary in color and form, with red to purplish or maroon tepals that may be slightly recurved or campanulate, and are streaked, checkered or mottled with yellow.

Flowers of *F. gentneri* can be distinguished from the similar appearing *F. recurva* by the careful observation of several floral characteristics. Flowers of *F. recurva* are narrower, have more distinctly recurved tepals, shorter nectary glands, and a less deeply cleft style than *F. gentneri*, although these characters exhibit some variation within each taxon (Amsberry and Meinke 2006). Due to morphological variability within *F. gentneri*, it is necessary to examine all the aforementioned traits together to accurately identify this species (Figure 2).



Figure 2. Many morphological characters of *F. gentneri* (middle) are intermediate between those of its parent taxa, *F. affinis* (left), and *F. recurva* (right). Photo by S. Meyers.

Both flowering and mature non-flowering individuals produce large numbers of small bulblets that are loosely attached to the parent individual (Pratt and Jefferson-Brown 1997, Gisler and Meinke 2002). Each bulblet may produce a single small leaf during the growing season, or may remain dormant underground, with no leaf emerging. These asexually produced bulblets (presumably genetically identical to the parent plant) create a homogenous “bulblet bank” of juvenile individuals which ensure population persistence in the absence of sexual reproduction and seedling recruitment (Figure 3). Undisturbed populations of *F. gentneri* (such as those at the Jacksonville Cemetery) have persisted since they were originally discovered over 50 years ago, indicating that this process is successful, and is probably the typical means of reproduction for this species.

Research summary

Fritillaria gentneri has been the subject of numerous studies since its description in 1951. Populations in the Jacksonville area have been monitored since 1991 (Knight 1991a, Knapp 1999,), and more recent monitoring studies have focused on collecting demographic data from a series of plots west of Merlin (Thorpe et al. 2006).



Figure 3. Bulblets are copiously produced by most mother bulbs, and can be harvested to provide propagules for outplanting. Photo by M. Carr.

Recovery-based research

projects have investigated the potential for reintroducing and augmenting populations of *F. gentneri*. Studies evaluating the effect of bulblet harvest and developing propagation protocols (Gisler and Meinke 2002) provided the basis for ongoing bulblet collection,

nursery cultivation, and field outplanting projects (Amsberry and Meinke 2004, Amsberry and Meinke 2005a, Amsberry and Meinke 2005b).

Ecological research provides a scientific basis for making management decisions that affect rare species, and studies on *F. gentneri*'s pollinators (Donham and Ferguson 2003), habitat preferences (Brock 2001), and life history (Thorpe et al. 2006) have assisted with recovery-based conservation planning. Initial reproductive ecology studies revealed the complex breeding system of this rare species (Amsberry and Meinke 2002), and molecular research focused on determining *F. gentneri*'s origin (Guerrant 1992, Carey and Jessup 2004, Amsberry et al. 2006). Despite this growing body of research, much is still to be learned about this unusual species, and the current report provides additional information that will assist in meeting recovery goals.

Objectives

- Evaluate the interfertility of *F. recurva* and *F. affinis* by cross-pollinating these two species, and documenting the ability of cross-pollinated flowers to produce capsules.
- Complete additional pollinations of plants of *F. gentneri* with pollen from flowers of the same species from sites other than the source site of the maternal parent.
- Determine the number of potentially viable seeds within hybrid capsules, and within capsules produced by *F. gentneri* x *F. gentneri* inter-populations crosses.
- Determine the germination rates of hybrid seed, and of seed produced by *F. gentneri* x *F. gentneri* inter-population crosses.
- Determine the viability of pollen of the three fritillary species.
- Determine the chromosome number of plants of *F. gentneri*, *F. affinis*, and *F. recurva* in various field sites.

Breeding system studies

Introduction

Understanding the breeding system of *F. gentneri* assists with conservation planning in two ways. Knowledge about the potential of the species to produce high quality seed for propagation and outplanting projects is critical to developing efficient and cost effective protocols for augmentation and reintroduction. Additionally, information regarding the fertility of this species provides insights into its origin, and aids in focused conservation planning.

Field pollination studies

Field studies in 2006-2007 focused on interspecific pollinations between *F. recurva* and *F. affinis* (the two presumed parental taxa of *F. gentneri*). As previous studies indicated that *F. gentneri* originated as a hybrid of *F. recurva* and *F. affinis* (Amsberry and Meinke 2002, Amsberry et al. 2006), we were interested in determining the fertility of this cross in field sites where the two species occur sympatrically. Flowers of *F. recurva* were with pollinated with pollen from *F. affinis* at both field sites, and the reverse cross (flowers of *F. affinis* pollinated with pollen from *F. recurva*) was also completed on multiple flowers at each site. In both cases, pollen from plants within the same field site was used for all pollinations.

Field pollination studies were conducted in two sites (Jacksonville and Siskiyou Pass) that support large populations of both *F. recurva* and *F. affinis* (Figure 4; see Appendices for specific site locations). The Jacksonville site is made up of two adjacent areas (Jacksonville Cemetery and the Britt Grounds) that we previously considered as two independent sites. These two areas are less than ½ mile from each other, and though currently separated by a road, were probably contiguous in the recent past. Both areas are within the same Recovery Unit, and a morphometric study completed by ODA in 2006 demonstrated that *F. gentneri* plants in these two sites are morphologically similar to each other (Amsberry and Meinke 2006).

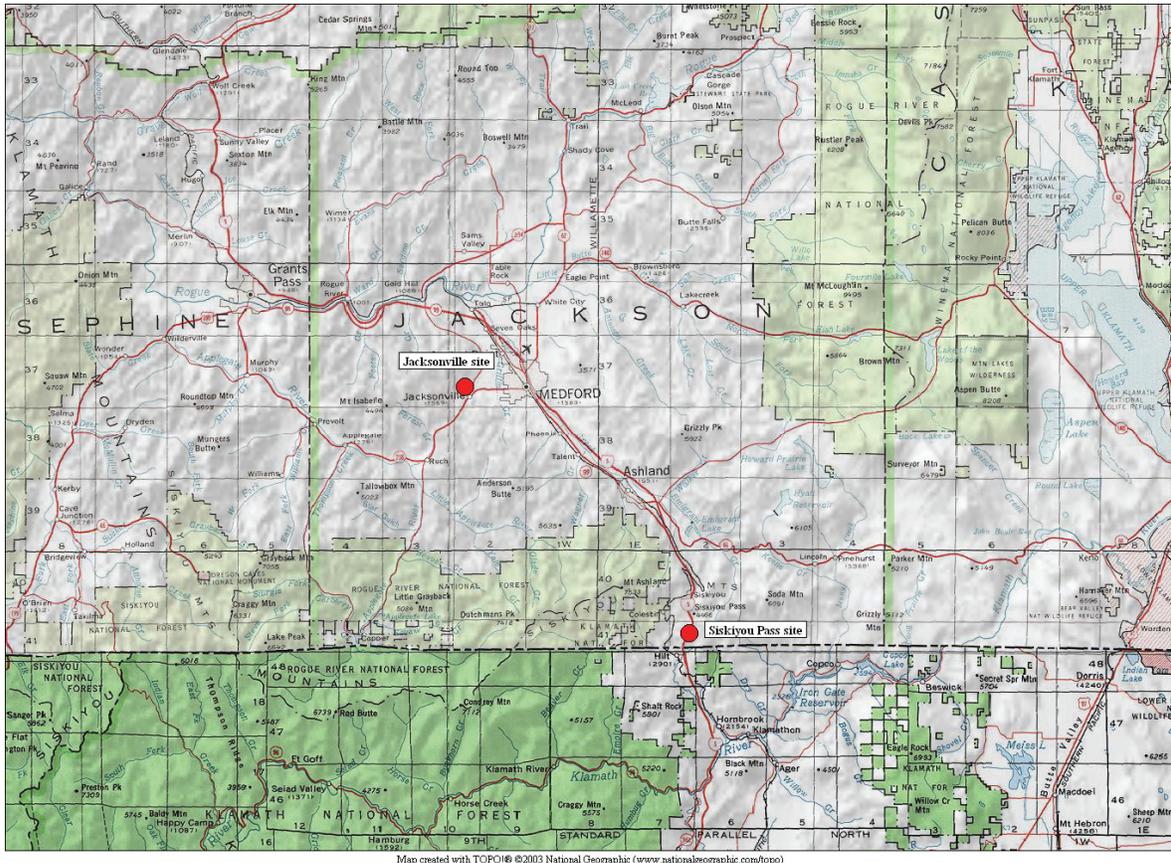


Figure 4. The Jacksonville and Siskiyou Pass study sites were used for field pollination work in 2006. Both sites have naturally occurring populations of all three fritillary species.

The Britt Grounds are located adjacent to the Britt Music Festival concert arena, on land owned by the City of Jacksonville and the Bureau of Land Management, and managed by the Jacksonville Woodlands Association (JWA). This site is partially shaded, with *F. recurva* (and *F. gentneri*) occurring in widely spaced groups under a canopy comprised of *Quercus garryana*, *Pinus ponderosa*, and *Arbutus menzesii*, as well as in open, grassy areas, where it is associated with *Festuca californica* and poison oak (*Toxicodendron diversilobum*). A few plants of *F. affinis* also occur here, but we selected plants for this study from the larger, more densely distributed population of this species located within the Jacksonville Cemetery.

The Jacksonville Cemetery (owned by the City of Jacksonville) is the site of a large and vigorous population of *F. affinis*, as well as one of the largest known populations of *F. gentneri*. The *F. affinis* plants chosen for this study occur along the east perimeter of the cemetery, in a grassy area interspersed with *Arbutus menzesii*, *Rubus discolor*, and *Toxicodendron diversilobum*. Much of the understory vegetation is weedy, and the site is mowed regularly. The elevation at the Jacksonville site is approximately 505 meters, and it is located in Recovery Unit 1.

The Siskiyou Pass site is at a higher elevation than most other *Fritillaria* sites (1126 meters), with the *F. affinis* population is located in an open meadow within stands of *Quercus garryana* var. *brewerii*. Plants occur in shallow soils that appear wet in spring, but dry thoroughly by midsummer. Associated species include *Ceanothus cuneatus* and *Calochortus greenii*. *Fritillaria recurva* plants occur southwest of the *F. affinis* population, in loamy soil under a canopy of *Quercus garryana*. Associated species include *Corallorhiza striata*, *Hydrophyllus capitata* and *Erythronium hendersonii*.

After emergence, but prior to full maturation, all study plants in both sites were enclosed in wire mesh cages to prevent damage from deer. A large, voracious population of blacktail deer in the Jacksonville area browse flowers and maturing capsules of all *Fritillaria* species, requiring the caging of study plants in wire mesh cages secured to rebar to prevent herbivory. *Fritillaria* plants in the Siskiyou Pass site are also subject to herbivory (although not at the levels seen in Jacksonville), so study plants in this site were also caged.

On April 24 -26, 2006, 62 flowers on 27 caged plants of *F. affinis* and *F. recurva* were pollinated at the Jacksonville site. Plants in the Siskiyou Pass site were pollinated later (May 3-5), as the higher elevation of this site results in later phenology. Seventy-two flowers on 31 plants were pollinated in this site. To prevent natural pollination prior to treatment, flowers were bagged in bud, then emasculated and rebagged before anthers dehiscid. Pollination was accomplished when flowers were completely open, and stigmas appeared receptive (Figure 5). Pollen was transferred by collecting entire flowers, storing them in egg

cartons to prevent damage, and using the pollen as soon after collection as possible (within an hour). Pollen was placed on the stigma by holding, with tweezers, an anther containing dehiscent pollen and brushing it over the stigma. Once pollination was complete, plants were carefully re-bagged, and bags secured to flower stems that were held upright with bamboo garden stakes. As capsules ripened (mid- July) bags were collected and returned to Oregon State University (OSU) for evaluation.

Pollination studies on cultivated plants

Reproductive studies beginning in 2000 documented very low

capsule production in response to intraspecific pollinations of *F. gentneri* plants, both in the field and in the nursery yard (Amsberry and Meinke 2002, Amsberry and Meinke 2005b). Our 2000 study in Jacksonville involved pollinating flowers of *F. gentneri* with pollen from other *F. gentneri* plants defined as near neighbor (plant closest to study plant), far neighbor (plant more than 50 meters away from study plant), or from “other population”.



Figure 5. All study plants were caged prior to the initiation of pollination studies, and plants were bagged before and after treatment. Photo by R. Meinke.

The “other population” used in this study is within ½ mile of the study plants and consists of plants morphologically similar to study plants. The two populations were probably contiguous prior to the construction of Highway 238. Subsequent years’ studies produced similar results, and by the end of 2005 we had pollinated 465 flowers of *F. gentneri* with conspecific pollen, with only three capsules formed. However, although conspecific pollinations continued to be sterile, backcrosses of *F. gentneri* with either parent yielded capsules (Figure 6).

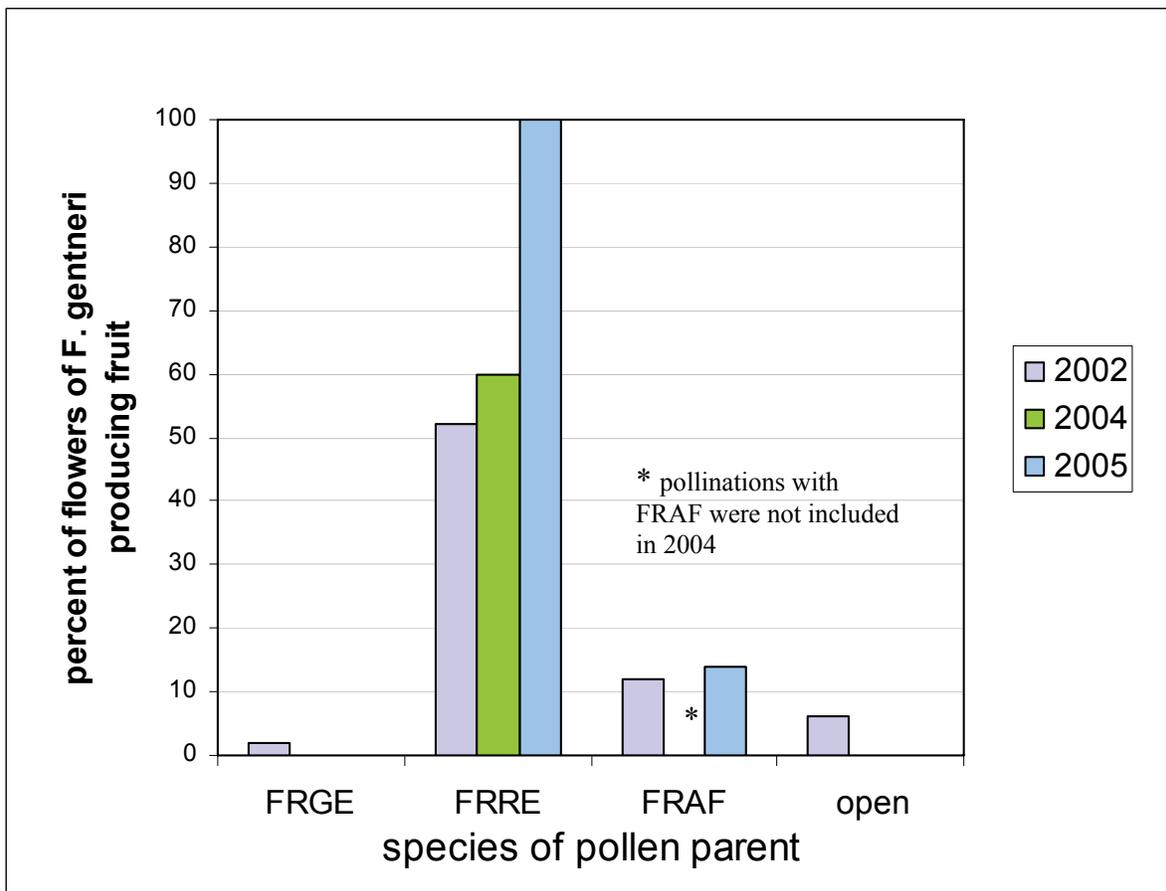


Figure 6. Prior to 2006, study plants of *F. gentneri* produced fruits best when pollinated with pollen from either parent, and only reproduced extremely rarely when pollinated with conspecific pollen. FRGE = *F. gentneri*, FRRE = *F. recurva*, FRAF = *F. affinis*, open pollinated plants were not bagged or treated.

Beginning in 2005, in addition to reproductive studies in our seven field sites, we initiated pollination work on cultivated plants in the nursery yard. Cultivated plants were collected as bulbs or “rice grain” bulblets beginning in 1999 from Jacksonville, Pickett Creek (near Merlin in Recovery Unit 2) and Pilot Rock (southeast of Ashland in Recovery Unit 4). Plants were quite vigorous by 2005, producing up to twelve flowers per plant. Working with these cultivated plants allowed for greater control of both environmental variables and the timing of pollination treatments (Amsberry and Meinke 2005b), and resulted in the production of 14 capsules from backcrossed pollinations. Eleven *F. gentneri* x *F. gentneri* pollinations (including two inter-population crosses) did not produce fruit. Cumulatively, these results suggested that *F. gentneri* is largely sterile.



Figure 7. Environmental variables that can affect fruit set were easily controlled in the nursery yard, and use of cultivated plants also allowed for more precise timing of pollination treatments. Photo by M. Carr.

However, before concluding unequivocally that *F. gentneri* is a sterile, hybrid entity unable to reproduce, we elected in 2006 to perform a series of conspecific, but inter-population, crosses on our cultivated plants in the nursery yard. As in 2005 studies, the ease of visiting the on-site nursery location for our 2006 pollination treatments was conducive to making repeated observations to evaluate stigma receptivity and the initiation of anthesis, and promoted completion of pollination treatments on an optimally timed schedule. Flowers on plants to be used as female parents were emasculated when mature (to avoid damaging stigmas in bud), but prior to anthesis. Flowers were emasculated at the end of March, with pollination treatments completed throughout the first week of April. These dates are slightly earlier than the average mid-April date for flower maturity in the naturally occurring Jacksonville populations, and considerably earlier than the late-April dates required for pollination of plants at the higher elevation Siskiyou Pass site. Flowers to be used as pollen donors were moved to an enclosed greenhouse prior to anthesis to prevent any opportunities for inadvertent pollen transfer. As fritillaries do not occur naturally in the Corvallis area, removal of pollen donors from the study area negated the need for bagging flowers, eliminating the potential for a “bag effect” on pollination treatment results. The ability to control post-treatment environmental conditions resulted in plants maturing intact in their pots, allowing for easy evaluation of capsule production.

As capsules ripened (in late June in the nursery yard and at the Jacksonville site, and in late July at Siskiyou Pass), they were collected, and opened carefully. Seeds were removed, sorted and counted. Large, filled seeds with obvious embryos were designated as “good”, thinner paler seeds were designated as “wispy”, and very thin, embryo-less bits of tissue were designated as “very wispy” (Figure 8). Only counts of good seeds were included in analyses, and germination trials were conducted exclusively with “good” seeds. The majority of capsules from most pollination treatments contained all three types of seeds, although several contained only those designated as “wispy”.

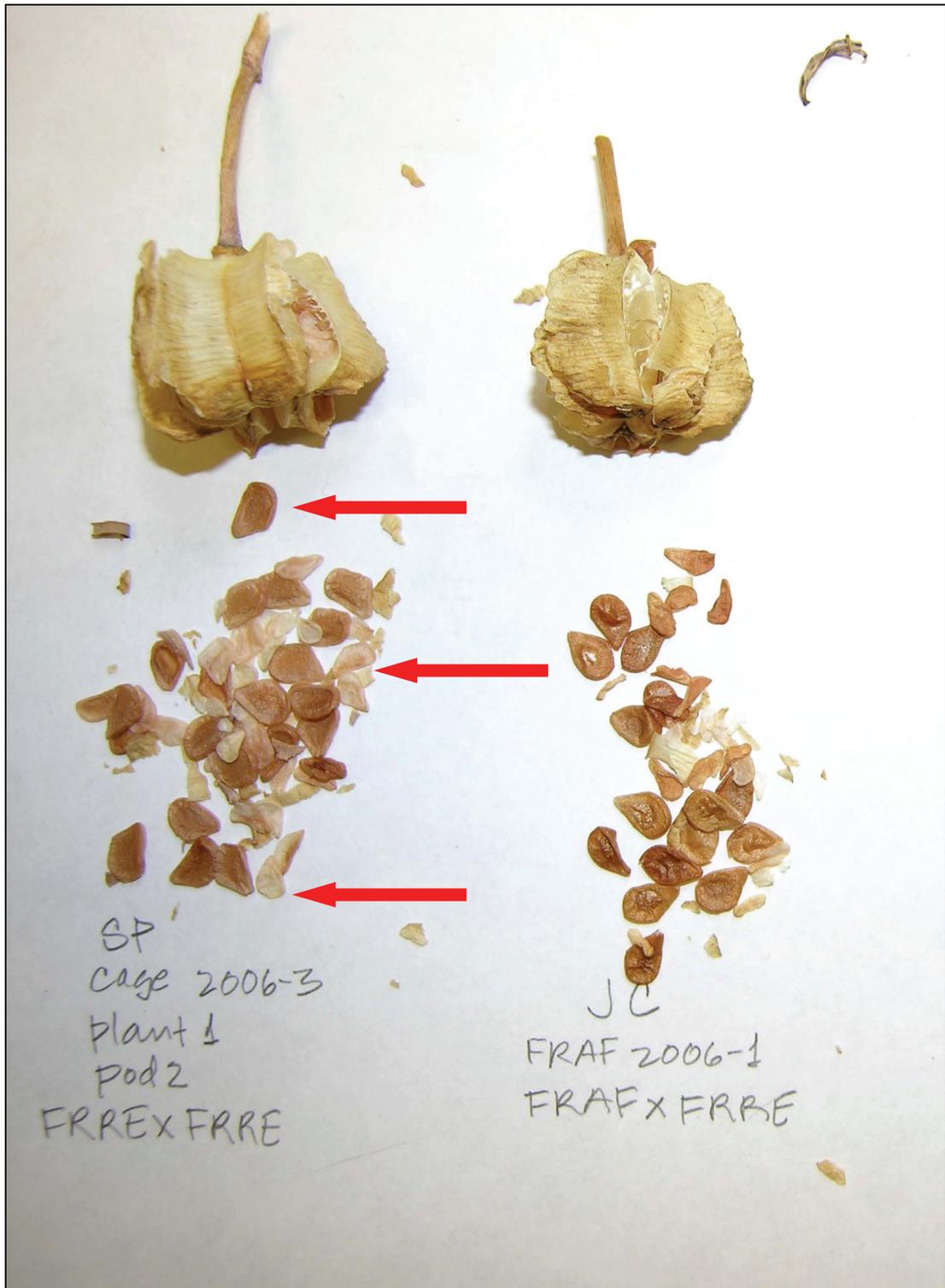


Figure 8. Capsules produced by most pollination treatments contained all three types of seeds; good (top arrow), wispy (middle arrow), and very wispy (bottom arrow). Photo by K. Amsberry.

Results

Surprisingly, in this year of our studies (2006), conspecific pollination of *F. gentneri* flowers was the most successful treatment, with 48.9% of these matings resulting in capsule production (Figure 9). Ninety-one percent of the conspecific pollination treatments used pollen from plants collected at sites other than the source site for the female parent; none of the few within-population crosses we completed resulted in capsule formation. As in previous years, backcrosses of *F. gentneri* with pollen from either putative parent resulted in capsules, and both interspecific pollination treatments ($\text{♀}F. recurva \times \text{♂}F. affinis$ and $\text{♀}F. affinis \times \text{♂}F. recurva$) produced fruits. Conspecific pollinations of *F. recurva* flowers produced fruit at a low level – pollen for these crosses was collected from plants within the same population (at Siskiyou Pass) as plants used as maternal parents.

Most capsules from most pollination treatments contained all three types of seeds. However, 95% of the capsules produced by $\text{♀}F. recurva \times \text{♂}F. affinis$ crosses contained only “wispy” and “very wispy” seeds; only 9 good seeds were produced by this treatment. A mean of 19.2 “good” seeds per pod was produced by all capsules combined; means of 35.5 “wispy” seeds, and 19.4 “very wispy” seeds per pod were produced by all capsules. Pollination treatment significantly affected the number of “good” seeds produced ($p < 0.05$ from ANOVA in Excel), with the most “good” seeds produced by the $\text{♀}F. affinis \times \text{♂}F. recurva$ cross (Figure 10).

Discussion

Field pollination studies. The first step in our attempt to artificially re-create *F. gentneri* by cross-pollinating the parent species was successful. Capsules were produced by pollinations of *F. recurva* with *F. affinis*, as well as *F. affinis* by *F. recurva*. As populations of all three species of *Fritillaria* occur in both sites where these pollination treatments were completed, the ability to produce fruit through cross-pollination lends credence to the hypothesis that *F. gentneri* could have originated independently in a series of sites as a hybrid between these two species. And, not only were capsules produced by hybrid pollinations, these capsules contained what appeared to be “good” viable seeds, in fairly large numbers. However, because flowers of *F. gentneri* are needed to confidently identify this species, the final

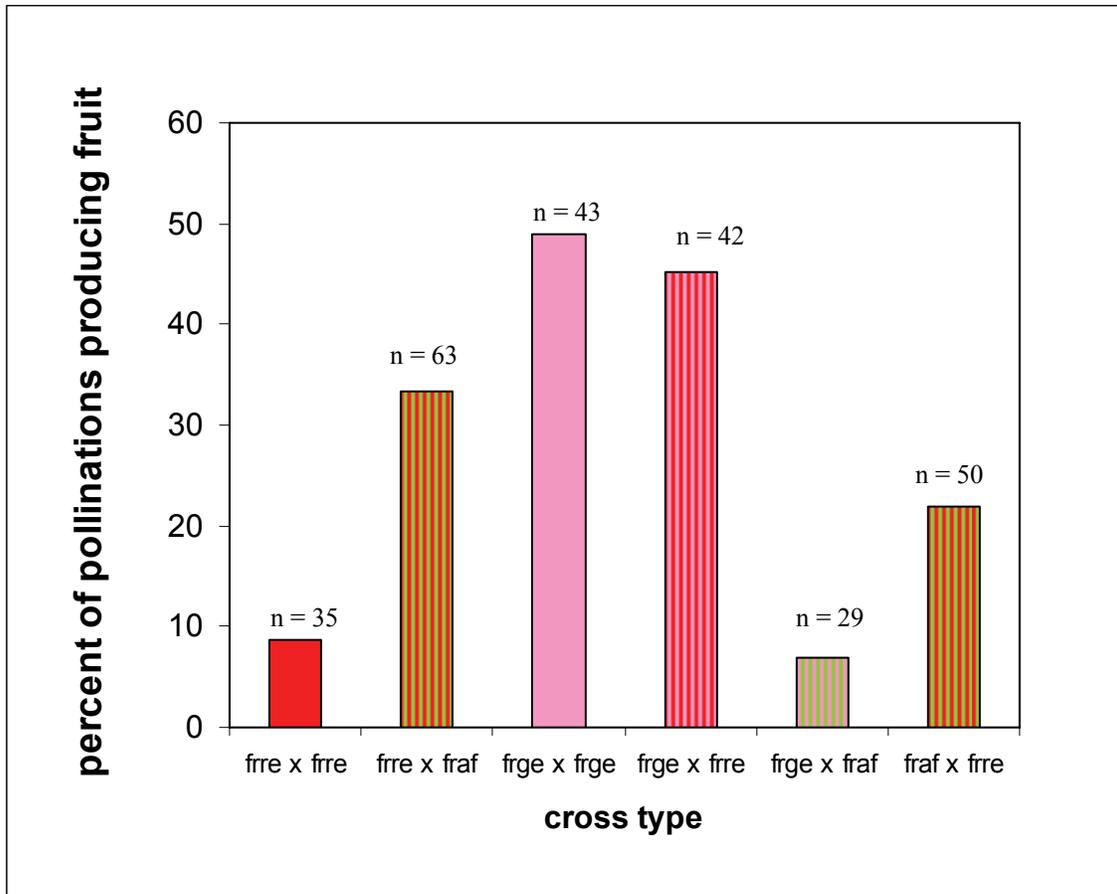


Figure 9. In 2006, a higher percentage of conspecific pollinations of *F. gentneri* produced pods than of any other pollination treatment. frre = *F. recurva*, fraf = *F. affinis*, frge = *F. gentneri*; the female parent in each mating is listed first.

evaluation of this study will be years in the future, when (and if) these seeds germinate, develop into mature plants, and eventually flower.

Hybridization, with subsequent vegetative reproduction, has been suggested as a mechanism for the origin not only of *F. gentneri*, but also of *Fritillaria eastwoodiae*, another North American species. The putative parents, of *F. eastwoodiae*, *F. recurva* and *F. micrantha*, are inter-fertile in controlled pollination studies, producing seed from both ♀*F. recurva* x ♂*F. micrantha* and ♀*F. micrantha* x ♂*F. recurva* crosses (Macfarlane 1978).

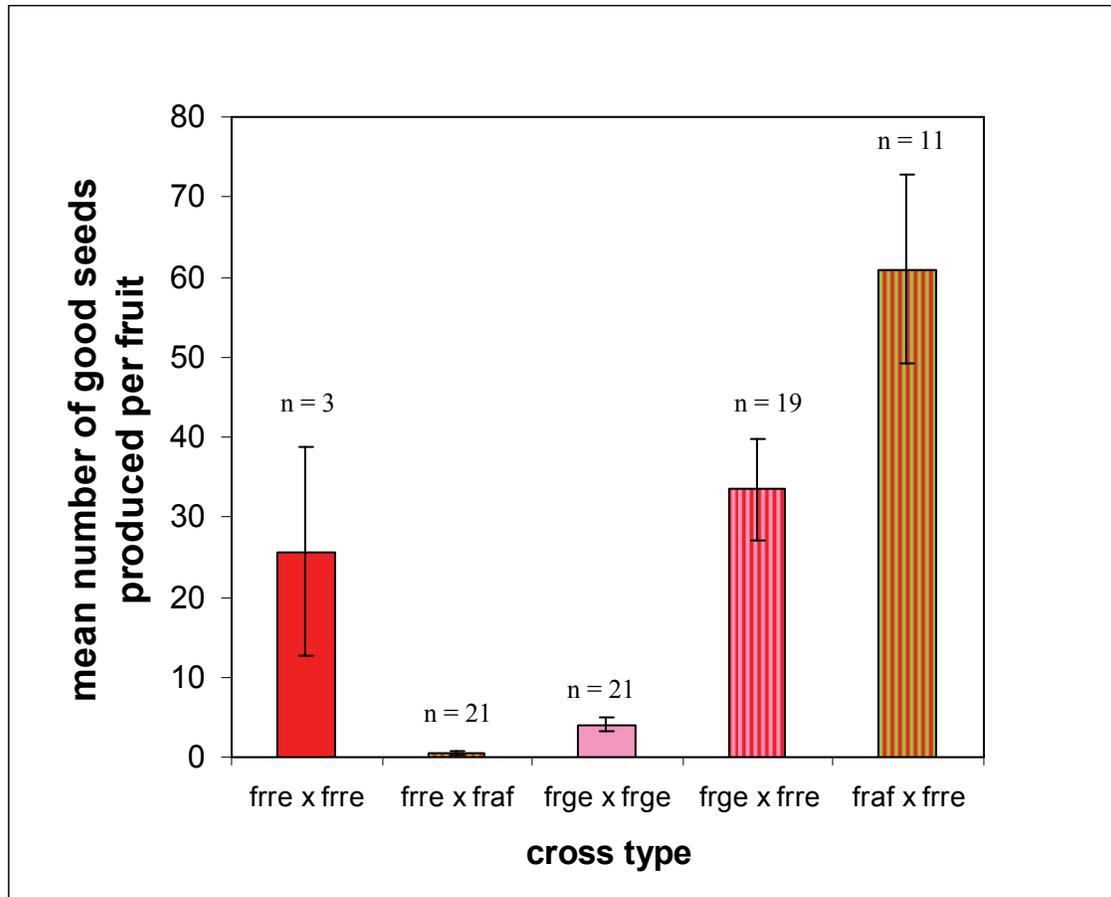


Figure 10. Fruits produced from most pollination treatments contained at least some good seeds. frre = *F. recurva*, fraf = *F. affinis*, frge = *F. gentneri*; the female parent in each mating is listed first. Error bars represent one SE above and below means.

Although considered intermediate in form between the two purported parents, populations of *F. eastwoodiae* exhibit a high degree of morphological variation, both within and among populations, with flowers reported as ranging from “greenish yellow or greenish orange to speckled red-yellow or red-purple in color, with sometimes flaring perianth parts” (BLM - Redding Field Office 2002). These morphological permutations probably occurred in independently created, sexually sterile, triploid individuals formed from unreduced gametes, which have subsequently persisted as vegetatively propagated clones. These clones, in combination with sympatric, apparently fertile diploid individuals, constitute the taxon *F. eastwoodiae* (MacFarlane 1978).

In addition to the two fritillaries, other species created by the process of recent diploid hybridization with subsequent persistence and dispersal of the new entity by clonal propagation, have been taxonomically recognized. *Opuntia kevinensis* (Kelvin cholla) consists of a series of largely sterile, clonally reproducing hybrid groups that have developed in the Gila River drainage in Arizona. Some of these groups (often called microspecies) are completely sterile, but due to later generation segregation, others produce a few fertile seeds. This combination of sexual and asexual processes is evidently a successful way of producing, and then multiplying, new adaptive types (Grant and Grant 1971), allowing the process of evolution to proceed.

Hybrid populations that possess recognizable character combinations, and which have spread by vegetative means throughout a definite geographic area, have attained the status of taxonomic species, even in the presence of limited sexual fertility (Grant 1981). These 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). *Helianthus paradoxus* (puzzle sunflower), a “stable and self-sustaining, biological unit” of documented hybrid origin (Riesberg et al. 1990) is listed as Threatened by the USFWS. Other land management agencies also recognize the need to conserve hybrid taxa. *Fritillaria eastwoodiae* is managed as a Sensitive Species by the US Forest Service (USDA Forest Service 2007), and was previously listed as “rare, threatened or endangered in California and elsewhere” (1B) by the California Native Plant Society (CNPS 2007). (The status of this species in California has recently been changed to List 3 – “need more information” – but is still considered “fairly endangered in California” and worthy of conservation concern.) Documentation of a recent hybrid origin for *F. gentneri* does not affect its taxonomic or conservation status. In fact, further research aimed at elucidating the origin of this rare species will help us better understand its unique biology and ecology, and provide information important to the development and implementation of appropriate conservation strategies.

Studies on cultivated plants. Self incompatibility systems in flowering plants prevent inbreeding and promote outcrossing, helping to retain intraspecific genetic variability (Darwin 1876, Ascher and Peloquin 1968, Dorken and Husband 1999). These systems operate by preventing the normal functioning of pollen tubes in styles of matched incompatibility genotype. The incompatibility reaction, which occurs after pollen tubes have penetrated into the style in species possessing gametophytically determined incompatibility, is specific, depending on identity of alleles in pollen and style. This system evolved in many angiosperms to insure cross-fertilization, and the lily family contains many self-incompatible species (Darwin 1876, Asher and Peloquin 1968). Pre-zygotic incompatibility is well studied in the horticulturally important species *Lilium longiflorum* and *Lilium martagon* (Ascher and Peloquin 1968, Dickinson et al. 1982), and has also been documented in the North American forest lily *Clintonia borealis* (Dorken and Husband 1999). In this study, mean fruit set was 0.22 for self-pollinated *C. borealis* flowers, and 0.54 for outcrossed flowers - this difference was largely attributed to the presence of a pre-zygotic self-incompatibility in this species. Although little information on compatibility in our northwestern species of *Fritillaria* is available, self-incompatibility is suspected for *F. recurva* (Jane McGary, personal communication), and has been documented in populations of *Fritillaria camtschaticensis* in Japan (Shimizu et al. 1998).

The results of our 2006 study indicate that *F. gentneri* may be self-incompatible, rather than completely sterile. *Fritillaria gentneri* is most likely of hybrid origin (See Molecular summary section below), and probably developed from multiple hybridization events across the range of the species. Morphology of individuals varies much more among populations than within them (Amsberry and Meinke 2006), indicating that plants within each population are probably closely related, and may have developed from a single hybridization, followed by perpetuation and proliferation by asexual reproduction through the production of rice grain bulblets. Because all individuals within a population are probably closely related, they may all contain the same incompatibility genotype - these matched incompatibility genotypes eliminate fertility of pollinations between individuals within a population. However, when pollen from plants collected from other populations was used, fruit set increased greatly, with

capsules readily produced by these matings. Each population may contain a different incompatibility allele, allowing the pollen tube of grains from one population to grow normally on the style of flowers from another population. Further investigation into this phenomenon will provide information on the extent to which interpopulation fertility occurs, and allow for evaluation of the suitability of this type of seed production for creating stock for outplanting projects. Production of seed by congeneric matings of *F. gentneri* individuals (even when these matings succeed infrequently) substantiates the status of this taxon as a “good” species, deserving of conservation efforts.

Seed germination

Introduction

In order to truly evaluate fertility, not only must capsules be collected and seeds counted, but the viability of the seed produced must also be tested. Germinability must be assayed in order to evaluate the ability of a species to reproduce, and to determine the ultimate results of pollination treatments. Early germination tests on seeds of *F. gentneri* at the Berry Botanic Garden in Portland, Oregon were not successful, and all seeds evaluated had “no or rudimentary embryos” (E.O. Guerrant Jr., Berry Botanic Garden, personal communication).

However, a successful germination protocol was developed during the course of several previous ODA studies. In these studies, we tested various stratification techniques and soil types, and determined that a “natural conditions” outdoor regime resulted in the best emergence of *Fritillaria* seeds (Amsberry and Meinke 2005). Purchased *F. recurva* seeds used in a pilot germination project completed in 2005 through 2006 germinated poorly, but results from this study provided additional insights into the development of the successful protocol described below. A few of the seedlings that germinated during this pilot project in 2006 re-emerged in 2007, providing the opportunity to document season to season development of fritillary plants (Figure 11). Seeds of our study species, like most lilies, produce one leaf each growing season. Photosynthate stored during growth allows for

development of an increasingly larger bulb each year, with emergent leaf size also increasing annually until reproductive maturity is reached (Shimizu et al. 1998).

Methods

In the 2006-2007 study, seeds were collected as fruits ripened in 2006, and stored in pollination bags at ambient temperature in our lab.

Capsules were collected from field sites on July 28 and 29, and from plants in the nursery yard on June 19-26.

Seeds were sorted in the lab, and only those determined to be “good” (described in Breeding system studies section above) were used for germination tests. On October 9, 2006, selected

“good” seeds from each pollination group were planted in rows in deep

nursery flats (15 3/4” x 15 3/4” x 5” deep, available from Anderson Die and Manufacturing, Portland, Oregon) filled with standard potting mix (bagged Professional Growers Mix SB40). Seeds were covered with a thin layer of sieved potting mix, watered well, and placed in the nursery yard. Flats were watered as needed throughout the winter, and seedlings began to emerge in early March 2006. Seedling emergence was monitored bi-weekly throughout the growing season; seedling emergence continued until early April. Each emerging seedling consisted of one grass-like leaf, which increased slowly in size until early May, and began to



Figure 11. This *F. recurva* plant is beginning to senesce after its second growing season. Outdoor conditions in field sites or the nursery yard are required for seeds to germinate. Photo by K. Amsberry.

senesce by late May (Figure 12). Flats were watered as needed throughout the growing season, and were fertilized each month with liquid fertilizer (Miracle-Gro®).



Figure 12. During the first growing season, fritillary seeds produce grass-like shoots. Seedlings emerged in the nursery yard in March and senesced in May. Rows of pure *F. recurva* seeds (right seedling row) were interspersed with seeds from our pollination treatments as controls. Photo by M. Carr.

Results

Despite their large, viable seeming appearance, seeds produced by the more prolific of the two interspecific crosses ($\text{♀}F. \textit{affinis}$ x $\text{♂}F. \textit{recurva}$) germinated poorly (Figure 13). The reverse cross ($\text{♀}F. \textit{recurva}$ x $\text{♂}F. \textit{affinis}$) produced very few viable appearing seeds, but these seeds germinated at a better rate. Seed from $\text{♀}F. \textit{gentneri}$ x $\text{♂}F. \textit{recurva}$ backcross pollinations germinated poorly, and despite poor capsule production and fairly low seed production by conspecific pollinations of *F. recurva*, the “pure” seed produced by this

treatment germinated very well. Again surprisingly, “pure” *F. gentneri* seed produced by inter-population population treatments also germinated well.

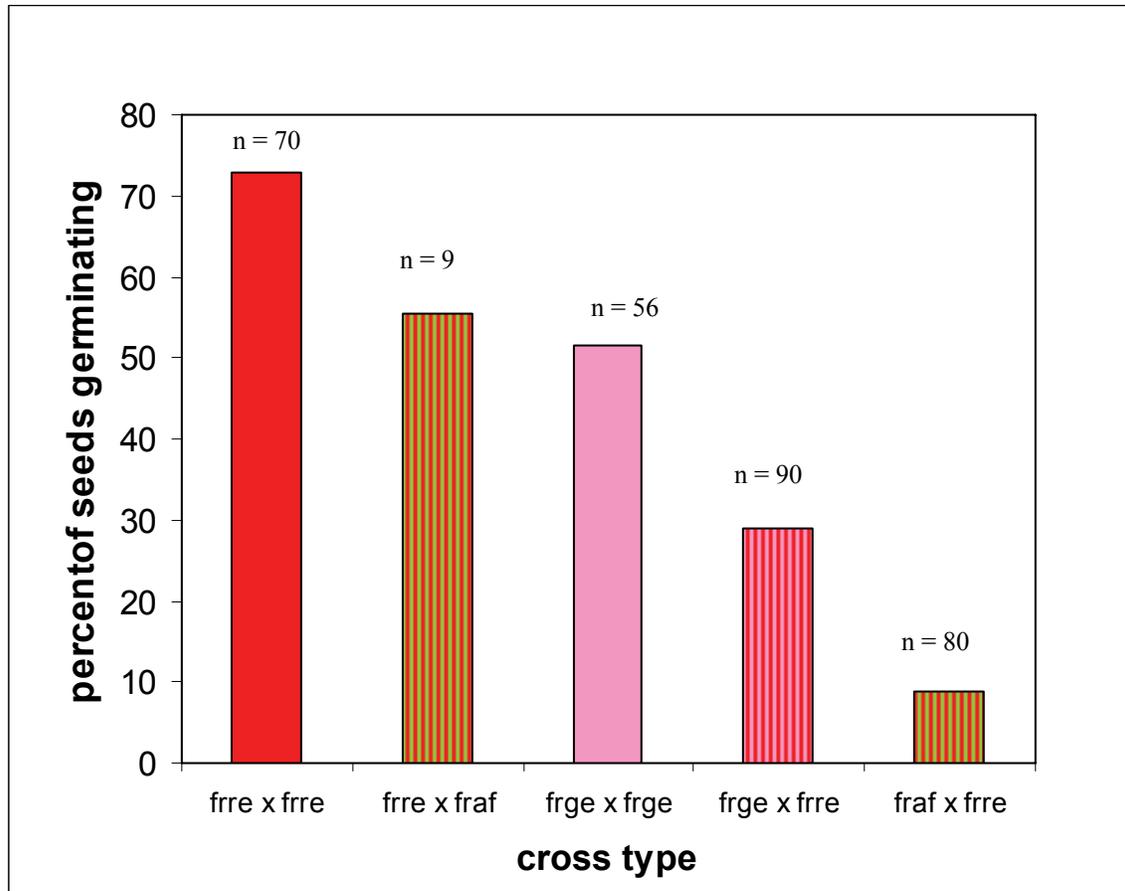


Figure 13. Although only nine seeds were produced (in 21 capsules) by ♀ frre x ♂frac matings, these seeds germinated well. The reverse mating (♀frac x ♂frre) produced many seeds, but these germinated poorly. Surprisingly, pure *F. gentneri* seed germinated well. frre = *F. recurva*, fraf = *F. affinis*, frge = *F. gentneri*; the female parent in each mating is listed first.

Discussion

Although overall production and germination of *F. recurva* x *F. affinis* hybrid seed was poor, with only 12 seedlings emerging, our study demonstrates that hybrid seed is viable, at least to the seedling stage. The existence of viable hybrid progeny, even in small numbers, supports our belief that interspecific hybrids of the purported parents of *F. gentneri* could have

developed *in situ*. Hybrids between these two species have previously been reported, although these hybrids were considered separate from the taxon *F. gentneri* (Cave 1970, Guerrant 1992). Unfortunately, specific information about regarding the origin and identity of these hybrids is not available. Specimens at the Jepson herbarium that were labeled “*F. recurva* x *F. lanceolata*” when used for by Cave for cytological work (1970) have been more recently annotated to a series of taxa and presumed hybrids, and documentation of a hybrid origin of specimens discussed by Guerrant (1992) could not be verified.

Further cultivation and monitoring of our plants of documented hybrid parentage will be necessary to evaluate their ultimate viability. And, as identification of *F. gentneri* depends on observation of floral characters, the extent to which these hybrids resemble “true” *F. gentneri* cannot be evaluated until progeny reach sexual maturity. Unfortunately, most lilies, including fritillaries, are notoriously slow to reach a blooming stage when grown from seed, making this evaluation a long-term project (Pratt and Jefferson-Brown 1997). Although cytology and molecular methods can provide additional information that is useful in evaluating the potential for a hybrid origin of existing taxa (see following sections), we plan to continue to cultivate our hybrid plants and document their growth, and ultimately observe their floral morphology.

The high germination rates of “pure” *F. gentneri* seeds indicate that this taxon is not sterile. Lack of within-population fertility may be due to a self-incompatibility mechanism occurring in closely related plants – see Breeding system studies section above. However, further evaluation of these plants as they mature and develop flowers will be needed to ultimately determine the fertility levels of this species. Data from our cytology and pollen viability work (presented below) are also relevant to the sporadic fertility observed – as has often been the situation with this cryptic species, newly discovered information provides more questions than answers, and the origin and reproductive system of *F. gentneri* remains poorly understood.

Our production of healthy seedlings from *F. gentneri* x *F. gentneri* crosses has practical value as well. Seed collection provides a potential alternative to bulblet harvest as a method for collecting propagules to be used for the creation of new populations. Seed collection requires less effort than bulblet harvest, and may impact donor populations to a lesser degree than the digging associated with bulblet extraction. Additionally, sexually produced progeny are more diverse, making them attractive for increasing the diversity of created populations. Planting seed introduces a large number of genotypes into a new population, an especially important consideration when creating populations in new environments (Guerrant 1996).

Introduction of genetically diverse plants grown from seed from inter-population crosses into populations that are currently sterile could also help increase sexual reproduction in these sites. Lack of variation in self-incompatibility genes has been demonstrated to contribute to poor reproduction and decline in rare plant populations (De Mauro 1993, Weekley et al. 2002), and could be contributing to the sterility we have observed in *F. gentneri*.

Introduction of compatible genotypes could result in an increase in sexual reproduction in these populations.

However, caution should be used when suggesting the incorporation of transplants from different Recovery Units into newly created or extant populations. In general, transplants into sites which currently support currently populations of rare species should represent, if possible, the genetic makeup of the existing population, or of geographically adjacent and ecologically similar populations (Reinartz 1995, Guerrant 1996). Plants in locally adapted populations may contain ecological adaptations specific to the sites where they occur. Creating new populations from this adapted stock increases the probability of success. Mixing stock from distant populations with local area plants should also be avoided. In addition to diluting local adaptations, this practice may promote outbreeding depression through the disruption of co-evolved gene complexes; both processes potentially contribute to population decline subsequent to transplant installation (Reinartz 1995, Van Andel 1998). Further study of the nature of the sterility observed in most populations of *F. gentneri*, as well as evaluation of the potential negative effects of genotype mixing, should be completed

prior to attempts to increase reproduction through the amalgamation of transplants from various Recovery Units.

Pollen viability analysis

Introduction

All three species of fritillaries in our studies occasionally exhibit female infertility, with cultivated plants as well as those in natural populations periodically developing one or more flowers that lack stigmas. This phenomenon has been documented by others in natural populations of *F. gentneri* (Knight 1991b), and also occurs in *Fritillaria camtschaticensis* (Shimizu et al. 1998). In the latter species, male flowers occurred more commonly than hermaphrodites, and male-only plants produced more bulblets than plants with cosexual flowers. The cause of this type of sterility, and the extent to which it occurs have not yet been studied. However, despite a high level of female sterility, both populations of *F. camtschaticensis* in this study consistently produced thousands of seeds per plot each year.

Male sterility can also contribute to low levels of seed production. Lack of pollen, or poor quality pollen can prevent adequate fertilization of available ovules, and reduce seed set even in populations of plants with adequate numbers of hermaphrodite flowers. Pollen germination and pollen staining document the amount of viable pollen produced by a species or population, and help elucidate the causes of observed low reproduction.

Method

Pollen germination. A pilot study evaluating several potential growing media for pollen germination demonstrated that a liquid modified Dickinson's medium (1.0g KNO₃, 0.1g H₃BO₃, 0.3g Ca(NO₃)₂, 20g sucrose in 1L distilled water, adjusted to pH 5.5) provided the best medium for *Fritillaria* pollen growth (Figure 14). Plated pollen was incubated for 12-24 hours prior to counting, and plates were scored by counting the number of pollen tubes that exhibited a "good response." A "good response" was defined as pollen tube growth many times longer than the pollen grain (Figure 15). Numbers of grains which exhibited a minimal

response (tube elongated slightly, but only 1-2 times as long as the pollen grain), and no response (no tube growth, with grains often shriveled in appearance) were also recorded. Pollen was collected from plants in the nursery yard, and from the Pilot Rock site (southeast of Ashland in Recovery Unit 4) and the Siskiyou Pass site.



Figure 14. Fritillary pollen germinated well on modified Dickinson's medium in ambient conditions in the lab. Photo by S. Meyers.

Pollen staining. Pollen viability can also be evaluated by staining. Mature pollen was collected from 11 *F. affinis* individuals from two sites, seven plants of *F. gentneri* from three sites, and nine individuals of *F. recurva* from two sites. Pollen was collected directly from plants currently occupying the Siskiyou Pass site (Figure 4) and from cultivated plants in the nursery yard that were previously collected from the Jacksonville and Pickett Creek sites as components of earlier research studies. The entire above-ground portions of plants from field sites were collected, placed in water-filled containers, and returned to OSU as quickly as possible. Pollen viability was evaluated by examining pollen grain size and stain reaction in cotton blue-lactophenol solution (Radford et al. 1974, Kim and Carr 1990, Motley and Carr

1998). Pollen grains were stained for a minimum of 24 hours prior to assessment, and at least 300 grains per individual were observed using a phase contrast microscope. Pollen grains that stained blue were considered viable, and stainability was calculated by dividing the number of apparently viable pollen grains by the total number of grains observed.

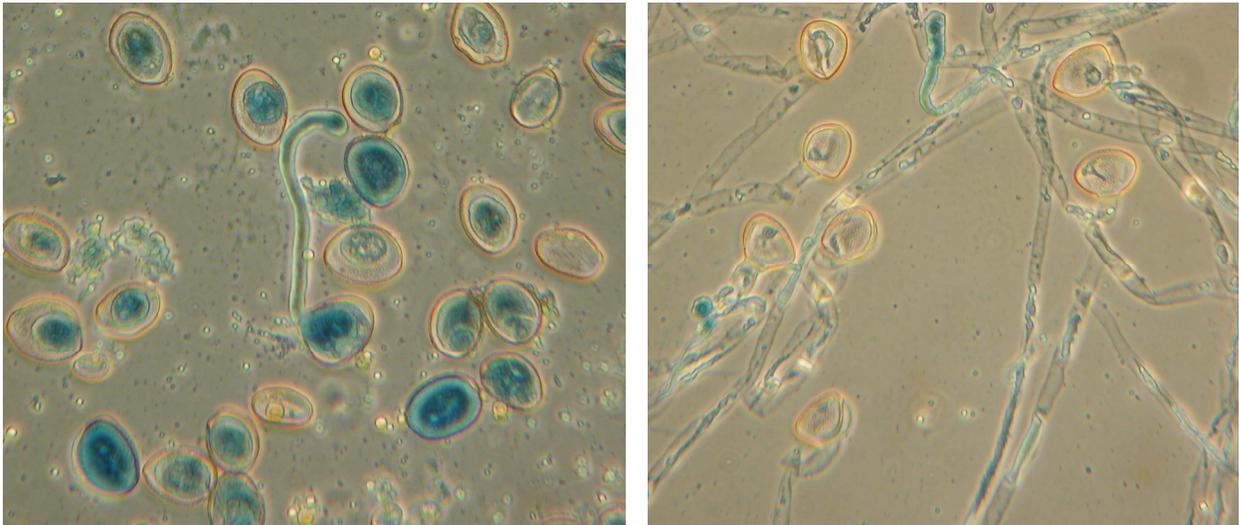


Figure 15. Pollen was scored as exhibiting no response (no pollen tube emerging - left photo), minimal response (short pollen tube - middle of left photo) or good response (long tube emerging - right photo). Grains were stained after germination . Photos by M. Carr.

Results

Pollen samples with stainabilities below about 70% exhibited large variation in size of both stained and unstained grains (Table 1). Moreover, variation in the uniformity and intensity of staining in these samples added to the ambiguity of scoring “stainable” pollen. This result is consistent with meiotic observations of variable numbers of chromosomes incorporated into the products of meiosis which leads to pollen grains of various sizes and quality of cytoplasm. It is likely that the scoring of stained grains grossly overestimates the actual fertility of pollen in individuals exhibiting these pollen characteristics. In contrast, pollen samples with stainabilities over about 70% typically exhibited sharp staining differentiation and relatively uniform size within the stained and unstained groups.

Pollen staining results were highly correlated with germination rates (correlation coefficient = 0.99), indicating that pollen staining provides a good estimate of germinability. Pollen source significantly affected stainability ($p < 0.05$ from ANOVA in Excel), with pollen from *F. recurva* and *F. affinis* staining better than *F. gentneri* (Figure 16; Table 1). Germination rates also varied among taxa, but high variability and low sample size prohibited statistical analyses of these data (Figure 17).

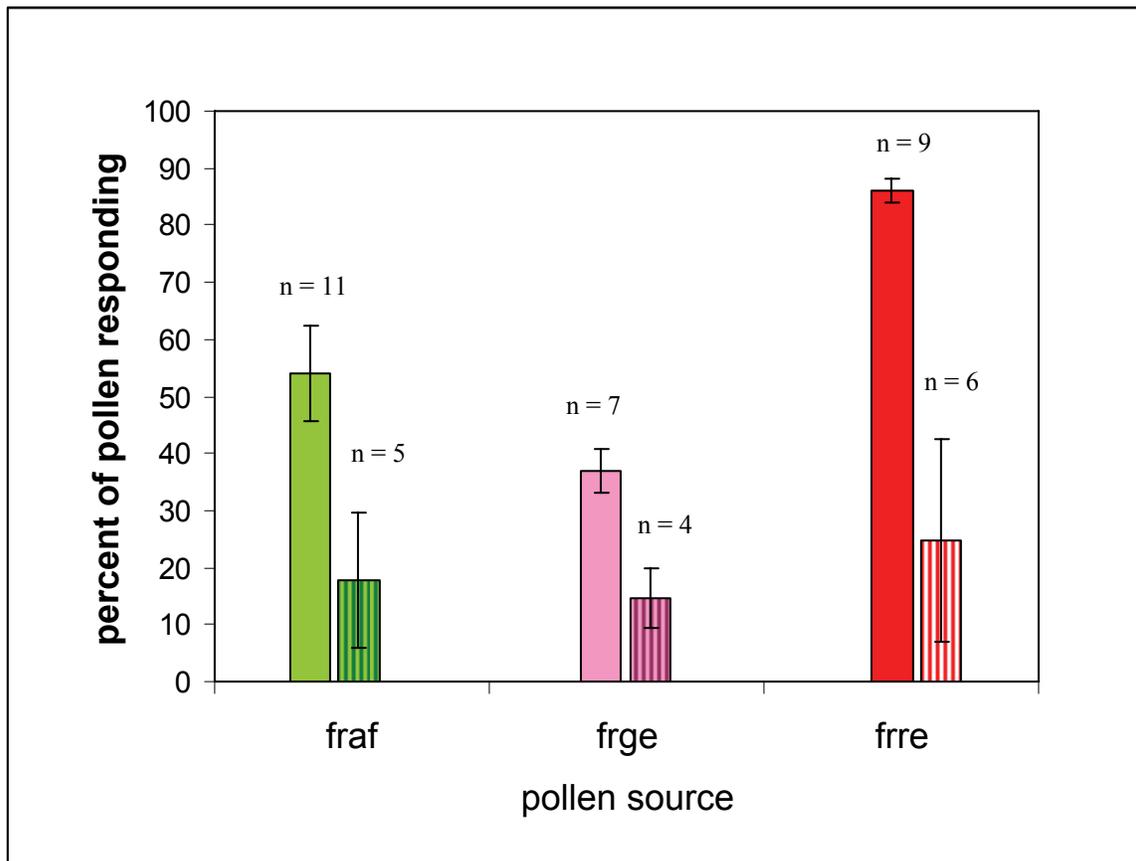


Figure 16. Mean pollen stainability (solid bars) and mean number of pollen grains exhibiting good germination response (striped bars) were correlated. Error bars represent one SE above and below the means. fraf = *F. affinis*, frge = *F. gentneri*, frre = *F. recurva*.

Table 1. Pollen stainability in *Fritillaria affinis*, *F. gentneri*, and *F. recurva*

<i>F. affinis</i>					
Sample	No. Stained	No. Unstained	Total No.	% Stained	Comments
FRAF 26JC 4/16/06	73	249	322	0.2267081	
FRAF 11JC 4/18/06	71	247	318	0.2232704	
FRAF 2006-1	250	58	308	0.8116883	1
FRAF 2006-2	263	54	317	0.829653	1
FRAF 2006-3	86	259	345	0.2492754	2
FRAF 2006-4 plt2	272	92	364	0.7472527	3
FRAF JC1	283	27	310	0.9129032	1
FRAF JC2	118	183	301	0.3920266	2
FRAF JC3	128	176	304	0.4210526	2
FRAF JC4	241	64	305	0.7901639	1
FRAF JC6	111	205	316	0.3512658	2

mean 0.5413873

<i>F. gentneri</i>					
Sample	No. Stained	No. Unstained	Total No.	% Stained	Comments
FRGE 67JC 4/18/06	98	215	313	0.313099	
FRGE 82SP 4/18/06	101	305	406	0.2487685	2
FRGE JC1 4/26/06	155	155	310	0.5	2
FRGE JC2 4/26/06	156	163	319	0.4890282	2
FRGE JC3 4/26/06	84	229	313	0.2683706	2
FRGE PC 2256	107	200	307	0.3485342	
FRGE PC 2301	128	174	302	0.4238411	

mean 0.3702345

<i>F. recurva</i>					
Sample	No. Stained	No. Unstained	Total No.	% Stained	Comments
FRRE BG-1 (new coll.)	260	43	303	0.8580858	
FRRE 81	305	15	320	0.953125	1
FRRE 72SP 4/17/06	270	33	303	0.8910891	1
FRRE BG3	271	37	308	0.8798701	1
FRRE BG7	237	77	314	0.7547771	1
FRRE BG8	276	27	303	0.9108911	1
FRRE BG9	261	69	330	0.7909091	1
FRRE BG11	252	48	300	0.84	1
FRRE BG14	258	45	303	0.8514851	1

mean 0.8589147

- 1 - Sharp staining differential, size uniform
- 2 - Poor staining differential, size variable
- 3 - Somewhat variable

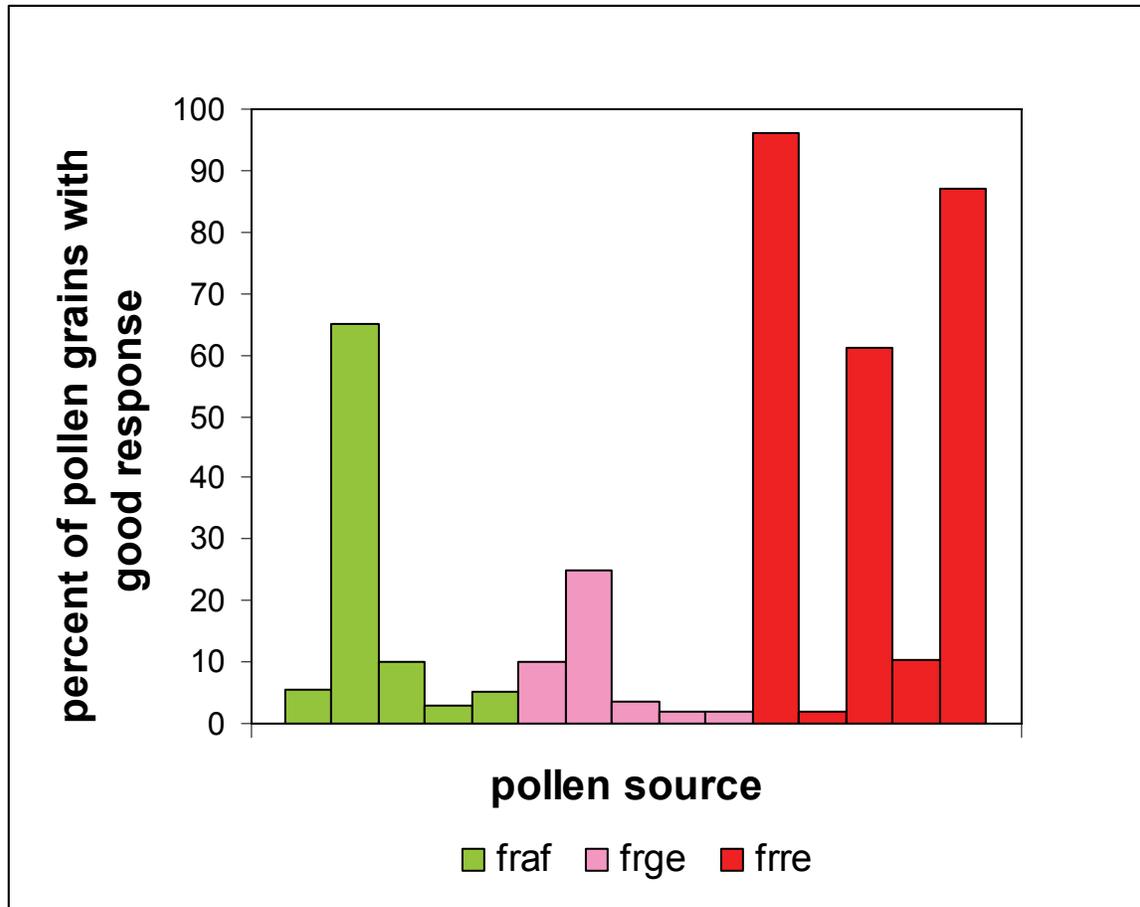


Figure 17. Pollen germination varied greatly among individuals, with most plants of *F. gentneri* producing poorly germinating pollen. Each bar represents one individual. fraf = *F. affinis*, frge = *F. gentneri*, frre = *F. recurva*.

Discussion

Low and variable pollen viability is a characteristic of polyploid hybrid taxa (Kim and Carr 1990), and interspecific hybrids within Liliaceae commonly exhibit pollen sterility (Karlov et al. 1999). Lack of chromosome pairing in these hybrids reduces the production of viable pollen grains, resulting in low germination and stainability, deformed or shrunken grains, and poor staining differential. Combined with the self-incompatibility mechanisms present in many lily species (Ascher and Peloquin 1968, Dickinson et al. 1982), pollen sterility reduces the ability to develop new hybrid varieties of ornamental lilies, and considerable horticultural research has focused on overcoming these barriers (Kim et al. 1998, Karlov et al. 1999, Obata et al. 2000). In addition to genetic contributions to male sterility, environmental

conditions can also affect pollen viability, and may be the source of much of the variability reported for pollen viability in experimental studies (Stone et al. 1995). However, although environmental conditions may be the cause of some of the variation we observed in our pollen viability assessments, the consistent lack of seed production for congeneric within-population crosses of *F. gentneri* point toward genetic causes for the sterility we report.

Despite variable results, mean stainability indices and germination percentages are indicative of poor pollen viability in *F. gentneri* relative to its parent species. Self-incompatibility mechanisms combined with chromosomal abnormalities associated with polyploid hybridization are probably the source of the low reproductive rates for *F. gentneri* reported in naturally occurring populations, and in our previous pollination experiments.

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 has not yet been completed for artificially created hybrids of *F. recurva* and *F. affinis*, (putative *F. gentneri*). Cytological studies provided an opportunity to collect additional information regarding the possibility of a hybrid origin for his species. A series of chromosome numbers were available in the literature for both parents (Table 2), and we wanted to verify the chromosome number of the parental taxa in our study sites. We also

wanted to document the chromosome number for *F. gentneri*, which had not been counted at the initiation of this study.

Table 2. Chromosome Numbers in *Fritillaria affinis* and *F. recurva*

<i>Fritillaria affinis</i>	
$2n = 24+B, 36$	Darlington, C. D. 1936 The external mechanisms of the chromosomes. Proc. Roy. Soc., Ser. B, Biol. Sci. 121, 823:264-319.
$2n = 24, 24+1-8B, 36, 48$	Beetle, D.E. 1944. A monograph of the North American species of <i>Fritillaria</i> . 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.
$2n = 24$	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. Sysesis 10:125-138.
$n = 12$	La Cour, L. F. 1978. The constitutive heterochromatin of chromosomes of <i>Fritillaria</i> 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 <i>Fritillaria</i> species. Chromosoma (Berl.) 67:67-75.
$2n = 24, 36$	Marchant, C. J. & R. M. Mcfarlane. 1980. Chromosome polymorphism in triploid populations of <i>Fritillaria lanceolata</i> Pursh (Liliaceae) in Calif. Bot. J. Linn. Soc. 81:135-154.
<i>Fritillaria recurva</i>	
$2n = 24+f, 36$	Darlington, C. D. 1936. The internal mechanics of the chromosomes, V. Relational coiling of chromatids at mitosis. Cytologia 7, 1-2:248-255.
$2n = 36$	Frankel, O. H. 1937. Inversions in <i>Fritillaria</i> . Jour. Genetics 34, 3:447-462.
$n = 12+1f$	Tischler, G. 1938. Pflanzliche Chromosomenzahlen. Tab. Biol. 16 (3):162-218.
$2n = 24$	Beetle, D.E. 1944. A monograph of the North American species of <i>Fritillaria</i> . Madrono 7:133-158.
$2n = 24+B, 36$	La Cour, L. F. 1951 Heterochromatin and the organization of nucleoli in plants. Heredity 5, 1:37-50
$2n = 24$	Beck, C. 1953. A gardener's introduction to the genus <i>Fritillaria</i> . London, Faber & Faber Ltd.: 1-96.
$2n = 24$	Dyer, A. F. 1963. Allocyclic segments of chromosomes and the structural heterozygosity that they reveal. Chromosoma 13, 5:545-576.
$n = 12, 12+f, 18+f$ [var. <i>coccinea</i>]	Cave, M. S. 1970. Chromosomes of California Liliaceae. Univ. Calif. Publ. Bot. 57:1-58.
$2n = 24$ [var. <i>recurva</i>]	Cave, M. S. 1970. Chromosomes of California Liliaceae. Univ. Calif. Publ. Bot. 57:1-58.
$2n = 24$	Schweizer, D. 1973. Differential staining of plant chromosomes with giemsa. Chromosoma (Berl.) 40:307-320.
$n = 12$	La Cour, L. F. 1978. The constitutive heterochromatin of chromosomes of <i>Fritillaria</i> sp., as revealed by giemsa banding. Phil Trans. Roy. Soc. London, Ser. B, 285:61. La Cour, L. F. 1978.

Methods

Chromosome counts for 17 individuals of *F. gentneri*, along with four individuals of *F. affinis* and six of *F. recurva* were completed for this study. Counts were completed on buds or on roots, depending on the material available (Figure 18). For meiotic counts, buds were collected and preserved in a mixture of 3 parts 95% ethanol: 1 part glacial acetic acid. Anthers were excised from preserved floral buds, squashed in acetocarmine, and mounted in Hoyer's solution for microscopic examination using phase contrast optics. Mitotic chromosomes in root tips were examined by treating collected roots in a saturated PDB (paradichlorobenzene) solution for 6-12 hours, and fixing in a 3 part 95% ethanol: 1 part glacial acetic acid solution. Root tips were then hydrolyzed in HCL, squashed in acetocarmine saturated in 45% acetic acid, and mounted in Hoyer's solution for microscopic examination using phase contrast optics.



Figure 18. Dr. Gerald Carr (emeritus professor from University of Hawaii) completed chromosome counts and pollen staining for our study. Photo by K. Amsberry

Results

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 2). Our counts of *F. recurva* and *F. affinis* corroborate previous reports of dipoidy and triploidy in these species (Table 3), and our documentation of both ploidy levels within the species *F. gentneri* is valuable in assessing the potential fertility and origin of this taxon.

Table 3. Chromosome counts for three fritillary species at five study sites.

site	<i>F. affinis</i>	<i>F. gentneri</i>	<i>F. recurva</i>
Jacksonville	2n=36, 2n=36	2n=36, 2n=36, 2n=36, 2n=36, 2n=36, 2n=36	
Siskiyou Pass	2n=24, 2n=24	2n=36, 2n=36	2n=24, 2n=24, 2n=36, 2n=36, 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 (Figures 19 - 21). The basic karyotype of diploid species in this genus with $n = 12$, found in N American as well as Mediterranean, consists of two nearly metacentric, and ten nearly telocentric chromosomes .

Based on pollen stainabilities and chromosome observations available, the populations of *F. recurva* sampled consist mostly of individuals with normal or near normal meiosis. In contrast, all pollen samples of *F. gentneri* exhibit high frequencies of abortive pollen consistent with abnormal meiosis. Most individuals of this species have been demonstrated to be triploid ($2n = 36$) based on chromosome determinations. The populations of *F. affinis* sampled appear to consist of a high frequency of individuals with abnormal meiosis. It is likely that some or all of the individuals with pollen stainability below 50% are triploid or suffer from other meiotic irregularities. On the other hand, at least some individuals of *F. affinis* have apparently normal pollen stainability, suggesting that they are likely diploid, or at least not odd polyploid.

Chromosome association in triploids was very high. Commonly, 12_{III} were observed during prophase and rarely were more than 2 or three unpaired chromosome seen before anaphase I. Unpaired chromosomes frequently remained on the equator or became isolated in other areas of the cells. Chromosomes of the trivalents also assorted irregularly such that most anaphase configurations exhibited unbalanced numbers of chromosomes. These irregularities are to be expected in triploids and they account for the impaired function and variation in size of derived pollen grains.

Chromosome observations so far offer little inherent support for the hybrid origin of *Fritillaria gentneri*. On the other hand, they are fully compatible with such an origin. Triploids appear to be common in *F. affinis* and are also known in *F. recurva*. Thus, the mechanism that has produced this condition in *F. gentneri* is well established in closely related species. The best arguments for hybridity of *F. gentneri* appear to be morphology, geography, and molecular research. Any hybrid with triploidy superimposed (whether concomitant with or subsequent to the hybridization event) would be well isolated from either parent and with an asexual means of propagation would be well on its way to species status.

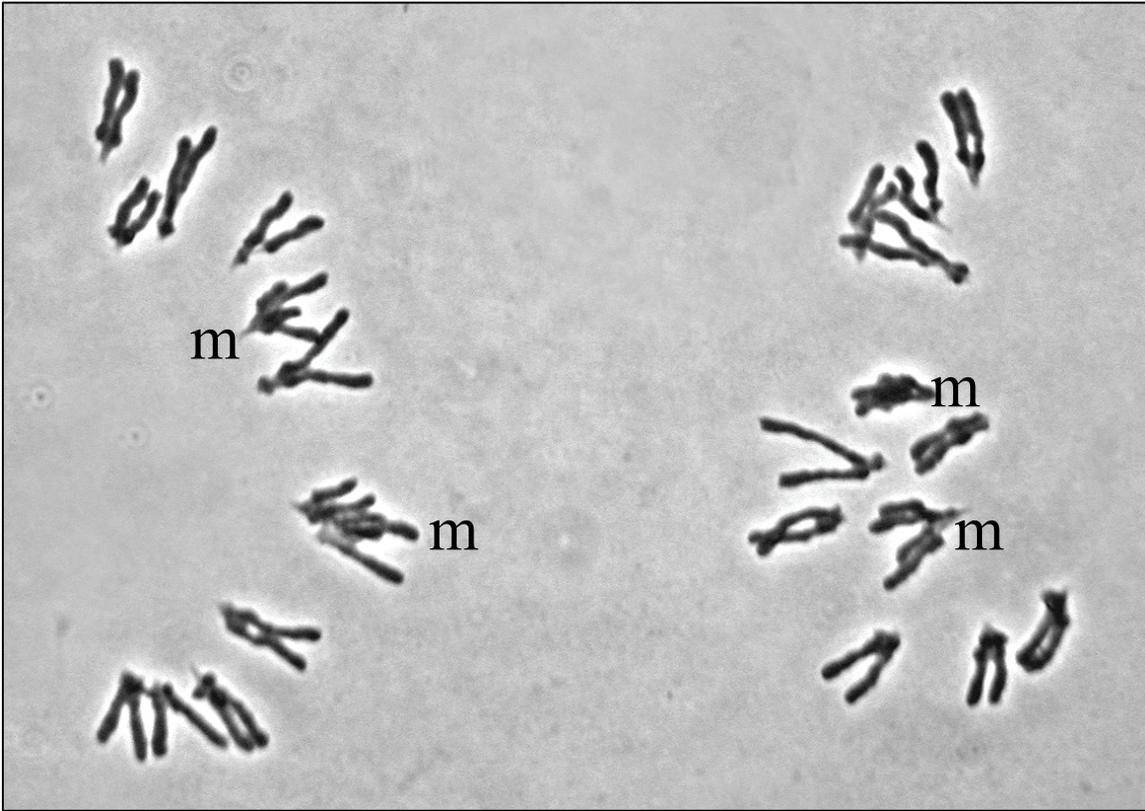


Figure 19. Normal 12-12 segregation in diploid at anaphase I in *F. recurva* – note chromatids distinctly visible, ten near teleocentric and two nearly metacentric chromosomes (m). Photo by Gerry Carr.

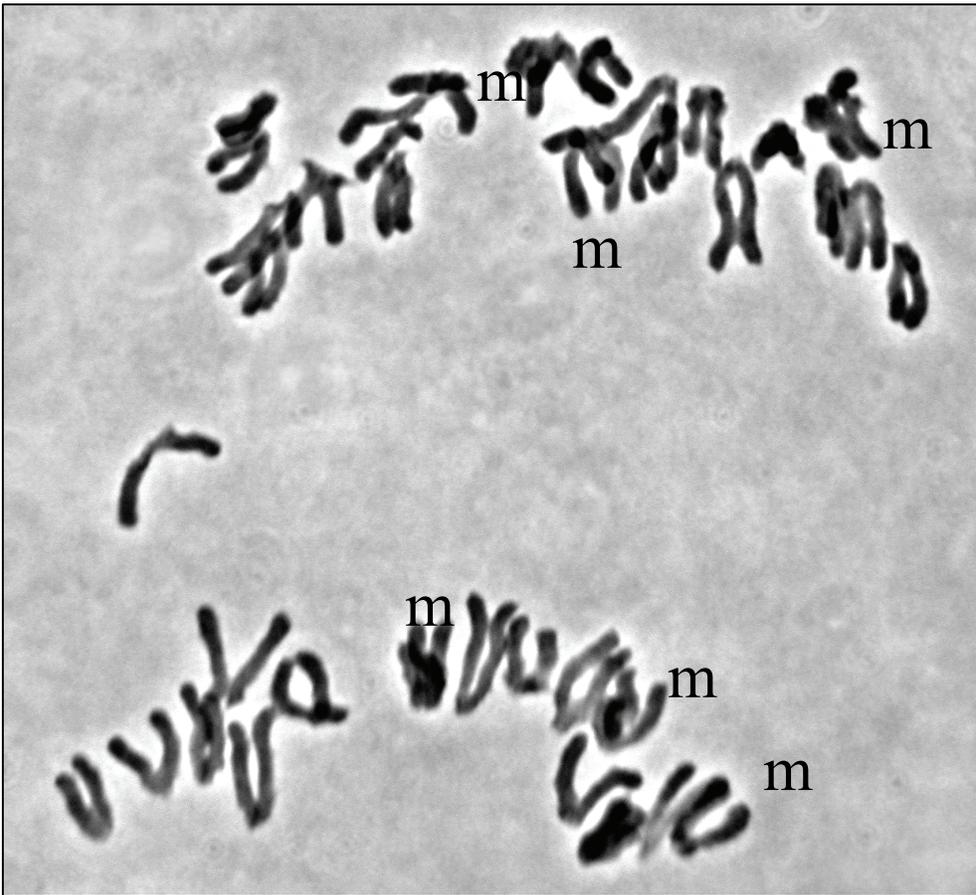


Figure 20. Meiosis in *Fritillaria affinis*. Triploids are frequent in the populations sampled. Note 12III in top cell and abnormal segregation in lower cell (15-1-20) Photo by Gerrv Carr

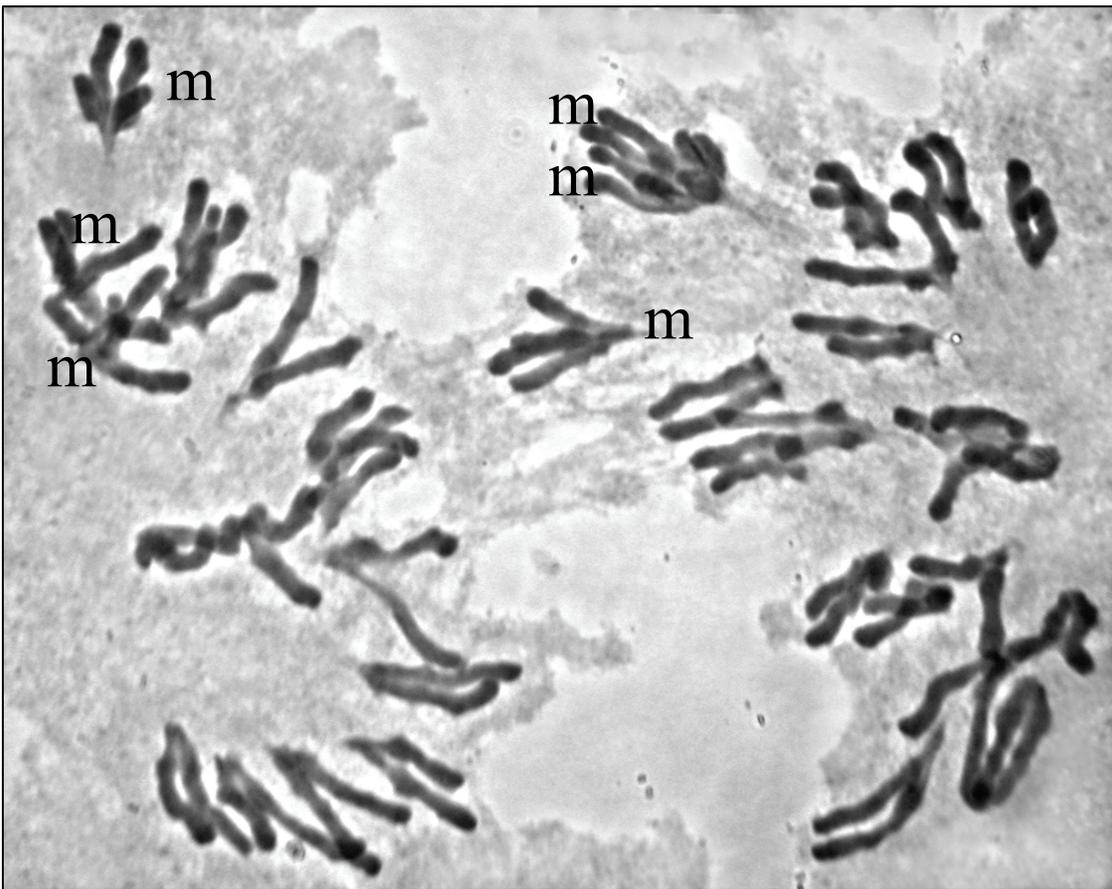
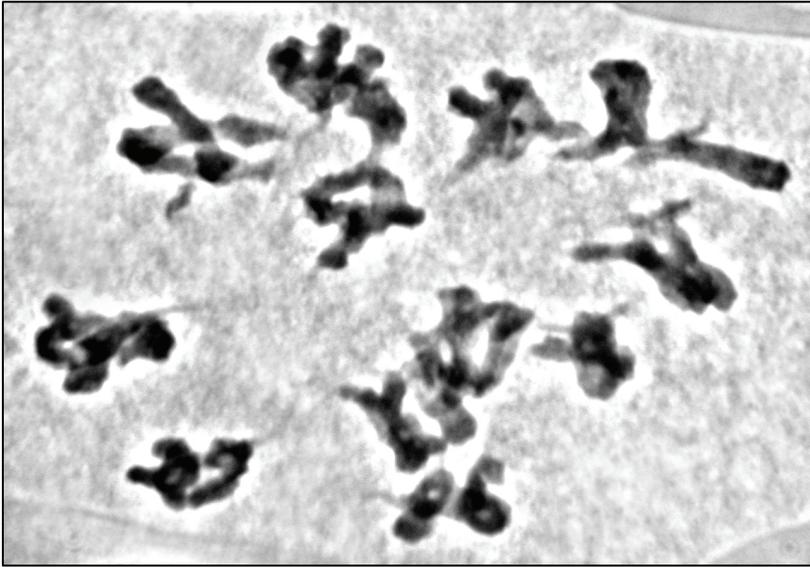


Figure 21. Meiosis in *Fritillaria gentneri*. Most individuals chromosomally determined are triploid. Note 12III in left cell below and abnormal segregation in lower right cell(16-20) Photo by Gerry Carr.

Molecular summary

Prior to our most recent studies, 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”, 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, we have chosen to focus our molecular research on unambiguous sequence data. We analyzed nuclear sequences and SNAPs (Superimposed Nucleotide Additivity Patterns) of the three 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, Whitall 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. Details of methods used and a summary of final results will be reported to USFWS at the completion of this portion of the study.

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* simply represents another example of a common speciation event in the plant kingdom.

Summary

- Interspecific pollinations of *F. recurva* and *F. affinis* produce capsules, and these capsules contain viable seeds. These seeds are capable of germination, and plants from these interspecific crosses are being cultivated at OSU. Although final evaluation of the morphology and fertility of these artificially created hybrid plants is well in the future, our study documents the potential for *in situ* creation of a hybrid taxon in sites where *F. recurva* and *F. affinis* occur sympatrically. Data from cytological and pollen viability analyses, as well as DNA sequencing, of the parent and hybrid taxa are consistent with expectations for a species of hybrid origin.

(Recovery Task 3.8)

- *F. gentneri* is not sterile, but instead produces capsules when pollinated with pollen from flowers of the same species from sites other than the source site of the maternal parent. Although further investigation is needed, a genetic self-incompatibility system may be causing the observed sterility in populations of closely related individuals. Additionally, documented chromosomal abnormalities typical of triploid hybrid taxa may be the cause of limited and sporadic pollen viability, resulting in lack of sexual reproduction in most sites. Although sporadic, documentation of fertility in this species demonstrates that it is capable of sexual reproduction, making it a “good” biological species. ***(Recovery Task 3.7)***

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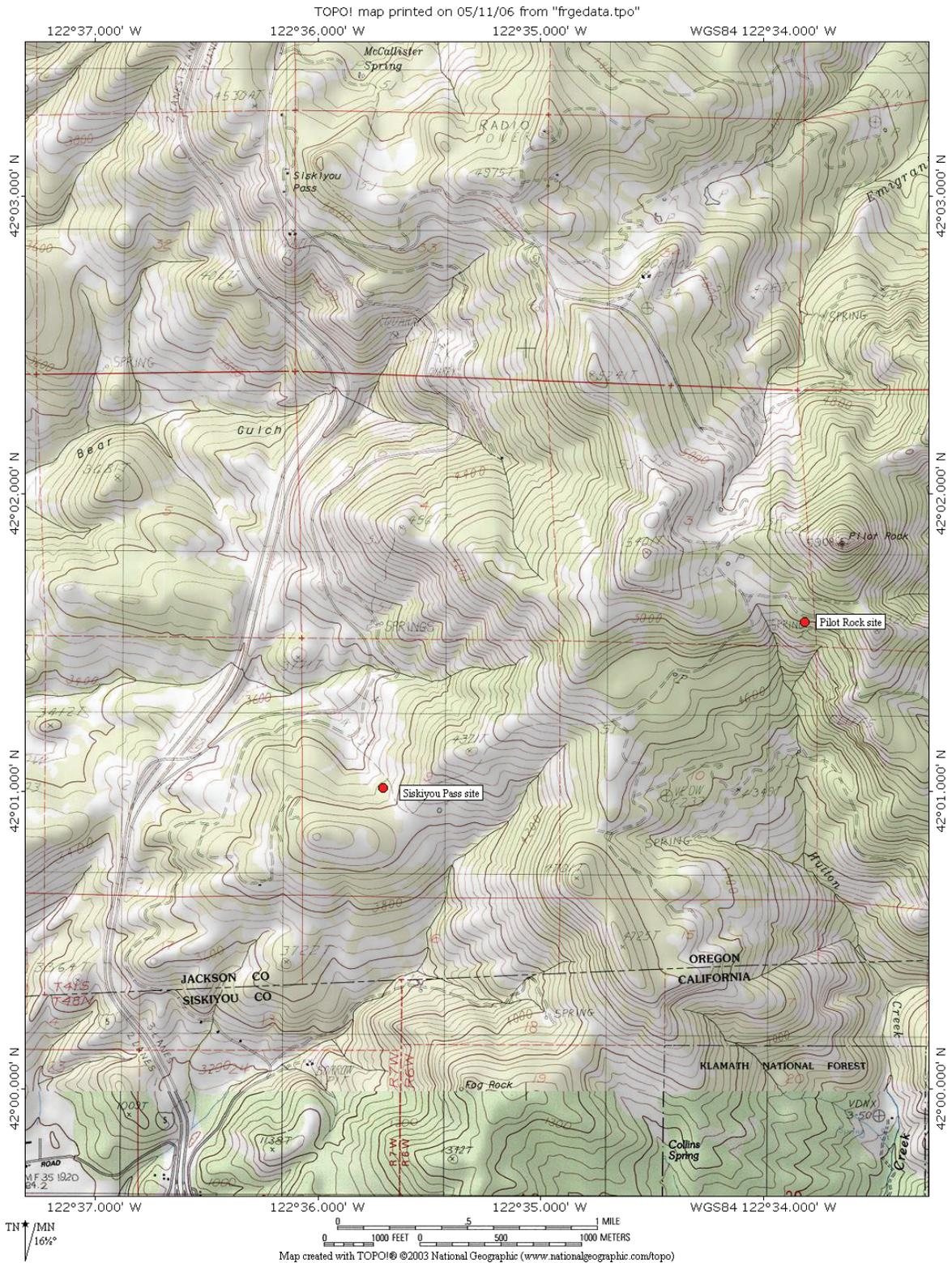
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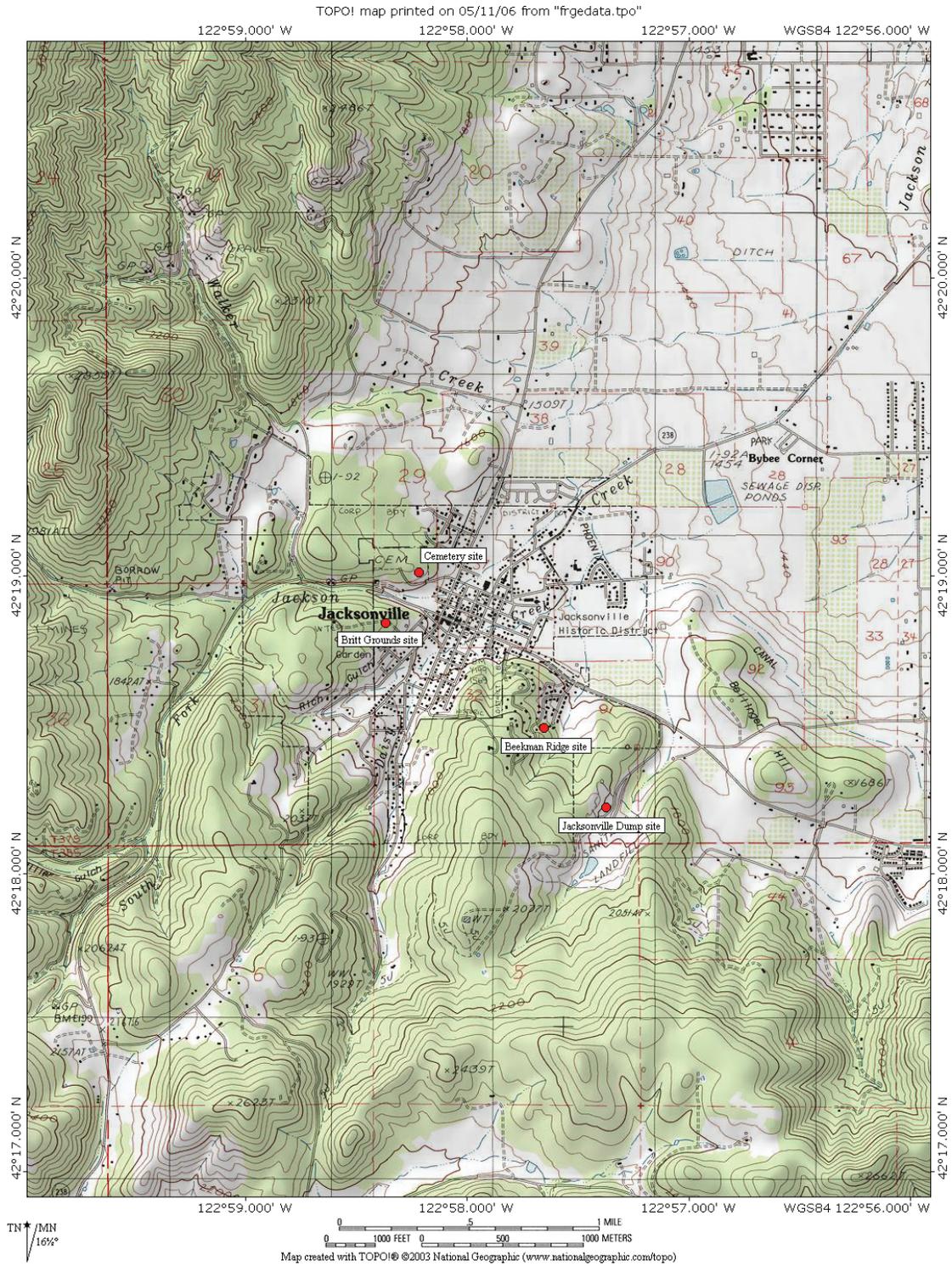
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Appendices



Appendix A. Location of the Siskiyou Pass site in Recovery Unit 4.



Appendix B. Location of the Jacksonville Cemetery site in Recovery Unit 1.