Reintroduction Plan for Golden Paintbrush (*Castilleja levisecta*)

Prepared for the US Fish and Wildlife Service
Western Washington Fish and Wildlife Office
through Section 6 funding, Region 1

Prepared by
Florence Caplow

Washington Natural Heritage Program
Washington Department of Natural Resources
PO Box 47014
Olympia, WA 98504-7014

2004

Executive summary

The recovery plan for *Castilleja levisecta*, or golden paintbrush, calls for twenty populations of at least 1000 plants on protected land (USFWS 2000b), and for the creation of a reintroduction plan. Reintroduction, in this case, is used broadly to refer to three distinct activities within the historic range of the species: *reintroducing* plants to sites from which they were formerly known, *introducing* plants to suitable sites within the historic range for which we have no evidence of past populations, and *augmenting* current populations to increase the population and/or its genetic diversity.

There are now eleven known extant populations of *Castilleja levisecta.* Half of these populations have less than 1000 plants, and only four populations are considered stable or possibly stable. Reintroduction will be a critical part of the recovery of *C. levisecta* . This document identifies the objectives, strategic and managerial considerations, the site election process, genetic and demographic considerations, ecological considerations, and technical considerations to be considered in the reintroduction process. Appendices include seed collection guidelines, recommendations from the genetic analysis, soils analysis of known sites, the site selection process, and suggested research prior to large-scale reintroduction.

This document recommends the following as essential to successful reintroduction.

- An emphasis on augmentation and protection of existing sites over the creation of new populations.
- The active participation of the technical team in reintroduction planning.
- A systematic approach to site selection.
- The protection through conservation agreements or easements of all sites used for reintroductions.
- The development of site specific reintroduction plans, including provisions for planting and experimental design, monitoring, site management and restoration, adaptive management and information sharing. These individual plans will be included as appendices to this reintroduction plan as they are completed.

Table of Contents

Appendices

1.0 Overview of reintroduction

The conservation and recovery of rare plants is a process that requires many strategies. Among these strategies are habitat protection and management, *ex situ* conservation, and increasingly, various forms of reintroduction. These include *reintroduction* to sites that were known to support populations in the past but from which the species has been extirpated, *introduction* to sites within the known range which were not known to support populations in the past, and *augmentation or enhancement* of existing populations. All of these activities, for the purposes of this document, are considered to be forms of reintroduction, though each has unique considerations. The Endangered Species Act (ESA) allows for reintroduction as a component of species recovery plans. The primary purpose of reintroduction efforts is to lessen the probability of extinction and encourage the recovery of a rare plant through the creation of new, self-sustaining populations (Pavlik 1996). However, the USFWS also states that controlled propagation of threatened and endangered species will be used as a recovery strategy only when other measures to maintain or improve the species' status in the wild have failed, are determined to be likely to fail, are shown to be ineffective, or would be insufficient to achieve full recovery (USFWS 2000a).

The *Recovery Plan for Golden Paintbrush (*Castilleja levisecta*)* specifically calls for the development and implementation of a reintroduction plan. The authors have determined that the establishment of additional populations is critical to the recovery of the species. The development and implementation of a reintroduction plan is a Priority 1 Task in the recovery plan (USFWS 2000b).

Despite current interest in reintroduction of rare plants, the biological understanding of the reintroduction of species and populations is poorly developed (Falk *et al*. 1996). Successful large-scale reintroductions are relatively rare, and there are concerns that reintroduction could be used *in lieu* of proper protection of existing populations. The policy of the International Union for Conservation of Nature and Natural Resources (IUCN 1987) notes that reintroduction and enhancement are powerful tools but notes that "like other powerful tools they have the potential to cause enormous damage if misused." Although several reintroduction experiments are described in the literature, to date no formal reintroduction plan for a rare plant has been published by the USFWS, despite the importance of systematic preparation before undertaking reintroduction. There are, however, guidelines for a rare plant reintroduction plan in Falk et al. (1996), and a number of reports on reintroduction attempts (Guerrant 1996, Cully 1996, McDonald 1996, Gordon 1996, Pavlik 1988, 1990, 1991a, 1991b, 1992, 1993, 1994, 1995, 1996, Pavlik and Espland 1991, DeMauro 1994). This document is based primarily on the guidelines in Falk (1996).

Central to the recovery plan and to this document is the understanding that the first priority for the recovery of *Castilleja levisecta* is the protection, management, and enhancement of the existing populations. Reintroduction of the species to additional sites throughout its historic range, while important, is understood to be an ambitious, long-term undertaking. This document contains guidelines that are applicable to both the creation of new populations and the augmentation or enhancement of existing populations. Each reintroduction site should have an individual plan which is tailored to the issues and concerns of the particular site.

The terms that are listed below are used throughout this document. The definitions are from Falk et al. (1996):

"Reintroduction" and " introduction" both refer to the addition or release of biological material. Whether or not a particular action is considered an introduction or reintroduction depends partly on scale. In general, "introduction" refers to the placement of biological material in a site that has never supported populations of the taxon, while "reintroduction" refers to the placement of biological material in a site that previously supported populations of the taxon. However, in many cases we don't know the precise past distribution of a taxon. For the purposes of this document we will refer to all additions of *Castilleja levisecta* to prairie habitats in the Puget Trough and Willamette Valley ecoregions as "reintroductions".

"*Augmentation or enhancement*" are used synonymously, and refer to the addition of individuals to an existing population, with the aim of increasing population size or genetic diversity to improve viability. Augmentation or enhancement can occur using plant material from the site or plant material from other sites, depending on the purposes of the action.

"*Outplanting*" refers to moving plants from an *ex situ* location (greenhouse, garden) to an *in situ* location (existing site or reintroduction site).

"*In situ conservation*" refers to the conservation of taxa in their native habitats.

"*Ex situ conservation*" refers to the conservation of taxa in seed banks, greenhouses, and garden environments outside of the native habitats of the taxa.

"*Natural populations*" are the existing populations in their native habitats.

"*Created populations*" are populations created in native habitats for the long-term conservation of the species.

"*Experimental populations*" are small populations created under strict experimental protocols to test reintroduction techniques and theories. These populations are not intended to serve as founders for a reintroduction effort, and may be destroyed or transplanted at the end of the experimental work. If, however, they become successfully established and meet the criteria for the site-specific reintroduction plan, they may be used as part of a larger-scale reintroduction effort.

2.0 Overview of the current status of *Castilleja levisecta*

Most of this overview of the current status of *Castilleja levisecta* is taken directly from the recovery plan (USFWS 2000b), with some additions of more recent information.

2.1 Listing history

The U.S. Fish and Wildlife Service began work on listing the golden paintbrush *(Castilleja levisecta*) as an endangered or threatened species in 1990 when they announced that sufficient information was available to proceed with listing this plant in a *Federal Register* notice of review published February 21, 1990 (55 FR 6184). After this announcement, a proposal to list *Castilleja levisecta* as a threatened species was published on May 10, 1994 (59 FR 24106), and a final rule listing it as threatened was published on June 11, 1997 (62 FR 31740).

A recovery plan was written in 2000 (USFWS 2000b) and at that time an informal technical team was established. The intention of a technical team is to provide biological advice to the USFWS. The Regional Director of the USFWS, not the technical team, exercises the Service's authority and responsibility (USFWS 1990). Personnel on a technical team are chosen for their knowledge of the species' biology, recovery planning, or other issues relevant to the recovery of the species. The current technical team is made up of staff from: the USFWS; the Washington Natural Heritage Program (WNHP); the Washington Natural Areas Program and the Washington Office of The Nature Conservancy (both of which manage populations of golden paintbrush and have participated in reintroduction research); the Berry Botanic Garden in Portland, Oregon; and the Institute for Applied Ecology in Corvallis, Oregon. The technical team is active in coordinating efforts for recovery of golden paintbrush. See Table 5 for more information on how to contact the USFWS, the WNHP, the technical team, or members of the team.

2.2 Species description

Castilleja levisecta is a perennial herb in the figwort or snapdragon family (Scrophulariaceae). *Castilleja levisecta* often has from 5 to 15 (up to 50) unbranched stems. The stems may be erect or spreading, in the latter case giving the appearance of being several plants, especially when in tall grass. Plants are up to 30 centimeters (12 inches) tall and are covered with soft, somewhat sticky hairs. The lower leaves are broader, with one to three pairs of short lateral lobes near the terminal third. The showy bracts are about the same width as the upper leaves, softly hairy and sticky, and are golden yellow. The bracts effectively hide the flowers.

2.3 Distribution and Collection History

Castilleja levisecta was first collected by Macoun in 1875, in Victoria, British Columbia. The specimen was labeled *C. parviflora*, but later annotated by Greenman (1898), who published a description of *C. levisecta* in that year. Piper (1906) designated a collection by Howell in 1880 from Mill Plain (Clark County, Washington) as the type specimen (K. Chambers, pers. comm.

cited in Sheehan and Sprague 1984). A specimen at Harvard is cited as the type specimen in the Harvard type specimen database (ID number 56156, Checked November 19, 1998).

Historically, *Castilleja levisecta* has been reported from more than 30 sites in the Puget Trough of Washington and British Columbia, and as far south as the Willamette Valley of Oregon (Hitchcock *et al.*, 1959, Sheehan and Sprague 1984, Gamon 1995). A 1984 assessment found that the plant had been extirpated from more than 20 historic sites (Sheehan and Sprague 1984, Gamon 1995). Many populations were extirpated because their habitats were converted for agricultural, residential, and commercial development. In Oregon, *Castilleja levisecta* occurred historically in at least six sites in the Willamette Valley, in Linn, Marion, and Multomah Counties. The species has been extirpated from all of these sites.

Two extant populations of *Castilleja levisecta* occur in British Columbia, Canada, on small islands near Victoria (Table 1 and Figure 1). Historically, *C. levisecta* was documented from nine sites on southeastern Vancouver Island, and on two adjacent islands. All but the two populations found on islands are extirpated or are of unknown status but are likely to have been extirpated (British Columbia Conservation Data Center 1993, Ryan and Douglas 1994).

Nine extant populations of *Castilleja levisecta* occur in Thurston, Island, and San Juan Counties in Washington (Table 1 and Figure 1). At least fourteen additional historic sites were in Island, San Juan, Clark , Pierce, King, Jefferson, and Skagit Counties (Gamon 1995).

Table 1. Summary of extant sites for *Castilleja levisecta* (Gamon et al. 2001, unpublished WNHP data).

Site Name	County (if In Wash.) / B.C.	Size of Flowering Population (year)	Area	10-Year Trend
Rocky Prairie	Thurston	5,493 (2002)	ca. 30 acres	Stable?
Bocker Environ. Reserve	Island	122 (2003)	ca. 1 acre	Declining
Fort Casey State Park	Island	307 (2003)	< 1 acre	Unknown
West Beach	Island	54 (2003)	< 1 acre	Declining
Forbes Point	Island	765 (2003)	< 1 acre	Declining
Ebey's Landing	Island	7,627 (2000)	ca. 1 acre	Stable?
False Bay	San Juan	269 (2002)	< 1 acre	Unknown
Long Island	San Juan	154 (2002)	< 1 acre	Unknown
Trial Island	Brit. Columbia	2,150 (2002)	5 acres	Stable
Alpha Islet	Brit. Columbia	800 (2002)	< 1 acre	Stable
San Juan	San Juan	7,528 (2003)	2	Increasing

2.4 Description of habitat

Castilleja levisecta occurs in the Puget Trough Physiographic Province of Washington (as mapped by Franklin and Dyrness 1973) and lower Vancouver Island at elevations from sea level to about 100 meters (330 feet) above sea level. It also historically occurred in the Willamette Valley Physiographic Province of Oregon, but has not been observed in Oregon for more than 50 years.

Castilleja levisecta occurs on generally flat grasslands, including some that are characterized by mounded topography, and on steep coastal bluffs that are grass-dominated. The coastal bluffs have a west or southwest aspect. Low deciduous shrubs are commonly present as small to large thickets. Many of the sites have been colonized by trees, (primarily Douglas-fir), shrubs (wild rose), and, more commonly, Scot's broom, (an aggressive nonnative shrub), and nonnative sod-forming grasses. In many cases this may be the result of fire suppression (Dunwiddie *et al.* 2001).

The sole mainland population in Washington occurs in a gravelly, glacial outwash prairie. Other populations occur on soils derived from either glacial drift or glacio-lacustrine sediments (in the northern end of the species' historic range). All of the extant populations are on soils derived from glacial origins. Historic populations also occurred on near-bedrock soils (Lighthouse Point), as well as clayey alluvial soils (in the southern end of its historic range). For more information on soils of the known sites, see Appendix D.

A vegetation map by Küchler (1966) shows the range of *Castilleja levisecta* associated with western red cedar – western hemlock – Douglas-fir forest in the northern part of its range and as a mosaic of the above type and Oregon oakwoods in the southern part of its range. A map by Bailey (1976) shows the range as Type 2410 Willamette – Puget Forest Province. Franklin and Dyrness (1973) map the range as the Puget Sound area of the Western Hemlock (*Tsuga heterophylla)* Zone and Interior Valleys of Western Oregon – Willamette Valley. The Puget Sound area of the Western Hemlock Zone differs from the rest of the Western Hemlock Zone by a number of features, among them the presence of prairies. Grasslands once covered much of the Willamette Valley, where forest communities were dominated by Douglas-fir (*Pseudotsuga menziesii*) or Oregon white oak (*Quercus garryana*). Grasslands have also declined in the Puget Trough, where currently there are less than 10 percent of the original grassland communities remaining (Crawford and Hall 1997). The composition of the remaining grasslands has, in most cases, been highly altered by the introduction of nonnative grasses, forbs, and shrubs.

2.5 Life history and ecology

Castilleja levisecta is a short-lived perennial herb. Individual plants generally do not survive longer than 5 to 6 years. Observations of individual tagged plants that were followed over time indicated that about 15 percent of the plants in one population were lost to mortality on an annual basis, but that another cohort lost an average of 35 percent annually (Dunwiddie *et al*., 2001). Biologists think this species reproduces exclusively by seed; vegetative spread has never been observed or reported. Evans *et al.* (1984) reported the following phenological information for the species from Rocky Prairie Natural Area Preserve (NAP): the plants emerge in early March; by mid-April, the plant is in bud; flowering generally begins the last week in April and continues until early June; fruits mature from June to mid-July; and by mid-July, the plants are senescent. Observations on other populations have documented blooming beginning as early as February on bluffs on Whidbey Island (P. Dunwiddie, personal comm., 2001). Occasional plants have been observed blooming as late as November. Capsules persist on the plants well into the fall.

Members of the genus *Castilleja*, like many others in the figwort family, may be parasitic or hemi-parasitic. Roots of paintbrushes are capable of forming parasitic connections to roots of other plants. These specialized connections are called haustoria. Heckard (1962) showed that although *Castilleja* plants could be grown in the greenhouse without host plants, they thrived better with hosts. *Castilleja levisecta* has also been shown to have the ability to germinate and develop in a greenhouse setting with and without a host plant (Wentworth 1994). Wentworth (1994) observed haustorial connections with the roots of a variety of host plants, although she also observed that *C. levisecta* will develop haustoria on its own roots when grown without a host, and is therefore a hemi-parasite rather than an obligate parasite

Two recent propagation experiments have tracked the effects of host plants. Sarah Reichard of the Center for Urban Horticulture (pers. comm. 2003) found that plants that were established with a *Festuca roemeri* host were more successful after outplanting than those not established with any host or with an *Eriophyllum lanatum* host. Tom Kaye of the Institute for Applied Ecology (2001), whose study did not include outplanting, found no significant effect of hosts on flowering rates of first year plants in the greenhouse. He did find a weak indication that host plant affected plant size: plants grown alone were the smallest, and plants grown with *Eriophyllum lanatum* were the largest. The advantage of *E. lanatum* as a host for greenhouse plants may have been due to the competitive effect of *F. roemeri* on plants in confined pots. A study of the effect of various hosts on another generalist hemi-parasitic *Castilleja* (Adler 2003) found that legume hosts increased seed production and pollinator visitation in comparison to other hosts.

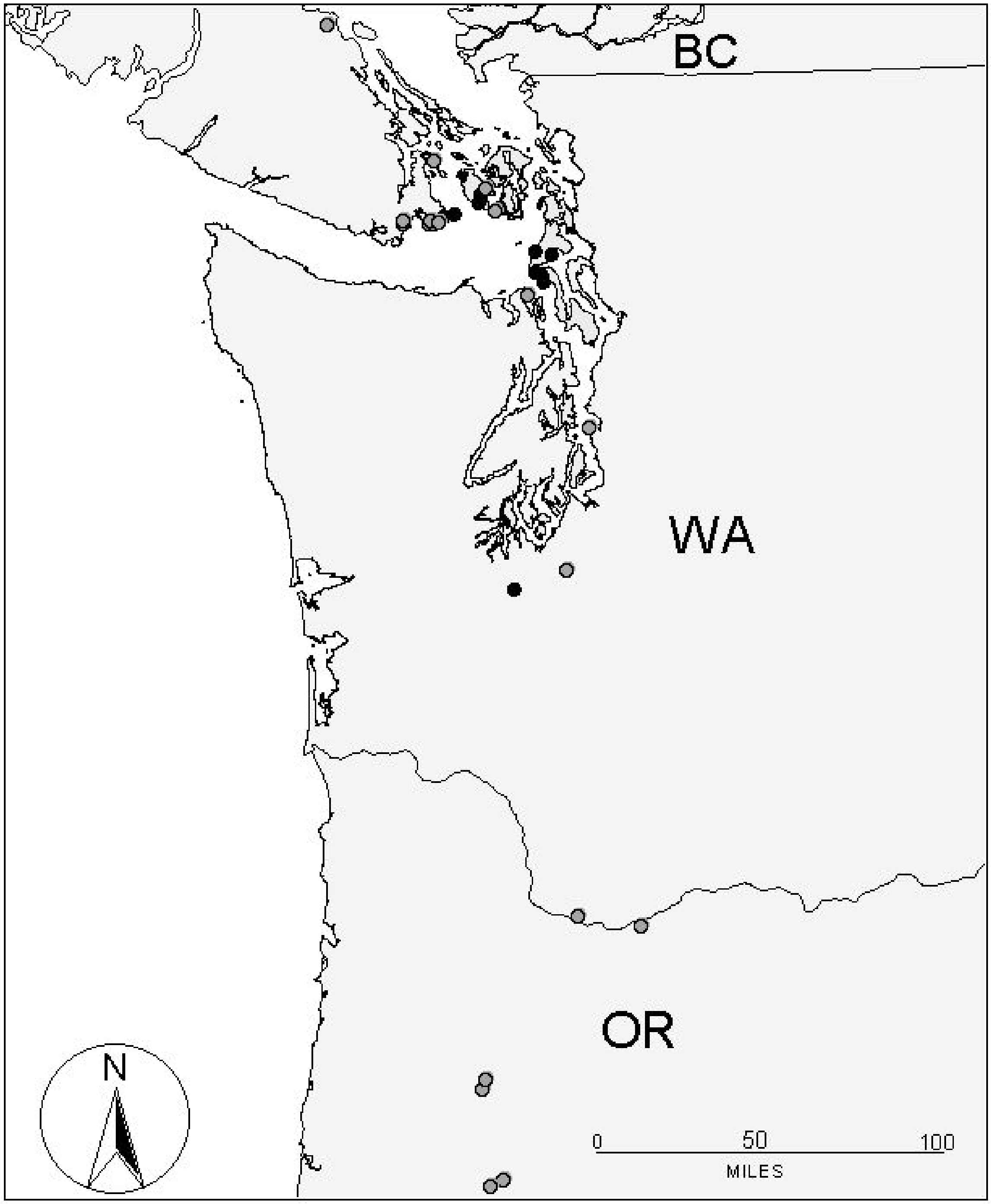


Figure 1. Golden paintbrush populations, historic and present. Gray circles are historic collection sites, black circles are extant populations.

The pollinators of *Castilleja levisecta* have not been thoroughly documented. Evans *et al.* (1984) reported that a species of bumblebee, *Bombus californicus*, was observed visiting *C. levisecta* . Pollen could be observed on the bee's head as it exited the inflorescence. Wentworth (pers. comm. 1995) also observed that *Bombus* sp. were the most frequent visitors to *C. levisecta* inflorescences. Tepedino (pers. comm. 2001) suggested that large species of *Megachile*, leaf cutter bees, can be mistaken by some for bumblebees, and two *Castilleja* species in the west (*C. aquariensis* and *C. christii*) were visited by large species of *Megachile.*

In a pollinator exclusion experiment, Wentworth (1994) found that fruits can be produced in the absence of pollinator visitation, but fruit set was almost five times greater in unbagged inflorescences compared to inflorescences bagged to prevent visitation to the flowers by pollinators.

In a study of the breeding system of *Castilleja levisecta* (Kaye 2003) found that the flowers are protogynous: the pistils extend beyond the galea and the stigma becomes receptive prior to anther dehiscence. *Castilleja levisecta* appears to be almost completely self-incompatible, and seed production increases as the genetic relationship between mated pairs becomes more distant. The highest seed set was between individuals from separate populations, and the lowest seed set was from self-pollinated individuals. Sibling and within-population crosses were intermediate in their seed production.

Evans *et al.* (1984) reported 75 % fruit set for all inflorescences. The percentage was calculated for each inflorescence as the ratio of flowers with seed capsules to the total number of flowers. Kaye (2003) found that fruit set ranged from 0.7% for self-pollinations, 33% for sibling crosses, 71% for within population crosses, and 80% for between population crosses.

Wentworth (pers. comm. 1995) calculated the mean number of seeds per capsule to be 165. Kaye (2003) calculated the mean number of ovules per flower to be 183 $\pm$ 5.8. Dunwiddie collected capsules over several years from Ft. Casey and Rocky Prairie. At Rocky Prairie one collection of 15 capsules had an average of 149 seeds per capsule; another collection of 37 capsules averaged 150 seeds per capsule, and another collection of 137 capsules had an average of 138 seeds per capsule. At Ft. Casey, 12 capsules had an average of 126 seeds per capsule. A second batch of 28 capsules had 158 seeds per capsule (P. Dunwiddie pers. comm. 2004). Seed viability varies widely, even between maternal lines collected within the same population at the same time (T. Kaye pers. comm.. 2004)

Germination studies on seeds collected from Rocky Prairie in Thurston County, Washington, were conducted at the Berry Botanic Garden in Portland, Oregon. After 6 weeks of chilling, 80 percent of the seeds germinated. Under laboratory conditions, Wentworth (1994) achieved 47 percent germination of first-year seeds and 13 percent for second-year seeds. Reichard (pers. comm. 2001) observed germination of seeds in flats in the greenhouse; rates varied by site, from 39% (Ebeys) to 78% (Ft. Casey) and 96% (Forbes Point). Germination rates also varied by researcher: Kaye (2001), who germinated seed from the same seed lots as Reichard, found germination rates from 64% (Ebey's) to 88% (Ft. Casey) and 74% (Forbes Point). Germination rates may vary depending on stratification techniques and may also vary at the same site depending on the time of year collected and the year collected (P. Dunwiddie, pers. comm.. 2004).

The germination rate appears to be much lower under natural conditions. Wentworth found that under natural conditions, first year establishment of seeds (from the seed bank for 2 different years) was 8 percent and 12 percent. Second year establishment was only 2 percent. Three year old seeds did not germinate (Wentworth 1994). A study by Pearson and Dunwiddie (2003) found no establishment in the field in multiple plots at two prairie sites seeded at a rate of 100 seeds per square meter. When the seeding rate was increased to 1000 seeds per square meter in 2003, 145 seeds established out of a total of 42,000 seeds. There may have been a greater number of germinants than were recorded in the study, since seedlings are small and may have germinated and died in between the times when the seedlings were counted (once every two weeks).

Although seed dispersal has not been directly observed, the seeds are probably shaken from the seed capsules and fall a short distance from the parent plant. The seeds are light and could possibly be dispersed short distances by the wind.

Seedlings are inconspicuous in the field. Most individuals familiar with the species suspect that seedling establishment is inhibited by an increase in grass and forb cover. Rhizomatous grasses may be a serious threat to seedlings. However, Pearson and Dunwiddie (2003) found no relationship between site preparation (burned, scarified, and control) and seedling germination, however, those plots with dense thatch, moss, and lichen cover did have lower numbers of seedlings. Under ideal conditions (*i.e.*, in pots in a greenhouse and occasionally in the field), seedlings can mature and flower in a single growing season (Wentworth 1994, T. Kaye, pers. comm. 2001, S. Reichard pers.comm. 2001, P. Dunwiddie pers.comm.. 2004).

Wentworth's (1994) population characteristics study of *Castilleja levisecta* found that the smallest plants had the greatest mortality. Individual plants sometimes regressed from a larger size class to a smaller one. Large reproductive plants were the most likely to regress in size. By regressing from reproductive size to a size too small for reproduction, individuals may be able to survive in years in which resources are limited. Regressing carries a certain risk, however, since small individuals suffer the greatest mortality (Wentworth 1998).

Threats to *Castilleja levisecta* include: invasion of habitat by woody trees and shrubs (as a result of fire suppression); invasion of habitat by nonnative plants; herbivory by mammals and invertebrates; roadside development for residential or commercial use; thatch, moss and lichen development (as a result of fire suppression); and trampling, picking, and collection at public sites (USFWS 2000b).

2.6 Population genetics

A study of the genetic structure of the existing populations of *Castilleja levisecta* was completed in 2003 (Godt *et al.* in manuscript). Material was gathered at all existing populations and allozymes were used to describe genetic diversity and structure in these eleven populations. The most significant results are summarized below. Further detail is available in Appendix C.

1) Despite its relatively narrow range, exceptionally high levels of genetic diversity are maintained in *Castilleja levisecta.*

2) Smaller populations tended to have fewer alleles and less genetic diversity.

3) There is a significant negative correlation between genetic identity and geographic distance, indicating reduced gene flow between populations, although this correlation was less strong when Rocky Prairie was removed from the analysis.

4) Rocky Prairie is one of the most genetically diverse and genetically divergent populations.

3.0 Objectives of reintroduction

In the recovery plan for *Castilleja levisecta* (USFWS 2000b), the authors state that the following conditions, among others, must be met before delisting can be considered:

1. There are at least 20 stable populations distributed throughout the historic range of the species. To be deemed stable, a population must maintain a five year running average population size of at least 1000 individuals

.

2. At least 15 of these populations must be on protected sites. In order for a site to be deemed protected, it must be either owned and/or managed by a government agency or private conservation organization that identifies maintenance of the species as the primary management objective for the site, or the site must be protected by a permanent conservation easement or covenant that commits present and future landowners to the conservation of the species.

One issue that was not resolved in the recovery plan is the exact definition of "1000 individuals". Non-flowering individuals are extremely difficult to count in the field. In most cases census data are based on number of flowering individuals, not on the number of plants. It may be most practical to assume that 1000 individuals are flowering individuals, which allows for a larger number of total individuals.

There are eleven known populations. Of those, only four currently meet the size criterion (if the size criterion is considered to be the number of flowering individuals) and of those, only three, Rocky Prairie, Ebey's Landing, and Trial Island, meet both the size and protection criteria (Table 1). Several sites have less than 200 plants. Lands owned by public agencies or conservation organizations have already been searched, so if new populations are found, they are likely to be on unprotected land. A combination of protection, habitat improvement, and reintroduction will be necessary to meet the requirements of the recovery plan. Protection and habitat improvement have been addressed in the recovery plan (USFWS 2000b). Augmentation, reintroduction, and introduction are discussed below.

3.1 Augmentation of existing populations

All of the existing populations under 1000 plants are priorities for augmentation, although issues of degree of protection and population viability must be considered prior to augmentation. In some cases, the land may not be adequately protected to justify augmentation. In addition to their lack of protection, several sites may also be too small to support populations of sufficient size and extent to be viable. However, protection and augmentation of existing populations should, in general, be a higher priority than the creation of new populations (IUCN 1987, USFWS 2000a). The genetic analysis (Appendix C) suggests that inter-population augmentation is not necessary at this time to increase the genetic diversity of the extant populations, which have maintained a relatively high degree of genetic diversity. The type of augmentation, methods, and monitoring plan should be clearly identified in the site specific reintroduction plan (Table 3) prior to placing plants or seeds on the site.

3.2 Reintroduction to sites within the historic range

Reintroductions of *Castilleja levisecta* to sites within its historic range fall into two categories: *reintroducing* plants to sites from which they were formerly known, and *introducing* plants to suitable sites within the historic range for which we have no evidence of past populations. We recommend the precedence of sites from which populations were formerly known over sites with no evidence of past populations, if other factors are equal. Subtle factors may be present at the historic sites that could increase the likelihood of success for the reintroduced population.

If all the known sites were to be protected and augmented, and no new sites were found, meeting the conditions of the recovery plan would require the creation of nine new and stable populations on protected land. Note that the recovery plan specifically calls for populations throughout the historic range of the species, which includes southwestern Washington and the Willamette Valley of Oregon. Since there are no extant populations south of Thurston County, Washington, reintroductions to southwestern Washington and the Willamette Valley of Oregon are necessary to meet the conditions of the recovery plan.

3.3 Definitions of reintroduction success

Although the recovery plan for *Castilleja levisecta* did not offer a definition of reintroduction success *per se*, the first recovery criterion specifies that a population will be considered stable (whether natural, augmented or reintroduced) if it has maintained a five year running average of at least 1000 individuals. Whether this number refers to all plants or flowering individuals is open to interpretation, though given the difficulties of census for all plants, it may be considered to practically refer to flowering individuals. The history of the known populations has shown that even relatively robust populations can experience precipitous declines within one or two decades (Gamon *et al*. 2001), which suggests that more than 1000 individuals would be preferable.

Pavlik (1996b) describes short-term reintroduction success as the point where a new population is able to carry on its basic life history processes of establishment, reproduction, and dispersal, such that the probability of complete extinction by random or chaotic forces is low. Long term success of a new population occurs when the population is as capable as its natural equivalent of integrating fully into ecosystem function and meeting the challenge of a changing environment through evolution or migration. He suggests that the objectives for reintroduction should consider **abundance** (establishment, increase in effective population size, fecundity, full life cycle can be completed), **extent** (increased spatial extent, establishment outside planted areas, satellite groups established), **resilience** (resistance to perturbation, seed bank density similar to natural populations), and **persistence** **(**utilization of multiple microhabitats, utilization of native pollinators**)** in the long term. This can be evaluated in both the short term (5-10 years) and the long term, though resilience and persistence cannot be fully evaluated in the short term.

3.4 Potential negative consequences of reintroduction attempts

The recovery plan for *Castilleja levisecta* specifies that the potential consequences of reintroduction must be addressed in this reintroduction plan (USFWS 2000b). Potential negative consequences of reintroduction include the following: a) a focus on reintroduction could supercede or dilute emphasis on the protection and habitat management of existing populations, b) reintroduction attempts could result in populations that appear stable after five years but are not stable or viable over longer time periods, and this could lead to premature delisting or downlisting of the species, or c) hasty reintroductions or augmentations without a thorough understanding of the genetics of the species could result in genetically depauperate new populations or genetically contaminated natural populations.

These potential negative consequences, if anticipated, can be controlled or diminished. The technical team (Table 5) should review all projects relevant to the recovery of *Castilleja levisecta* on an annual basis, and make recommendations that renew the primary commitment to the protection and habitat management of natural populations. The technical team can choose to extend the period of evaluation of all populations beyond five years before making recommendations for downlisting or delisting; and the technical team can request the destruction or flower removal of any experimental population to prevent the dispersal of unwanted genetic material.

4.0 Strategic and managerial considerations

4.1 Precedence of natural populations over the creation of new populations

The recovery plan and the literature on reintroduction both emphasize the importance of protecting and managing existing populations. Reintroduction is too uncertain to be the primary mode of recovery. To that end the technical team should review all projects relevant to the recovery of *Castilleja levisecta* on an annual basis, and make recommendations that renew the primary commitment to the protection and habitat management of natural populations.

The precedence of natural populations includes the precedence of augmentation of existing populations over the reintroduction of new populations, if the existing populations meet minimum standards of protection and habitat viability.

4.2 Experimental populations and full-scale reintroductions

"Experimental populations" are small populations created under strict experimental protocols to test reintroduction techniques and hypotheses, and which may be neither essential to nor necessarily intended to meet recovery objectives. These populations may be destroyed or transplanted at the end of the experimental work. Research plans should clearly specify the time period of research and the planned fates of the plants on the site. We recommend early coordination with the technical team (Table 5) to improve the design and feasibility of reintroduction experiments. NEPA consultation may be required in some cases (Section 4.4). The site should be re-evaluated at the end of the experiment by the researcher, project funders (if appropriate) and/or members of the technical team. We would hope that all researchers would conform to the guidelines in Table 2.

Most experimental populations will not need to be designed and placed according to the stricter reintroduction guidelines in this document. A primary issue, however, is preventing genetic contamination of existing populations or potential reintroduction sites with inappropriate genetic material. If there is some risk of genetic contamination, a researcher may be asked to prevent the experimental population from flowering and/or setting seed. Another issue is the critical importance of documenting all placements of seed or plants into natural habitats. Depending on the state or province in which the activity is occurring, the WNHP, BC Conservation Data Centre (BC CDC), or the Oregon Natural Heritage Program (ORNHP) should be notified and supplied with a map, a description of the project, and a research plan. Before the placement of any plant material as part of an experimental population, there should be a written agreement between the researcher, the landowner or land manager and (if needed) the USFWS, specifying:

1) Purpose of the experiment
2) Duration of the experiment
3) Plans for plants at the end of the experiment
4) Plans for the site at the end of the experiment

It may be important to include in such an agreement an understanding that golden paintbrush plants and seeds will not be taken off the site by the landowner, and that sale of plants and/or seed is illegal.

Table 2. Guidelines for experimental populations

1. Ideally, the research addresses one of the high priority questions in Appendix E. If another question is identified as a high priority, please contact the WNHP and/or the USFWS.
2. The research plan has been reviewed by the *Castilleja levisecta* technical team (Table 5).
3. The research has been reviewed and approved by the landowner/manager, and an agreement has been signed between the researcher, the landowner, and, if needed, the USFWS.
4. A copy of the research plan and map of the experimental population has been filed with WNHP (or BC CDC in British Columbia or ORNHP in Oregon) and the USFWS.
5. Seed collecting guidelines (Appendix A) are followed.
6. If appropriate, provisions are made and followed to prevent genetic contamination of the site by the experimental population (clipping inflorescences, etc.)
7. The results of the research are published or distributed as soon as possible.
8. The researchers clearly state the ultimate fate of plants and/or seed from the experimental population.
9. An annual report and population map is sent to the WNHP (BC CDC or ORNHP) and the USFWS.

In some cases, successful experimental populations may be used as part of a larger reintroduction attempt. However, if experimental populations are used as founders in a larger reintroduction attempt they should conform with a site specific reintroduction plan.

In order to optimize the chances of success for reintroductions that meet the objectives of the recovery plan, there are a number of critical research questions that must be answered. A list of high-priority research questions is in Appendix E. Many of these questions are best answered using experimental plantings. Note that experimental populations on federal land do fall under the jurisdiction of the ESA.

“Full-scale reintroductions”, on the other hand, are intended to meet the objectives of the recovery plan. They are likely to be much larger and more labor-intensive than experimental populations, and they should be carefully designed to maximize population growth and persistence. No full scale reintroductions should be planned prior to the completion of the site selection process and the appropriate site specific reintroduction plan.

At the conclusion of the experiment (as identified in the original agreement and plan) the plants used in the experiment should be either removed or used as partial founders for a full scale reintroduction, if the site meets the criteria for a full scale reintroduction. At that time a site plan should be written and the site should be fully protected. If the plants are removed, they could be used elsewhere as founders, used in other experiments, or destroyed. Issues of weed seeds and genetic contamination should be considered when moving plants from one site to another.

4.3 Conservation agreements and easements

Protection for a reintroduction site should be secured prior to reintroduction efforts. The economic and biological costs of reintroduction are too high to risk losing reintroduced populations after a change of management or ownership. The language in the recovery plan is quite specific: "In order for a site to be deemed protected, it must be either owned and/or managed by a government agency or private conservation organization that identifies maintenance of the species as the primary management objective for the site, or the site must be protected by a permanent conservation easement or covenant that commits present and future landowners to the conservation of the species" (USFWS 2000b). Permanent conservation easements, possibly held by the USFWS, would provide the most protection. Potential sites on federal land will be a high priority for reintroductions, since the ESA provides direct protection for listed plants on federal land.

4.4 NEPA

Researchers, land managers, and others planning introductions, reintroductions or experimental introductions should be aware that golden paintbrush projects receiving federal funds and/or projects taking place on federal land are subject to National Environmental Protection Act (NEPA) regulations. NEPA utilizes a systematic, interdisciplinary approach to planning and decision making for any project that may have an impact on the human environment. For any of these projects to proceed, an Environmental Assessment will be developed to address the need for the proposal, account for the environmental impacts and to consider alternatives to the proposed action. Public disclosure of the proposed actions and possibly public hearings may be required and will include input from stakeholders and any interested individual. Anyone planning a project that involves *Castilleja levisecta* may require NEPA and should coordinate with the USFWS as early in the planning process as possible, to avoid NEPA related delays or other difficulties

4.5 Site specific reintroduction plans

Each site proposed for reintroduction should have a site-specific reintroduction plan in place prior to the introduction of any material other than those used for experimental work. A thorough site-specific reintroduction plan would include all the information in Table 3. This plan should be reviewed by the technical team (Table 5) prior to implementation.

Table 3. Information to be considered in site selection and in site specific reintroduction plans

Site name
Site location
Persons responsible for completing reintroduction
Reasons site was chosen
Priority of site in the site selection process
Habitat characteristics
Disturbance
Level of herbivore activity
Degree of protection
Management
Size of site
Access (nearby roads, trails, etc.)
Ownership and protection
Landscape context of site
Nearby prairie and bluff habitats
Nearest population of *Castilleja levisecta*
Nearest population of other *Castilleja* congeners
Future development potential adjacent to site (zoning, etc.)
Reintroduction design
Target population size
Number and type (seed, nursery-grown) of propagules to be placed on site
Seed source
Site preparation
Spatial design of reintroduction (map)
Plan for repeated placement of propagules if necessary
Description of experimental design, if applicable
Monitoring plan
Type of monitoring
Monitoring techniques
Number of years of monitoring
Frequency of monitoring
Site management and restoration
Long and short term management and restoration goals
Planned management and restoration
Evaluation and monitoring of management activities
Adaptive management and information sharing
Interval between reports and re-evaluation of plan
Anticipated reports and other products

4.6 Time frame for reintroduction planning and implementation

Reintroduction is a long-term process. We anticipate that no large-scale reintroductions will be attempted prior to the completion of the site selection process, site specific reintroduction plans, and site protection.

Modeling has suggested that it may 20 or more years for reintroduced populations to develop demographic patterns similar to those of natural populations (Guerrant and Fiedler, 2004). A reintroduction and monitoring plan would ideally include at least a 20 year time frame.

4.7 Community restoration and management

Open natural habitats in western Washington and Oregon are either maintained through disturbance or through local scale environmental factors. All prairie and bluff systems have experienced some degree of invasion by exotic species and/or conversion to nonnative dominated systems (C. Chappell, pers. comm. 2001.). Community restoration and/or community management are necessary in any open system, and will need to be integrated into site specific reintroduction planning. Restoration of native vegetation is generally thought to increase the probability that the site will be able to support reintroduced self-sustaining populations (Sutter 1996). Mowing and burning are frequently used in grassland systems for community restoration or management, and may be beneficial for *Castilleja levisecta* as well, if conducted at the appropriate time of year (see Section 7.5).

The reintroduction of *Castilleja levisecta* will often be taking place within the context of other prairie restoration efforts. Although community restoration does not have to be integrated into the site-specific reintroduction plans, there should be active contact between personnel involved with reintroduction and those involved with restoration in order to prevent conflicting management directives, such as the following: a prairie restoration plan might call for the seeding of *Castilleja hispida*, which could potentially hybridize with *C. levisecta* and cause damage to the reintroduced population.

4.8 Experimental design and monitoring

Most reintroductions will have multiple objectives. The primary objective, in the case of a full-scale reintroduction, is the creation of a viable, self-sustaining population that meets the requirements of the recovery plan. The primary objective, in the case of an experimental population, is resolving the primary question posed by the researcher(s). However, in both cases it's likely that there will be secondary objectives as well. For instance, one may undertake a full-scale reintroduction and yet also be interested in the effects of fire on the establishment of seedlings. The primary and secondary objectives help determine the design of the project and largely determine the type of monitoring that will occur. However, it is critical to determine one's primary and secondary objectives prior to the commencement of the project, and to design the project and its monitoring accordingly.

Monitoring should be considered to be a component of introduction. Monitoring will be the most time consuming and expensive portion of any reintroduction, but without appropriate monitoring there will be no way of evaluating the success of the reintroduction and it will be difficult to identify factors that contributed to the success or failure of the project. The following recommendations are taken from Sutter (1996) and Pavlik (1996).

There are both short term and long term monitoring goals for reintroduction monitoring. In the short term, monitoring can document establishment of the population, survivorship, and basic life history processes. Long term monitoring can document responses to management activities, occurrence of recruitment, and natural trends and temporal variability in population size and processes. *Adaptive management* is an important component of monitoring: the identification of thresholds beyond which management activities will be re-evaluated.

There are many types of monitoring, from simple census or monitoring the result of a single management activity to full-scale demographic monitoring in which the fates of individual plants are followed through the entire life-cycle. In each case one will be weighing the costs and time of monitoring against the importance of the additional data. In general, we recommend at a minimum tagging the original cohort of plants so that maternal line survival and fecundity can be measured.

There are at least six criteria for successful monitoring:

1) a design which appropriately addresses the objectives for the monitoring
2) known and acceptable levels of precision,
3) repeatable and consistent data collection techniques,
4) a long enough period of data collection to capture important natural processes,
5) a design which is feasible, realistic, and inexpensive enough to be maintained over the long term, and
6) a design which minimizes the deleterious impacts of the monitoring itself to the monitored population and its habitat.

There are numerous resources in the literature for experimental and monitoring design and implementation (Sutter 1996, Elzinga *et al.* 1998). For reintroduction monitoring it is critical that the monitoring address the measures of success discussed in section 3.3: **abundance, extent, resilience** and **persistence.** Obviously, a reintroduction is not successful if plants are not able to reproduce and establish successive generations, so at a minimum a monitoring plan must be able *to identify the establishment of a second and subsequent generations, determine changes in the area occupied by the population, and determine trends in population size and number of seeds produced.* A simple annual count of flowering individuals will not provide all of the information needed to evaluate the success of the reintroduction, although it may be one portion of a monitoring plan. At a minimum, annual monitoring should include spatial extent of the population; sub-sampling to identify whether regeneration is taking place and the abundance of non-flowering individuals; and measures of vigor of all or a subsample of plants in the population, including number of stems, number of capsules, and average number of seeds per capsule.

It may also be appropriate to have some level of community monitoring, if community management or restoration is taking place. Finally, if populations begin to decline, one may want to undertake genetic monitoring of the remaining individuals to rule out a genetic cause for the decline.

How long does a reintroduced population need to be monitored? Sutter (1996) writes, "Only under the most optimistic situation – when a population has expanded, recruitment is common, and the natural processes in the community are active – will monitoring ever end. In most situations, monitoring will only be reduced in intensity or frequency."

5.0 Site selection

5.1 Site selection protocol

Although it is tempting to focus reintroductions on sites that are already protected or "seem" like good habitat, several reintroduction efforts have been highly successful in identifying reintroduction sites systematically, based on similarities to known successful sites (DeMauro 1994, Pavlik and Heister 1988). In general, potential reintroduction sites should match as closely as possible the habitat conditions of the source populations (Huenneke *et al.* 1986).

Demauro's method for the reintroduction of the lakeside daisy (1988) was a three step hierarchical screening process. Potential sites were initially identified on the basis of the appropriate geological substrate within the species' historic range. Disturbed areas (pasture, row crops, developments) were eliminated. The remaining areas were field surveyed and evaluated by comparing specific habitat characteristics to a site with natural populations: geology, soils, topography, aspect, hydrology, plant community type, and range of microhabitats. The sites identified through that process were then evaluated for size, amount of suitable habitat, habitat quality, and protection status.

Pavlik and Heister (1988) developed habitat factors for the natural populations, including slope, aspect, soils, level of disturbance, and community type. The technical team then visited the natural populations and subjectively ranked the relative importance of each of the habitat factors to the vigor of the population. Geographic boundaries were established that reflected the known historic range for the species. Areas that didn't conform to the most important habitat factors (in this case, soils, certain aspects, slope location, slope steepness, and degree of woody cover) were eliminated. The potential reintroduction sites that were identified were then further evaluated on the bases of the following criteria: land use history and ownership, road access (generally considered positive for logistical purposes), predominant aspect, disturbance and proximity to development, and size. For each site, each criteria was worth 0-3 points, with 3 being perfect and 0 being not acceptable. All sites were then visited and compared to the known populations, including soil testing. The owners of the sites that qualified were contacted and asked if they would be willing to have a reintroduction take place on their land. Four successful reintroduction sites were found through this process.

Because the habitat characteristics vary between portions of the historic range of *C. levisecta*, there will need to be a different site selection plan for each portion of the range: South Puget Prairies, Island and San Juan Counties, Southwest Washington, and the Willamette Valley. Site selection plans, as they are completed, will be on file with WNHP, ORNHP (where appropriate) and the USFWS.

5.2 Appropriate size and buffers

Studies have shown that fragmented areas can have reduced species richness and relative abundance of both vascular plants and pollinators (Jennerston 1988). Fire and other management tools become more difficult in small areas surrounded by development. Many nonnative plants invade open areas along the boundary between disturbed and native habitats, so adequate buffers must be in place to protect reintroduced populations from weed invasions. Prairies are also vulnerable to native tree invasion from forest/prairie boundaries.

A small, fragmented area of appropriate habitat will be less suitable for a long-term reintroduction attempt than a large, contiguous area, but we are not recommending a minimum size for a reintroduction site, given that some existing populations of *C. levisecta* have thousands of plants in an area of less than 2 acres. Moreover, in some portions of the historic range there may not be suitable large areas for reintroduction.

5.3 Native versus nonnative vegetation in the reintroduction site

Many of the extant populations of *Castilleja levisecta* occur in areas that are dominated by nonnative plants. Given a small number of suitable reintroduction sites, it may be appropriate to consider sites for reintroduction that are not currently dominated by native plants. If a site that is dominated by nonnative vegetation is chosen as a reintroduction site, it may be appropriate to include restoration and management of the plant community as part of reintroduction.

5.4 Landscape context in site selection

A reintroduction site occurs within a landscape matrix, so reintroductions should take into consideration the larger landscape. Are there other prairie or bluff areas nearby? How far away are they? How far is the nearest natural population of *Castilleja levisecta*? How far away are the nearest plants of any other species of *Castilleja*? What is the present and future level of development adjacent to the site?

6.0 Genetic and demographic considerations

6.1 Genetic structure of existing populations

A study of the genetic structure of the existing populations of *Castilleja levisecta* was completed in 2004 (Godt *et al.* in manuscript). Material was gathered from all existing populations and allozymes were used to describe genetic diversity and structure in these eleven populations. Further detail is available in Appendix C.

Allozyme studies alone cannot identify possible inbreeding depression, which is particularly likely in small populations or populations which have experienced a bottleneck event in the past. Allozyme studies are also unable to identify the possible effects of crossing plants from disparate populations, including the break-up of co-adapted gene complexes and subsequent outbreeding depression.

Tom Kaye (2003) conducted a study that examined possible inbreeding and/or outbreeding depression in *Castilleja levisecta*. He hand-crossed individuals and followed the progeny from pollination through 89 days of growth. The crosses were between self, sibling, within-population, and between-population. He found evidence of inbreeding depression in seed set and in plant growth and flowering. The highest seed set, greatest plant growth, and greatest rate of flowering was between individuals from separate populations, and the lowest seed set and lowest plant growth was from self-pollinated individuals. Sibling and within-population crosses were intermediate in their seed production and plant growth.

These results suggest outbreeding advantage rather than outbreeding depression, but as Kaye pointed out in his discussion, other studies have shown that expression of outbreeding depression may not be apparent in the F1 generation (Kaye 2003).

6.2 Genetic structure of reintroduced populations

The goal of reintroduction is to establish resilient, self-sustaining populations that retain the genetic resources necessary to undergo adaptive evolutionary change. There are two, seemingly contradictory strategies for the best kind of genetic structure for reintroduced populations: encouraging initially high levels of genetic diversity and heterozygosity in the reintroduced population, and/or taking advantage of co-adapted gene complexes which, in effect, refer to a well adapted internal genetic environment. Most reintroduction literature focuses on the first strategy, since even a reintroduction site in close proximity to a seed source site may have subtly different characteristics that require adaptation in the reintroduced population. **However, the ideal is to find a seed source which is BOTH genetically diverse and from a habitat that closely matches that of the reintroduction site.**

The primary reason that the literature emphasizes the importance of initial high levels of genetic diversity is to allow the new population to adapt to the unique characteristics of its site and be able to withstand unusual events or changes after planting (drought, insect infestations, and other calamities). If co-adapted gene complexes develop over time, one would expect some declines in genetic diversity in the decades after reintroduction, but these would be much less serious than initially low levels of genetic diversity.

Initial high levels of genetic diversity can be obtained through using seed sources from populations of high genetic diversity, through equalizing founder representation, through overlapping generations of founders, and through mixing seed sources from more than one site (Guerrant 1996b). However it is realized, the goal is to maximize the frequency of alternative alleles in the founding population (Pavlik *et al*. 1993). Tracking of maternal lines from seed collection through outplanting is usually recommended, since studies have shown that equalizing founder representation results in lower inbreeding and higher genetic variation than random founder representation. It may also be possible to further lower the risk of inbreeding by overlapping generations, i.e., by repeated founder events, rather than one single founder event (Loebel *et al*. 1992, cited in Guerrant 1996b). We strongly recommend that any full-scale reintroduction design includes this last technique.

Co-adapted gene complexes develop in response to a specific habitat (Guerrant 1996b). Choosing a reintroduction site that most closely matches the source site increases the likelihood that reintroduced plants will be genetically well-adapted to the site (Pavlik *et al*. 1993). Co-adapted gene complexes can be broken up by cross-breeding of plants from different populations, which can lead to outbreeding depression.

Guerrant (2003) suggests using caution in mixing populations in augmentation or reintroduction within the current range of *C. levisecta*. However, mixing seed from two or more populations may be appropriate in developing suitable material for reintroduction into the Willamette Valley and/or SW Washington. A common garden study in the Willamette Valley, utilizing seed from all Washington populations, is currently underway through the Institute for Applied Ecology (T. Kaye, pers. comm. 2003).

6.3 Number of founders for reintroduced populations

Minimum population size at founding is an important factor driving genetic losses. Smaller populations are more vulnerable to genetic bottlenecks, inbreeding depression, the effects of random genetic drift, environmental stochasticity and catastrophic losses (Barrett and Kohn 1991, Menges 1991). Since the goal in most reintroductions is rapid population growth, maximizing the number of founders is essential.

Modeling by Guerrant and Fiedler (2004) based on empirical data from reintroduction attempts shows a drop in population size after founding for four to 30 years, depending on life-cycle type. The degree of loss after founding also varied widely between species and type of propagule used in the reintroduction (seed, seedling, juvenile, and various size classes of adult). The most successful reintroductions would have required three to four times as many propagules as the target population (3000-4000 propagules for a stable population of 1000 plants). The least successful introductions would have required 500 times as many propagules as the target population (500,000 propagules for a target population of 1000 plants). In general, reintroduction projects that used young transplants had the lowest mortality and the greatest post-planting population growth.

These figures emphasize the importance of pre-reintroduction experiments to determine the mean losses after seeding or transplanting specifically for *Castilleja levisecta*. Nonetheless, it cannot be overemphasized that the establishment of stable populations of 1000 or more individuals may require a very large number of founders, particularly if seeds are the primary propagule.

6.4 Size-class of founders for reintroduced populations

Guerrant (1996b) has an extensive discussion and modeling of the use of various size classes in reintroductions. In general, populations founded with individuals of the smallest size class (i.e., seeds) were at a significantly greater risk of extinction than were those in any other size class. One of the goals of successful reintroductions is to create a population that grows as rapidly as possible, and populations founded with seeds grew least rapidly.

Because there is a risk of genetic alteration in *ex situ* populations in response to selection pressures and inbreeding (Templeton 1991, Pavlik *et al*. 1993), if transplants are used they should be from wild seed, unless the source population is too small to produce the necessary seed. Two potential problems with transplants, especially if used for augmentation, is the possibility of introducing new pathogens to wild populations or introducing foreign congeneric genes as a result of unintended cross-pollination (Guerrant 1996b). Greenhouse growers should make sure that *Castilleja levisecta* is isolated from all other species of *Castilleja* in the greenhouse, that plants from separate populations are isolated from each other, and that potting mixes are as sterile as possible.

Despite our recommendation against using seeds as founders, we recommend experimental seeding at all potential reintroduction sites prior to full-scale reintroductions. This will provide some assurance that seeds can successfully germinate on the site. Pearson and Dunwiddie (2003) had some success on Thurston County prairies with high rates of seeding (1000 seeds per meter). However, even in this case only 0.3% of the seeds germinated in the field.

6.5 Distance between populations

Due to the possible dangers of outbreeding depression, reintroduced populations should not be within range of pollen flow of populations that were <u>not</u> seed sources for the reintroduction (Guerrant 2003). Estimates of pollen flow distance have varied widely in the literature (Ellstrand

1992 cited in Guerrant 1996b). NatureServe considers a minimum separation distance between "element occurrences" (populations) to be greater than 1 km. (The Nature Conservancy *et al*. 1999). Bumblebees, the presumed primary pollinator of *C. levisecta*, generally forage within 5 km of the nest, but distances as far as 20 km have been recorded (Smith 2004). This suggests that experimental and reintroduced populations should be at least 3 miles from the nearest natural population which was not used as a seed source, and preferably at least 5 miles from the nearest natural population which was not used as a seed source.

It may be appropriate to create a metapopulation dynamic within areas used for reintroductions, or between reintroduced populations and seed source populations. Multiple subpopulations derived from the same seed source(s) that are within pollination distance of one another reduce the risk of catastrophic loss of a reintroduction attempt and reduce the effects of random genetic drift. Each subpopulation will lose some alleles randomly, but they are less likely to lose all the alleles at a particular locus than would a single, freely breeding population (Guerrant 1996b).

6.6 Spatial distribution within reintroduced populations

We recommend experimental plantings to test a variety of distributions. However, there are several advantages to multiple dense clusters. *Castilleja levisecta* can grow in high densities in natural populations. Fencing, tagging, and tracking plants are easier in clustered subpopulations, and pollinator foraging may be enhanced. There may be some positive metapopulation effects as well (see above).

6.7 Seed sources for reintroduction

In general, an ideal natural seed source for reintroductions is one that is:

1) the closest geographically,
2) from a population whose habitat matches that of the reintroduction site,
3) genetically diverse, and
4) can be collected without undo harm to the natural population.

In actuality, it may be necessary to compromise on one or more of these criteria. "Collecting without undo harm to the natural population" is the one which is least likely to be compromised except under extraordinary circumstances, since the recovery plan (USFWS 2000b) is specific that the protection and management of existing populations takes precedence over the creation of new populations. Appendix A contains seed collection guidelines.

6.8 Seed sources for augmentation

For augmentation of existing populations, the seed source will be from the existing population itself, in order to prevent outbreeding depression caused by the break-up of co-adapted gene complexes. In some cases it may be desirable or necessary to use seed from other sources, particularly if the existing population is shown through experimental work to be suffering from inbreeding depression or is no longer producing seed.

6.9 Appropriate range

The appropriate range for reintroduction projects is the historic range of the species. This range would include prairie and bluff habitats (and to a lesser extent, balds) in the Puget Trough and Willamette Ecoregions in Oregon, Washington, and British Columbia, to the southern and northern extent of historic collections. At the discretion of the technical team this range could be further narrowed or increased on the basis of soils, climate, or political boundaries. If the potential for reintroduction success is high at a site somewhat outside the historical range, the technical team could consider an expansion of the range.

7.0 Ecological considerations

7.1 Physical and ecological characteristics of sites

All known sites need to be carefully evaluated for their physical and ecological characteristics, prior to site selection (Table 4). This information will then be used in the site selection process.

Table 4. Physical and ecological characteristics of natural populations

- Soils
    - Texture and degree of compaction
    - Chemistry
    - Mapped type
- Substrate
    - Depth to bedrock
    - Bedrock type
- Hydrology and soil moisture regime
- Wind
- Salt spray
- Plant associates
- Aspect
- Elevation
- Latitude
- Slope and slope stability
- Cryptogam cover
- Open soil
- Past natural disturbance processes (fire, slope movement, herbivory)

7.2 Pollinators

Little is known about the pollinators of *Castilleja levisecta* . Lack of pollinators could inhibit reintroduction attempts. If possible, observations and identifications of pollinators in natural populations should take place. At this point, *Bombus* has been the only observed pollinator (Evans *et al.* 1984). *Bombus* requires grassy thatch and abandoned rodent burrows for nesting, so habitat management that includes mowing and/or burning should take into account the importance of maintaining areas for potential nest sites.

7.3 Host plants and mycorrhizal associates

The role of host plants in the propagation and outplanting success of *Castilleja levisecta* is still being investigated (see section 2.5 in this document). Nonetheless, preliminary evidence suggests that host plants may increase the size and outplanting success of *C. levisecta*. Reintroduction experiments should consider including the use of host plants, at least as one possible treatment, to clarify the importance of this variable in enhancing the success of reintroductions.

Some native plant restorationists have begun using mycorrhizal inoculation for plants that will be used for restoration. Healthy fungal colonization can increase outplanting success through increased nutrient uptake, reduced transplant shock, lowered drought stress and root disease, improved soil chemistry and rhizosphere environment (Bitterroot Restoration 2001). Reintroduction experiments should consider including mycorrhizal inoculation by material from natural populations as one possible treatment to clarify the importance of this variable in enhancing the success of reintroductions.

7.4 Herbivory

Herbivory has been observed at all sites, and in some cases is one of the most important factors affecting current *Castilleja* survival. Herbivory by deer, rabbits, voles, and invertebrates has been observed. Fencing and vole trapping have had a positive effect in reducing herbivory (P. Dunwiddie, pers. comm. 2001). The presence of high concentrations of herbivores on a site may make it less feasible as a reintroduction site without a commitment to extensive and long-term herbivore control. Herbivore control should be considered for all site-specific reintroduction plans.

7.5 Ecosystem processes

Ecosystem processes are dynamic processes that include natural disturbance (fire and slope movement), climate variation, long term climate change, and natural succession.

Fire: Three studies by Dunwiddie *et al*. (2001) examined the effects on *Castilleja levisecta* of burning at Rocky Prairie over an 11 year period. They found that populations generally respond positively for several years following burning. Increased survival rates, increased germination and/or seedling survival, increased flowering, and increased seed production were observed after burning. Population increases were observed for 6 or more years after burning, although populations had declined again by the 11^{th} year after burning. Various conditions appear to affect the response of the species to burning, including the timing of the burn and post-burn climatic conditions and herbivory. Some noxious weeds and/or nonnative species may increase after burning. These results suggest that carefully designed burning may be an important aspect of population management in both existing and reintroduced populations.

An accidental fire on July 4, 2001 at the Ebey's Landing population resulted in extensive mortality in approximately 1/3 of the spatial extent of the population, and there has been no regeneration to date (P. Dunwiddie, pers. comm. 2004). This negative response may have been due to the intensity or the timing of the burn. Experimental burning has not taken place in mid-summer.

Mowing: Mowing may be a more practical alternative at sites which cannot support prescribed burning. Studies in Oregon prairies have shown that mowing can be used as a tool to shift grass-dominated communities toward native grasses (Wilson and Clark 2001). There is some evidence from a golden paintbrush population in San Juan County that fall mowing may spread golden paintbrush seed and encourage dispersal within appropriate habitat. Timing of mowing in golden paintbrush populations is crucial : mowing between early March and mid-August is likely to lead to some loss in reproductive capacity, while fall mowing may encourage the spread of seed.

Slope movement: Slope movement has not been directly studied in any of the San Juan or Island County populations, but augmentations or reintroductions into similar habitats may need to consider slope stability. An experimental planting at Perego's Lagoon in 2001 on a steep, sandy slope did experience small scale erosions on the slope, and second year mortality was high (P. Dunwiddie, pers. comm. . 2004) On the one hand, the steep, unstable slopes are a barrier to colonization by woody vegetation. On the other hand, a slope failure could result in the catastrophic loss of a reintroduced population and slopes are high stress environments for seedlings and transplants. Movement across steep slopes for planting and monitoring could also cause increased erosion and instability. Bluff sites may need to be evaluated by a geomorphologist for potential for failure before reintroduction is attempted.

Climate variation: Natural climate variations can have catastrophic effects on reintroduced populations (see DeMauro 1994). Site specific reintroduction plans should have plans for monitoring and supplemental watering in case of extreme drought, or be prepared for potentially high mortality in the population.

Long term climate change: Current models of global warming cannot predict its effects on local areas. However, general warming, more severe climate oscillations, and more severe storm events are expected (Kuttner and Morse 1996). Populations at the southern end of a species range or on exposed, droughty soils may be particularly at risk. This raises concerns about reintroductions to the Willamette Valley and to droughty sites throughout the range of *Castilleja levisecta*. Maintaining high levels of genetic variability may be important in buffering populations from losses due to climate change. Weeds may also increase as a result of climate-induced disturbance and disruption of natural communities.

Succession: In the recovery plan for *Castilleja levisecta*, the authors state, "Historic loss of prairie and grassland habitat in the Puget Trough reduced the range of *Castilleja levisecta*, and habitat loss continues to be the primary threat to remaining populations. Currently, the primary cause of habitat modification is encroachment by native and nonnative woody species." (USFWS 2000). The conversion of prairies to shrubland or forest as a result of fire suppression is widespread. Invasive nonnative shrubs, particularly Scot's broom (*Cytisus scoparius*), have complicated the management of prairie habitats. Any site reintroduction plan will need to provide for specific, long-term control of native and nonnative woody vegetation. Various management techniques are possible, including fire, mowing, herbiciding, and pulling. Herbicides are not recommended in the immediate vicinity of *C. levisecta* plants.

8.0 Technical considerations

8.1 Seed collection

Seed collection guidelines have been developed for *Castilleja levisecta* (Appendix A). The primary purpose of these guidelines is to conserve the known populations of *Castilleja levisecta* . If we collect only the seed we need and if we are able to track the levels of seed collection that occur in each population over a number of years we can minimize the risk of inadvertently over collecting. Secondly, these guidelines are designed to standardize the sampling of genetic material throughout a population. Since many researchers will be left with extra seed and/or plants at the end of an experiment, it is helpful to collect and document at a higher level than may be called for by a particular experimental design**.** It is important that any time seed is collected, on public or private land, documentation is filed with the Washington Natural Heritage Program and the U.S. Fish and Wildlife Service, in order to track seed and degree of collecting from natural populations. At this point we recommend collecting and propagating seed in such a way that maternal line can be tracked through outplanting.

8.2 Propagation protocols

Propagation of *Castilleja levisecta* has been successfully accomplished with seeds collected from wild populations. Kaye (pers. comm. 2001) suggests the following propagation protocols.

Germination
The following germination procedure was developed with seeds collected from all US populations of C. levisecta, and proved highly successful (28%-88%) for seeds from most sources (Kaye 2001).

1. Seed germination is highest from seeds collected within one year of germination (Wentworth 1994), and appears to be higher from seed collected before October (based on the results of 2000 and 2001 seed collections).

2. Place seeds on fine, loose organic potting soil (in pots or flats), sand, or on germination paper.

3. Cold-stratify seeds at 5 °C for up to eight weeks to break dormancy (some seeds may germinate during cold stratification). Shorter periods of stratification may also work, and any temperature between 1 °C and 10 °C may be adequate.

4. After stratification, place seeds in a warm environment, such as alternating 15 °C/25 °C.

Ex situ *cultivation*

1. After germination, seedlings are very small and fragile. Care should be taken to handle them gently and keep them moist.

2. Plant seedlings in loose potting soil in flats or individual pots.

3. Plants grow well in a heated greenhouse under lights. Seedlings remain very small (<0.5 cm) for up to two months, then begin to grow more rapidly.

4. Because the species is a hemi-parasite, it may be advisable to grow the plants in pots with suitable host plants. Host plants used to date include *Festuca roemeri* and *Eriophyllum lanatum*. Paintbrush plants grown with *E. lanatum* were larger than those grown alone or with *F. roemeri* in one experiment (Kaye 2001). In another experiment (Reichart, unpublished data), plants grown with *Festuca* had higher survival after out-planting than those grown with *Eriophyllum* or alone. Therefore, companion planting with a host plant is recommended both for short and long-term planting success. Dunwiddie (pers. comm. 2004) suggests that planting with a host should perhaps be delayed until after seedlings have grown for some time, to minimize early competition with a host plant.

5. Plants in pots have been grown successfully in greenhouse, shadehouse, and full sun environments. Plants should not be kept in pots any longer than necessary, and if possible should be outplanted in the fall after the first season of growth. High mortality and competition with hosts has been observed in plants left in pots for more than one year, and there may be selection for plants that thrive in pots.

Reichard (pers. comm. 2001) used these protocols:

1. The seed is cleaned and sown on top of a damp standard seedling mix, covered with a propagation dome to keep moist and placed at 40^{o} F for 8 weeks.

2. After stratification the flats are put into the greenhouse.

3. The plants are fertilized with Petersons All Purpose fertilizer two times a week for normal plant growth. Some of the plants are watered every time with the fertilizer (high nitrogen treatment).

4. Once the seedlings are up to an inch tall they are pricked out and potted up in individual 4-inch pots and placed in a soil with high pumice content for good drainage.

5. They are placed in the shade house for at least one week, then moved to a protected area that receives full sunlight.

6. Haustorial connections form in September and October.

7. All fertilizer treatments are stopped in September to allow the plants to go dormant.

8. Increased nitrogen, with or without a host, appears to increase the plants' survival after outplanting.

8.3 Planting considerations

Outplanting experiments in 2001 through 2003 have shown that fall outplanting leads to higher survival rates of transplants than spring outplanting (P. Dunwiddie, pers.comm.. 2003). Mortality also increases if plants are left in pots for longer than one year (T. Kaye, pers. comm. 2003). In gravelly prairies, frost heaving may push transplants out of the ground, so plants should be planted with the caudex well below ground level (S. Pearson, pers. comm. 2003). Host plants should be from seed collected at or near the planting site.

8.4 Post-planting maintenance

Not much is known about post-planting maintenance. Possible causes of mortality include drought stress and competition from grasses and weeds, so decreasing these stresses may increase survival of transplants. Exclosures may be necessary to reduce herbivory and mowing and/or burning may decrease thatch and competition (see section 7.5).

9.0 Summary of Recommendations

General

- A combination of protection, habitat improvement, and reintroduction will be necessary to meet the requirements of the recovery plan.

- The appropriate range for reintroduction projects is the historic range of the species.

- Since there are no extant populations south of Thurston County, Washington, reintroductions to southwestern Washington and the Willamette Valley of Oregon will be necessary to meet the conditions of the recovery plan.

Augmentation

- All the existing populations under 1000 plants are priorities for augmentation.

- Protection and management of existing populations should be a higher priority than the creation of new populations.

- The type of augmentation, methods, and monitoring plan should be clearly identified in the site specific reintroduction plan prior to placing plants or seeds on the site.

- The precedence of natural populations includes the precedence of augmentation of existing populations over the reintroduction of new populations, if the existing populations meet minimum standards of protection and habitat viability.

Technical team

- The technical team should review all projects relevant to the recovery of *Castilleja levisecta*, and make recommendations that renew the primary commitment to the protection and habitat management of natural populations.

- The technical team can choose to extend the period of evaluation of all populations beyond five years before making recommendations for down listing or delisting.

Experimental populations

- Until the appropriate site specific reintroduction plans are completed, all reintroduced populations will be considered experimental populations.

- The WNHP (or ORNHP or BC CDC) and the USFWS should be notified and supplied with a map and a description of the project.

- We recommend that the technical team review and approve all experimental populations.

- If experimental populations are used as founders in a larger reintroduction attempt they should conform with a written and approved site specific reintroduction plan.

- We recommend experimental seeding at all potential reintroduction sites prior to full-scale reintroductions, to provide some assurance that seeds can successfully germinate on the site.

- Those designing reintroduction experiments should consider including the use of host plants, at least as one possible treatment, to clarify the importance of this variable in enhancing the success of reintroductions.

- Those designing reintroduction experiments should consider including mycorrhizal inoculation by material from natural populations as one possible treatment to clarify the importance of this variable in enhancing the success of reintroductions.

- The technical team may request the destruction or flower removal of any experimental population to prevent the dispersal of unwanted genetic material.

- A written agreement should be drawn up with the land owner/manager, the researcher, and the USFWS (if needed) prior to the placement of any material on the site.

- NEPA may be required. Contact the USFWS as early in the process as possible.

Full-scale reintroductions

- No full scale reintroductions should be planned prior to the completion of the appropriate site selection process, and the site specific reintroduction plan.

- The WNHP and the USFWS should be notified, allowed to review the reintroduction plan, and supplied with a map and a description of the reintroduction project.

- Protection for a reintroduction site should be secured prior to reintroduction efforts.

- We recommend that the technical team review and approve all full-scale reintroductions.

- NEPA may be required. Contact the USFWS as early in the process as possible.

- A written agreement should be drawn up with the land owner/manager, the researcher, and the USFWS (if needed) prior to the placement of any material on the site.

Site-specific reintroduction plans

- All known sites need to be carefully evaluated for their physical and ecological characteristics, prior to site selection.

- Each site proposed for reintroduction should have a site-specific reintroduction plan in place prior to the introduction of any material, which should be reviewed by the technical team and on file with the Washington Natural Heritage Program.

- A reintroduction and monitoring plan would ideally include at least a 20 year time frame.

- There should be active contact between personnel involved with reintroduction and those involved with restoration in order to prevent conflicting management.

- If a site that is dominated by nonnative vegetation is chosen as a reintroduction site, it may be appropriate to include restoration and management of the plant community as part of reintroduction.

- If possible, observations and identifications of pollinators in natural populations and reintroduction sites should take place.

- Site specific reintroduction plans should have plans for monitoring and supplemental watering in case of extreme drought

- Any site reintroduction plan will need to provide for specific, long-term control of native and nonnative woody vegetation.

- Herbivore control should be considered for all site-specific reintroduction plans.

Monitoring plans

- A monitoring plan must, at a minimum, be able to identify the establishment of a second and subsequent generations, determine changes in the area occupied by the population, and determine trends in number of seeds produced.

Site selection

- Potential reintroduction sites should match as closely as possible the habitat conditions of the source populations.

- We recommend the precedence of sites from which populations were formerly known over sites with no evidence of past populations, if other factors are equal.

- Carefully designed burning may be an important aspect of population management in both existing and reintroduced populations.

- Bluff sites may need to be evaluated by a geomorphologist for potential for failure before reintroduction is attempted.

Genetic considerations

- The goal of reintroduction is to establish resilient, self-sustaining populations that retain the genetic resources necessary to undergo adaptive evolutionary change.

- A seed source is ideally both genetically diverse and from a habitat that closely matches that of the reintroduction site.

- Garden experiments to test for inbreeding depression are highly recommended, and garden experiments for outbreeding depression are essential before plants or seed from different populations are placed on the same site. However, garden experiments should be designed to prevent seeding of any potential reintroduction site with inappropriate seed.

- Reintroduced populations should be shielded from possible pollen sources of other species of *Castilleja*, either through choosing sites which are far from known populations of congeners, or through the removal of congeners before reintroducing *C. levisecta*.

- It may be possible to further lower the risk of inbreeding by overlapping generations, i.e., by repeated founder events, rather than one single founder event. We strongly recommend that any full-scale reintroduction design includes this technique.

- Since the goal in most reintroductions is rapid population growth, maximizing the number of founders is very important.

- Because there is a risk of genetic alteration in *ex situ* populations in response to selection pressures and inbreeding (Templeton 1991, Pavlik *et al*. 1993), if transplants are used they should be from wild seed, unless the source population is too small to produce the necessary seed.

- Greenhouse growers should make sure that *Castilleja levisecta* is isolated from all other species of *Castilleja* in the greenhouse, and that plants from different populations *Castilleja levisecta* are isolated. Potting mixes and greenhouse plants are kept free from weed seeds and exotic insects.

- Due to the dangers of outbreeding depression and genetic contamination, reintroduced populations should not be within range of pollen flow of populations that were <u>not</u> seed sources for the reintroduction. Five miles should be an adequate separation distance to prevent any pollen flow,

- It may be appropriate to create a metapopulation dynamic within areas used for reintroductions, or between reintroduced populations and seed source populations.

Seed source and seed collection guidelines

- In general, an ideal natural seed source for reintroductions is one that is: the closest geographically, from a population whose habitat matches that of the reintroduction site, genetically diverse, and can be collected without undo harm to the natural population.

- For augmentation of existing populations, in most cases the seed source will be from the existing population itself, in order to prevent possible outbreeding depression caused by the break-up of coadapted gene complexes.

- Follow the seed collection guidelines in Appendix A.

- It is important for the recovery of this species that any time seed is collected, on public or private land, documentation is filed with the Washington Natural Heritage Program and the U.S. Fish and Wildlife Service.

- Seed collection from public land will require a permit from the appropriate agency(s).

Propagation protocols

- Seed germination is highest from seeds collected within one year of germination.

- Because the species is a hemi-parasite, it may be advisable to grow the plants in pots with suitable host plants. Host plants that are used for transplanting should be from seed collected at or near the transplant site.

- Plants should not be kept in pots indefinitely, and if possible should be outplanted in the fall after the first season of growth.

Table 5. Appropriate entities to contact for information, advice, or to share information on golden paintbrush

Question	Program	Contact information
Where do I report finding a population of golden paintbrush?	**The Washington Natural Heritage Program***	**Florence Caplow, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**
I would like to engage in research involving golden paintbrush.	**Technical team**	**Florence Caplow, botanist, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**
I would like to reintroduce golden paintbrush to my land or to land I manage.	**Technical team**	**Florence Caplow, botanist, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**
I would like to write a grant to do golden paintbrush research or reintroduction.	**Technical team**	**Florence Caplow, botanist, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**
I'm interested in obtaining seeds or plants of golden paintbrush in order to benefit the species.	**The U.S. Fish and Wildlife Service, Lacey office**	**Ted Thomas or current Recovery staff member, 360-753-9440**
I will be doing seed collection of golden paintbrush.	**The U.S. Fish and Wildlife Service, Lacey office** **and the Washington Natural Heritage Program***	**Ted Thomas or current Recovery staff member, 360-753-9440.** **Florence Caplow, botanist, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**
I was given seed of golden paintbrush and I'm not sure what to do with it.	**The U.S. Fish and Wildlife Service, Lacey office**	**Ted Thomas or current Recovery staff member, 360-753-9440**
I'd like a copy of the recovery plan for golden paintbrush.	**The U.S. Fish and Wildlife Service**	**http://ecos.fws.gov/docs/ recovery_plans/2000/000823.pdf**
I'd like another copy of this document.	**The Washington Natural Heritage Program**	**http://www.dnr.wa.gov/nhp refdesk/plan/cale_plan.pdf**
I would like to contact the technical team or individual member of the team.	**Technical team**	**Florence Caplow, botanist, 360-902-1600 or go to http://www.dnr.wa.gov/nhp/ for current staff contacts**

For work in Oregon, contact Sue Vrilakis, Oregon Natural Heritage Program, (503)-731-3070; *for work in BC, contact the BC Conservation Data Centre, (250) 356-0928*

10.0 References

A. Literature Cited

Adler, L.S. 2003. Host species affects herbivory, pollination, and reproduction in experiments with parasitic *Castilleja*. Ecology 84(8):2083-2091.

Bailey, R.G. 1976. Ecoregions of the United States. USDA Forest Service, Ogden, Utah. Map.

Barrett, S.C.H. and Kohm, J.R.1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A. and Holsinger, K.E., editors. *Genetics and conservation of rare plants*. Oxford University Press, New York. pp 225-238.

Bitterroot Restoration Inc. [October 24, 2001] . Customized mycorrhizal inoculation. http://www.bitterrootrestoration.com/BRIWeb/plant_prop.html#1.

British Columbia Conservation Data Centre. 1993. Native vascular plant tracking lists. Copy on file in the office of the Washington Natural Heritage Program, Olympia, WA.

Center for Plant Conservation. 1996. Guidelines for developing a rare plant reintroduction plan. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp 453-490.

Crawford, R.C. and Hall, H. 1997. Changes in the South Puget prairie landscape. In: P. Dunn and Ewing, K. editors. *Ecology and conservation of the South Puget Sound prairie landscape*. The Nature Conservancy, Seattle, WA.

Cully, A. 1996. Knowlton's cactus (*Pediocactus knowltonii*) reintroduction. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 403-410.

DeMauro, M. M. 1994. Development and implementation of a recovery program for the federal threatened Lakeside daisy (*Hymenoxys acaulis* var. *glabra*). In: Bowles, M.L. and Whelan, C.J. *Restoration of endangered species*. Cambridge University Press, Cambridge UK. pp. 298-321.

Dunwiddie, P.W., Davenport, R. and Speaks, P. 2001. Effects of burning on *Castilleja levisecta* at Rocky Prairie Natural Area Preserve, Washington: a summary of three long-term studies. In: Reichard, S.H., Dunwiddie, P.W., Gamon, J., Kruckeberg, A.R. and Salstrom, D.L., editors. *Conservation of Washington's native plants and ecosystems*. Washington Native Plant Society, Seattle, WA. pp 161-172.

Ellstrand, N.C. 1992. Gene flow among seed plant populations. New Forests 6:241-256.

Elzinga, C.L., Salzer, D.W., and Willoughby, J.W. 1998. *Measuring and Monitoring Plant Populations.* U.S. Department of the Interior, Bureau of Land Management, National Applied Resource Sciences Center, Denver, CO. Report number BLM/RS/ST-98/005+1730.

Evans, S., Schuller, R., and Augenstein, E. 1984. A report on *Castilleja levisecta* Greenm. at Rocky Prairie, Thurston County, Washington. Unpublished report to The Nature Conservancy, Washington Field Office, Seattle, WA.

Falk, D.A., Millar, C.J., and Olwell, M., editors. 1996. *Restoring Diversity*. Island Press, Washington D.C..

Franklin, J.F. and Dyrness, C.T. 1973. *Natural vegetation of Oregon and Washington*. USDA For. Serv. Gen. Tech Rep. PNW-8. Pacific Northwest Forest and Range Experiment Station, Portland, OR.

Gamon, J. 1995. Report on the status of *Castilleja levisecta* Greenman. Unpublished report, Washington Natural Heritage Program, Dept. of Natural Resources, Olympia, WA.

Gamon, J., Dunwiddie, P.W., Thomas, T, and Rush, T. 2001. Assessing the viability of golden paintbrush (*Castilleja levisecta*). . In: Reichard, S.H., Dunwiddie, P.W., Gamon, J., Kruckeberg, A.R. and Salstrom, D.L., editors. *Conservation of Washington's native plants and ecosystems*. Washington Native Plant Society, Seattle, WA. pp 161-172.

Gordon, D.A. 1996. Apalachicola rosemary (*Conradina glabra*) reintroduction. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp.417-422.

Godt, M.J., Caplow, F. and Hamrick, J.L. 2004. Allozyme diversity in federally threatened golden paintbrush, *Castilleja levisecta*, (Scrophulariaceae). In manuscript.

Greenman, J.M. 1898. Some new and other noteworthy plants of the Pacific Northwest. Bot. Gaz. 25: 261-269.

Guerrant, E.O. 1996a. Experimental reintroduction of *Stephanomeria malheurensis.* In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 399-402.

Guerrant, E.O. 1996b. Designing populations: demographic, genetic, and horticultural dimensions. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 171-208.

Guerrant, E.O. 2003. Recommendations for reintroduction of *Castilleja levisecta* based on patterns of genetic diversity as revealed by allozyme data. Report on file with WNHP.

Guerrant, E.O. Jr., and Fiedler, P.L. 2004. Accounting for Sample Decline During Ex Situ Storage and Reintroduction. Pages 365-385 *In* Guerrant, E.O. Jr., K. Havens, and M. Maunder (eds.) *Ex Situ Plant Conservation: Supporting Species Survival in the Wild.* Island Press. Covelo, CA.

Heckard, L.R. 1962. Root parasitism in *Castilleja*. Bot. Gaz. 124:21-29.

Heunneke, L.F., Holsinger, K., and Palmer, M.E. 1986. Plant population biology and the management of viable plant populations. In: B.A. Wilcox, Brussard, P.F., and Marlot, B.G., editors. *Management of viable populations: theory, applications, and case studies*. Stanford University, Palo Alto, CA.

Hitchcock, C.L., Cronquist, A., Ownbey, M., and Thompson, J.W. 1959. *Vascular plants of the Pacific Northwest, Part 4: Ericaceae through Campanulaceae*. University of Washington Press, Seattle, WA.

Jennerston, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2(4):359-366.

Kaye, T.N. 2001. [December 26, 2001]. Restoration research for golden paintbrush (*Castilleja levisecta*), a threatened species. http://www.appliedeco.org/Reports/Cale research.PDF

Kaye, T.N. 1992. Ethics and guidelines for rare plant reintroductions (and a policy statement from the Native Plant Society of Oregon). Hortus Northwest 3:12-14.

Kaye, T.N. and B. Lawrence. 2003. Fitness effects of inbreeding and outbreeding on golden paintbrush (*Castilleja levisecta*): implications for recovery and reintroduction. Report on file with WNHP and Institute for Applied Ecology.

Kaye, T.N., Amsberry, K.A., Gisler, S.D., and Menke, R.J. 1998. The challenges of endangered plant reintroduction. Hortus West 9(1):5-11.

Küchler, A.W. 1966. Potential natural vegetation of the United States, including Alaska and Hawaii. In: U.S. Department of Interior, Geological Survey. National Atlas of the United States. pp. 89-92 [Map, in color, republished (1978) by USDA For. Serv. Washington, D.C.]

Kuttner, L.S. and Morse, L.M. 1996. Reintroduction in a changing climate. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 23-48.

Loebel, D.A., Nurthen, R.K., Frankham, R., Briscoe, D.A., and Craven, D.O. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equalizing founder representation. Zoo Biology 11:319332. Cited in Guerrant, E.O. 1996b. Designing populations: demographic, genetic, and horticultural dimensions. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 171-208.

McDonald, C.B. 1996. Texas snowbells (*Styrax texana*) reintroduction. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 411-416.

Mills, J.N. and Kummerow, J. 1988. Root parasitism in Indian paintbrush. Fremontia, October.

Pavlik, B. 1988. Nutlet production and germination of *Amsinckia grandiflora*. I. Measurements from cultivated populations. Unpublished report, California Department of Fish and Game. California Department of Fish and Game. Sacramento, Cal.

Pavlik, B. 1990. Reintroduction of *Amsinckia grandiflora* to Stewartville. Unpublished report. California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1991. Management of reintroduced and natural populations of *Amsinckia grandiflora*. Unpublished report, California Department of Fish and Game. Sacramento, CA..

Pavlik, B. 1991. Reintroduction of *Amsinckia grandiflora* to three sites across its historic range. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1992. Inching toward recovery: Evaluating the performance of *Amsinckia grandiflora* populations under different management regimes. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1993. Current status of natural and reintroduced populations of *Amsinckia grandiflora* under different management regimes. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1994. Tales of success and failure: Trends in natural and reintroduced populations of *Amsinckia grandiflora* under different management regimes. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1995. And the beat goes on: populations of *Amsinckia grandiflora* under different management regimes. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1996a. Populations of *Amsinckia grandiflora* (Large-flowered fiddleneck): Growth and decline under different management regimes. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. 1996b. Defining and measuring success. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp. 127-156.

Pavlik, B. and Espeland, E. 1991. Creating new populations of *Acanthomintha duttonii*. I: Preliminary laboratory and field studies. Unpublished report, California Department of Fish and Game. Sacramento, CA..

Pavlik, B. and Espeland, E. 1993. Creating new populations of *Acanthomintha duttonii*. III. Enhancement at Pulgas Ridge. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. and Espeland, E. 1994. Creating new populations of *Acanthomintha duttonii*. IV. Demographic performance at Pulgas Ridge and Eastwood Park. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B. and Heisler, K. 1988. Habitat characterization and selection of potential sites for establishment of new populations of *Amsinckia grandiflora*. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pavlik, B., Nickrent, D.L. and Howard, A.M. 1993. The recovery of an endangered plant. I. Creating a new population of *Amsinckia grandiflora*. Conservation Biology 7(3):510-531.

Pavlik, B., Espeland, E. and Wittman, F. 1992. Creating new populations of *Acanthomintha duttonii*. II: Reintroduction at Pulgas Ridge. Unpublished report, California Department of Fish and Game. Sacramento, CA.

Pearson, S.F., and Dunwiddie, P. 2003. Experimental seeding and outplanting of golden paintbrush (Castilleja levisecta) at Glacial Heritage and Mima Mounds, Thurston County, WA. Progress report on file with WNHP.

Piper, C.V. 1906. *Flora of the State of Washington*. Contrib. U.S. National Herbarium vol. 11; Bulletin (U.S. National Museum). U.S. Govt. Print. Office, Washington, D.C. .

Ryan, M. and Douglas, G.W. 1994. Status report on the golden paintbrush *Castilleja levisecta* Greenm. Unpublished, draft report prepared by the British Columbia Ministry of Environment, Lands and Parks. Victoria, B.C.

Sheehan, M. and Sprague, N. 1984. Report on the status of *Castilleja levisecta*. Unpublished report submitted to the U.S. Fish and Wildlife Service, Portland, Oregon.

Smith, L. 2000. [January 2, 2004). Foraging preferences. http://www.mearns.org.uk/mrssmith/bees/foraging.htm

Sutter, R.D. 1996. Monitoring. In: Falk, D.A., Millar, C.J. and Olwell, M., editors. *Restoring diversity*. Island Press, Washington D.C. pp 235-264.

Swenerton, K.K. 2003. Soil suitability and site preparation techniques for *Castilleja levisecta* restoration on Whidbey Island, Washington. Masters Thesis, Center for Urban Horticulture, University of Washington. Seattle, WA..

Templeton, A.R. 1991. Off site breeding of animals and implications for plant conservation strategies. In: Falk, D.A. and Holsinger, K.E., editors. *Genetics and conservation of rare plants*. Oxford University Press, New York. pp. 225-238.

The Nature Conservancy, the Association for Biodiversity Information, and the Network of Natural Heritage Programs and Conservation Data Centers. 1999. *Draft Element Occurrence Data Standards*.

U.S. Fish and Wildlife Service. 1990a. Endangered and threatened wildlife and plants: review of plant taxa for listing as endangered or threatened species. Federal Register 62: 6184-6229.

U.S. Fish and Wildlife Service. 1990b. Policy and guidelines for planning and coordinating recovery of endangered and threatened species. U.S. Department of the Interior.

U.S. Fish and Wildlife Service. 1994. Endangered and threatened wildlife and plants; Proposed threatened status for golden paintbrush (*Castilleja levisecta*). Federal Register 59(89): 24106-24111.

U.S. Fish and Wildlife Service. 1997. Endangered and threatened wildlife and plants: determination of threatened status for *Castilleja levisecta* (Golden Paintbrush). Federal Register 62(112): 31740-31748.

U.S. Fish and Wildlife Service. 2000a. Policy regarding controlled propagation of species listed under the Endangered Species Act. Federal Register 65: 56916-56922.

U.S. Fish and Wildlife Service. 2000b. Recovery plan for golden paintbrush (*Castilleja levisecta*). U.S. Fish and Wildlife Service, Portland, OR. .

Wentworth, J. B. 1994. The demography and population dynamics of *Castilleja levisecta*, an endangered perennial [thesis]. University of Washington, Seattle, WA.

Wentworth, J. B. 1996. Conservation Recommendations for *Castilleja levisecta* in Washington. Unpublished report, Washington Department of Natural Resources, Natural Heritage Program. Olympia, WA.

Wilson, M.V. and Clark, D.L. 2001. Controlling invasive *Arrenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegetation Science 4: 129-138.

B. Personal communication

Dunwiddie, P. 2004. The Nature Conservancy, Seattle, Washington.

Chappell, C. 2001. Washington Natural Heritage Program, Olympia, Washington.

Kaye, T. 2001-2003. The Institute for Applied Ecology, Corvallis, Oregon.

Pearson, S. 2003. Washington Natural Areas Program, Olympia, Washington.

Reichard, S. 2001. The Center for Urban Horticulture, Seattle, Washington.

Tepedino, V. 2001. Utah State University, Logan, Utah.

Wentworth. J. 1995. King County Noxious Weed Board, Seattle, Washington.

APPENDICES

Appendix A. Seed Collection Guidelines
for *Castilleja levisecta*
Reintroduction Experiments
October 2001

These guidelines have been developed to provide a framework for researchers who need to collect seed from natural populations of *Castilleja levisecta*. This process may seem tedious and time consuming, but it is designed to protect the future viability of *Castilleja levisecta* at a time when there is great interest in seed collection from our few remaining populations.

The primary purpose of these guidelines is to conserve our known populations of Castilleja levisecta. If we collect only the seed we need and if we are able to track the levels of seed collection that occur in each population over a number of years we can minimize the risk of inadvertently over collecting.

Secondly, these guidelines are designed to standardize the sampling of genetic material throughout a population. Since many researchers will be left with extra seed and/or plants at the end of an experiment, it is helpful to collect and document at a higher level than may be called for by a particular experimental design.

Thirdly, this document provides a structure for thinking through all aspects of seed collection, seed sources, fate of the plants grown from any seed, and data collected, BEFORE collecting any seed. We hope that this will be helpful for researchers and lower the amount of unnecessary seed that is collected

Fourthly, by keeping a database of all seed and reintroduction experiments, we can avoid duplication of effort among researchers and pool our knowledge of the biology of the species.

The process is divided into several steps. We request that anyone collecting seed from *Castilleja levisecta* populations follow these steps, document the answers to each question, and share their documentation and data with the Washington Natural Heritage Program (WNHP) and the land owner or manager of the populations from which you collect.

Step 1. Questions to answer before seed collection begins

These are derived from the questions suggested by Guerrant *et al*. (2003) and by the Center for Plant Conservation (1991). We suggest sitting down and writing out the answers to these questions, and giving the WNHP a copy of your answers for our files. Some answers will be quantitative and will be needed for calculating sample sizes.

A. Is it legal to collect seeds from my proposed site? Are permits required?

B. How much material (i.e. how many plants) do I need for my reintroduction experiment, after losses due to germination, transplanting, etc.?

C. What kinds of experiments will I be doing?

D. What will I do with any “extra” material? Seeds can be placed in long-term storage with both the Berry Botanic Garden and the Center for Urban Horticulture (CUH). Seeds or plants may be useful for other researchers.

E. What is the long term fate of the material I grow? Am I prepared to destroy plants at the end of my experiment, or do I hope to use them as part of a larger reintroduction attempt? Am I prepared to keep them from producing seed if they are new genetic material at a site?

F. Are there seeds and/or plants already available that I can use for my study?

G. Am I prepared to document every step of my work?

H. In what form will I publish or share my data?

For each population from which I wish to collect:

What are the known greenhouse germination rates for this population? This information may be available from the WNHP, CUH and/or The Institute for Applied Ecology (IAE). Most populations have had some germination testing.

What is the size of the population?

Are there any known monitoring or experimental plots within the population that I need to exclude from my sampling area? This may require checking with WNHP or with the land manager. In general, plants that are part of a demographic monitoring project should not be used as a seed source.

Is there topographic or other variation within the population?

What is the past collecting history and possible future collecting needs from the population(s)?

What are the population dynamics of this population (increasing, stable, declining)? This information may be available from the WNHP.

Step 2. Arriving at an appropriate sample size:

There are three general rules for sampling from small populations of 500 or fewer individuals (Menges *et al*. 2003):

Collect samples from 10-50 individuals per population, following a stratified random sampling regime.

Harvesting 10% of seeds in 10% of years is generally safe.

Harvesting 50% of seeds in 50% of years is generally unsafe.

Keep in mind that we may need to make large collections from some or all of these populations within the next five years for large-scale reintroduction efforts. Successful reintroduction efforts may require putting out as many as 10^3 propagules in relation to your target stable population size (DeMauro 1994). That means that if you want a population of 1000 plants you may have to put out as many as 100,000 propagules, and will almost certainly need to put out as many as 10,000 propagules. Direct seeding may require even more propagules. Most populations were used as seed sources in 2000, and several populations were also used as seed sources in 2001. This may influence your choice of populations to sample. Smaller populations are more vulnerable to over collecting.

If the main purpose of your study is to test germination and propagation protocols, consider using small collections of seed from the largest and most stable population.

For developing reintroduction protocols, begin with the smallest collections necessary to address the management questions being posed in the experimental reintroductions. Collect and maintain seed from each maternal line. Only in this way can representation of the different founders be known, and controlled intentionally (Guerrant and Fiedler in press).

Modeling of collecting from various populations shows that collecting from populations of greater than 500 plants (unless it is complete collecting) is generally not harmful. Less intense, frequent harvests are safer than more intense, infrequent harvests (Menges *et al*. 2003).

To identify the number of propagules you need to collect from the population, identify how much material (i.e. how many plants) you need for the reintroduction experiment, after losses due to low germination, transplanting, or other events. Multiply that number by the percent germination in the greenhouse of seeds from that population, and any other percent loss data you may have. If you want to have 100 plants and you know that there is a 20% germination for the population, you will want to collect at least 500 seeds. If you are collecting from 50 plants, that would be 10 seeds per plant. Estimates range from 135-165 seeds per capsule and 3-12 capsules per inflorescence (see section 3.0 in the Reintroduction Plan). Number of inflorescences can be as few as one per plant and as many as 46 per plant on extremely robust plants.

We do have greenhouse single year estimates of germination rates from many populations. These rates may differ from year to year, season of collection, and germination technique. We will know more as researchers continue to document germination rates.

There may also be grown out material at both CUH and at the Institute for Applied Ecology in Oregon, and seed stored at the Berry Botanic Garden. Consider using this material if possible.

Based on the above, what is your sampling size?

Step 3. Sampling design

A system of stratified random sampling combined with mapping the location of plants from which seed was collected is strongly recommended (CPC 1991, Guerrant and Fiedler in press). If mapping using tapes and a grid is too time consuming, consider using a GPS unit to map the plants from which you collected. This may be very helpful in experiments which are testing the fitness or germination variation within a population (and remember, your sample may be used by others). The larger and more diverse the spatial sampling area the better, for most purposes. If there is topographic variation within the population, make sure to include it in your sampling.

Based on the above and the particulars of the population, what is your sampling design?

Step 4. Documentation

Document, document, document!

At this point we believe that it is important to keep track of maternal lines. Unless seed from each maternal line is kept separate during collection, it will be impossible to equalize the contribution of different parent plants during a reintroduction experiment, or to understand the variation within the population. Future genetic work may show that this is not necessary, but for now we request everyone to keep track of maternal lines in seed collection and storage, and any experiments in which plants are grown out in the greenhouse. Tracking maternal lines in direct seeding is more difficult, but consider whether your experiment can be designed to keep track of maternal lines.

For each plant from which seeds are collected, record:
date of collection

location within stratified random grid
microsite characteristics
number of fruits collected
unique number

The unique number should follow seeds from collection through germination, transplanting, outplanting, and any further work.

Step 5. Determining the fate of plants

We recommend identifying the final fate of all material prior to collecting. If you have extra seed, how will it be handled and stored? If you have extra plants not used for outplanting, where will they go? What will happen to any outplanted experimental plants? Will they be allowed to flower? Will they be allowed to set seed? Will they be dug up and transplanted? Will they be destroyed? Will they be made available to other researchers?

Step 6. Data collection and data sharing

We ask that anyone who undertakes seed collection be meticulous in the collection of data on germination rates, growth, mortality, and effects of various treatments. Furthermore, we ask that anyone working with *Castilleja levisecta* publish or distribute the results of their work as soon as possible, so that their results may benefit long-term reintroduction efforts. Ideally, any work would be published in a peer-reviewed journal. The Washington Natural Heritage Program will keep files of all seed collections, raw data, and reports, and these will be available to other researchers. Copies will be sent to the Center for Urban Horticulture, the Washington chapter of The Nature Conservancy, and the U.S. Fish and Wildlife Service.

Literature cited

Guerrant, E.O. and P. L. Fiedler. 2003. Revised genetic sampling guidelines for conservation collections of rare and endangered species. In: *Ex situ plant conservation: supporting species survival in the wild.* Eds. E. O. Guerrant Jr., K. Havens, and M. Maunder. Island Press. Covelo, CA.

DeMauro M.M. 1994. Development and implementation of a recovery program for the federal threatened lakeside daisy (*Hymnoxys acaulis* var. *glabra*). In *Restoration of Endangered Species.* Eds. M.L. Bowles and C.J. Whelan. pp. 298-321. Cambridge University Press. New York..

Center for Plant Conservation. 1991. Genetic sampling guidelines for conservation collections of endangered plants. In *Genetics and Conservation of Rare Plants.* Eds. D. A. Falk and K.E. Holsinger, pp. 225-238. Oxford University Press. New York.

Menges, E.S., E.O Guerrant, and S. Hamze. 2003. What is the effect of seed collection on extinction risk of perennial plants? In: *Ex situ plant conservation: supporting species survival in the wild.* Eds. E. O. Guerrant Jr., K. Havens, and M. Maunder. Island Press. Covelo, CA.

Appendix B. Site selection process
For *Castilleja levisecta*

STEP 1 Area Identification

There are three primary areas within the range of *Castilleja levisecta*: 1) the islands in Puget Sound, 2) the gravelly prairies of southern Puget Sound, and 3) the Willamette valley area, including southwestern Washington. Each area should be evaluated separately.

STEP 2 Existing site characterization

Within each of the three areas, characterize the known or historic sites based on the following:

annual precipitation
elevation
slope
aspect
salt spray influence
geologic substrate
soils
level of disturbance
community type
degree of woody cover

STEP 3 Boundary identification for each area

Identify the outer boundaries of each area, based on environmental or political factors (i.e. counties of historic collections, area of appropriate precipitation, elevation, etc.).

STEP 4 GIS analysis

Conduct a GIS analysis of the identified areas, based on, at least, soils and community type and identify a list of candidate sites.

STEP 5 Priority lists for each area

Refine and develop a priority list of candidate sites for each area based on the following:

size
proximity to roads
disturbance
potential for development adjacent to site
current protection level of site
current ownership of site
similarity to known sites
A numeric ranking system for various environmental and political factors may be appropriate.

STEP 6 Field visits

Visit each high-priority site and evaluate further.

STEP 7 Protection process

Begin the protection process for the site(s).

STEP 8 Site specific reintroduction plan

Write a site-specific reintroduction plan for the site(s).

STEP 9 Implementation

Implement the site specific reintroduction plan.

Appendix C. Recommendations based on genetic analysis

Recommendations for reintroduction of *Castilleja levisecta* based on patterns of genetic diversity as revealed by allozyme data .

April 10, 2003

Submitted to Washington Department of Natural Resources Natural Heritage Program

Prepared by

Edward. O. Guerrant Jr.
Berry Botanic Garden
11505 SW Summerville Avenue
Portland, OR 97219

503 636-4112 ext. 29

edguerr@rdrop.com

Recommendations for reintroduction of *Castilleja levisecta* based on patterns of genetic diversity as revealed by allozyme data.

I. Additional perspectives on the genetic data.

Before considering specific recommendations about the possible conservation implications of Godt and Hamrick's findings about genetic (allozyme) diversity in *Castilleja levisecta* (CALE), I am going to discuss briefly the findings themselves, and also incorporate additional data on geographic distances among populations provided by WNHP.

Data displayed graphically in Figures 1-5 are taken from Godt and Hamrick's (2002) Tables 1 and 2. Figures 1-5 show for each of the populations the relationships between various measures of diversity (genetic diversity, percent polymorphic loci, alleles per polymorphic locus, allelic richness, and effective number of alleles per locus) and two measures of population size. One measure is the 5-year average population size, and the other is effective population sizes (N_e). Note that the symbols used to indicate the various populations are coded so as to draw attention to their location and protection status. The two populations found in Canada, and thus not under WADNR jurisdiction, are indicated with the symbols +, and ×. Populations found in Washington State are indicated by a geometric shape (circle, square, diamond or triangle). Washington populations on private property are indicated by solid symbols, and thus easily distinguished from those enjoying some protected status, which are indicated by open symbols.

The two population size measures each tell us different things about the population sizes. Both measures attempt to reduce observed variation in population size to a single number, against which various measures of genetic diversity can be compared. The 5-year average is a simple arithmetic mean value, emphasizing the most recent data, and provides an intuitively clear general indication of recent population sizes. Effective population size is based on all available data, some of which goes back 20 or more years. Effective population size can be approximated by the harmonic mean of population sizes, and as such is more sensitive to periods of low population sizes than it is to higher values. It is a theoretical construct that was developed to allow comparison of a real population with "an ideal population of size N in which all partents have an eeuql expectation of being the parents of any idividual." (Hedrick, 1985). The concept of effective population size can be viewed as an attempt to gain insight into how susceptible a population is to random genetic drift (smaller populations are more susceptible than large). There are three populations, especially, for which the N_e is dramatically higher than the 5-year mean population size: Ebey's Landing (2.3x), False Bay (1.9x), and Long Island (1.8x). Thus, from a genetic point of view, the much lower effective population sizes of these three populations may indicate that they are more at risk of random genetic drift than their 5 year average sizes might lead us to expect The graphical display in Figures 1-5 makes it apparent that except for the San Juan Valley population, all other populations on private land are small. Conversely, among the four protected populations, three (Forbes Point, Ebey's Landing, and Rocky Prairie) are relatively large, and only one, Ft. Casey, is relatively small. Viewed in this way, it appears that the genetic legacies of the four small, privately owned populations (Bocker, West Beach, Long Island and False Bay) are particularly vulnerable. Despite the generally high levels of genetic diversity overall, and a general positive correlation between population size and various aspects genetic diversity, some large populations (especially Ebey's Landing and to a lesser degree, Forbes Point) are relatively depauperate in some measures, such as allelic richness, and alleles per polymorphic locus. Among the smaller populations, Ft. Casey and Bocker and West Beach are generally less diverse than Long Island and especially False Bay.

In addition to the generally very high levels of genetic diversity found in *Castilleja levisecta*, Godt and Hamrick emphasize the statistically highly significant negative correlation between genetic identity and geographic distance between populations. Genetic identity, (also called genetic similarity) is a measure of how similar any two populations are to one another. varies between 0 (nothing in common) to 1 (genetically identical.) They note that this outcome was strongly influenced by the distant and diverse Rocky Prairie population, but add even without Rocky Prairie, the correlation between genetic and geographic distance remained significant. All of this is true, but tabular (Table 1) and graphical representation of this relationship (Figure 6) show this generality conceals considerable variation.

If all of the data are considered (*i.e*. Rocky Prairie included in the data set) geographic distance 'explains' about half the variation in genetic identity values (the correlation coefficient, or r-value of –0.72, translates into a squared multiple R-value of 0.51). The squared multiple R-value (which also can vary from 0 to 1) indicates the proportion of the variation in the dependent variable (in this case, genetic identity between pairs of populations) that is explained by the independent variable (i.e. geographic distance between those pairs of populations). When the disjunct Rocky Prairie is omitted from the analysis, the correlation coefficient, or r-value, drops to –0.40 (which gives rise to a squared multiple R-value of 0.16). Thus, without the disjunct and distinctive Rocky Prairie population, geographic distance among populations explains only about 16% of the documented variation in genetic identity. In other words, although the effect of geographic distance on genetic identity values between populations is still statistically significant ($p=0.006$ as opposed to $p<0.001$) 84% of the variation in genetic identity values among populations remains unexplained. Regression analysis may not, strictly speaking, be properly applied in this instance, because the data points are not truly independent of one another. Bottom line: there is a lot more going on than simply distance when it comes to understanding potential causes of genetic differences among populations.

Genetic differences among populations can have either random or adaptive causes, or more likely some combination of the two. The degree to which genetic differences among populations have an adaptive basis should affect how far and where germplasm can successfully be moved, and also the optimal composition of founding populations in different areas. For as revealing as they are, molecular genetic data, unfortunately, do not speak directly to the genetic bases and patterns of adaptive differences and their relationships to available habitats.

II. General considerations for conservation, introduction, augmentation, reintroduction, and seed collection in *Castilleja levisecta* populations.

a. Do no harm

First, do no harm. When thinking about reintroduction (in the broad sense, to include all forms, augmentation, introduction, etc) it is important to keep in mind that the goals of scientific inquiry and conservation are not always the same, or even compatible. This is not to say that they are necessarily incompatible. They are not, but it is worth noting that outplanting material that has been held or propagated off site can have detrimental consequences for wild populations. These potential effects should be considered and evaluated on a case by case basis.

b. Mixing genetic material

CALE's high genetic diversity overall, and within even small populations, means that we are not faced with the difficult issue of the need to mix genetic material from different populations as source material for reintroduction or augmentation. In general, and especially in the extant part of the species' range, it seems to me that in the absence of clear reasons to mix sources in a reintroduction, the conservative, or default position should be single source population founders. In other words, I would not recommend mixing sources of population founders within extant populations or within anything that could reasonably be seed or pollen dispesal distance from extant populations. As a practical matter this may well mean not using mixed source founding populations within the extant range of *Castilleja levisecta* except possibly as part of a larger scientific study of the ecological genetics of the species – and then with the caveat that it be done as far away from extant populations as can be managed and still find suitable habitat .

Where the potential negative impact of a mixed source founder population on extant populations is minimal to absent (*e.g.* in the portion of the range from which *CALE* has been extirpated.), there may be good scientific and conservation reasons to use mixed source founding populations It is possible, especially for introductions attempted far away from extant populations, for there to be beneficial adaptive outcomes that result from mixed source founding populations. For example, mixed source founding populations could produce novel genetic combinations that are adaptively superior in the new habitat to any single source population. The management and policy challenges are to explore possible benefits in a way that minimizes possible negative results, especially on existing populations, but also on future populations that might result from such introductions. Potentially undesirable consequences of introducing ‘foreign’ propagules into existing populations (i.e. for use in augmentation) include genetically ‘polluting’ the extant population by introducing maladapted alleles or co-adapted gene complexes, thus reducing the ability of a population to survive and flourish. Potential negative consequences of mixed source introductions (i.e. to currently unoccupied sites) include the creation of poorly adapted populations that might persist for a long time, and which could then cross, thus making the area less suitable for later introductions with more well adapted founders.

c. The fate of small, private populations.The recovery plan (USFWS 2000) calls for all populations to be managed for viability. In principle, who can argue with this? In practice, is this possible, not to mention necessarily reasonable, or even prudent? It is important for managers and agency decision makers to make ‘objective’ assessments of which existing populations can reasonably be expected to be viable and contribute toward recovery *in situ*, and perhaps more important, which are not. From a policy perspective, what then becomes the fate of the genetic material in populations judged to be non-viable? Is there a role for populations to be managed primarily for seed production (for collection)? If so, for what purposes should that seed be used (to genetically enrich other populations to be used to re-establish the same genetic population in another location)?

> There is little likelihood that genetic augmentation will become an issue in the foreseeable future, given the high genetic diversity in all extant populations, so I will not discuss it further. Ifthe goal is to relocate a small, non-viable population from its native location on non-protected private property to another biologically suitable and administratively protected site, the implications go beyond the biological to the policy realm. Biologically, the primary concern is whether or not the reconstituted population is sufficiently well adapted to the new site to be viable. If it is not viable, and there is no other back up, the lineage could be lost. The science of reintroduction is still very young, and the long term viability of reintroduced populations is not well established. The broader issues raised by the potential relocation of small populations on private property judged to be non-viable to new, better protected sites are philosophical, and from a legal/policy perspective, any effort to do so might establish a potentially counterproductive precedent..
>
> Even if the genetic legacy of demonstrably non-viable populations on private land can be established a ‘better’ site, is it wise to go down that path? It seems clear to me that acting in a way so as to set a precedent for moving rare plant populations from where their future is bleak to where they are more likely to survive is not on balance in the

conservation interest of rare plants generally. In part, the danger is potential misuse of the tool of translocation in situations where the reason for relocation is more for economic or administrative convenience than as a last ditch effort to save what for ecological reasons is apparently doomed. That said, neither is it in the conservation interest either simply to watch a population judged to be non-viable simply drift into oblivion, or to sink tremendous resources into a situation that is unlikely to have a positive outcome. The best is, I think, to make honest and concerted efforts to conserve all CALE populations *in situ*, while at the same time placing a high priority on using the germplasm from those populations judged to be in the greatest peril as the source founders for introductions as close as possible to their native sites in suitable, protected habitat. In effect, these 'new' populations would become another form of *ex situ* conservation, and serve as a complement not alternative to attempts to conserve the populations *in situ*.

d. Seed collection

None of the remaining populations is particularly large, in absolute terms, so I recommend that a comprehensive collection plan be developed, such that *ex situ* collections be made of all populations. This should be a high priority, and is discussed in the section below on what could be done in the next few years.

III. Founder population composition and phytosanitary considerations

It is important in all reintroductions to assemble the founder population from the greatest number of donor individuals possible, and to equalize founder contribution (Guerrant, 1996). This would be especially important in augmentation projects involving small populations initially, where genetic material from a population is propagated off site and returned to its original habitat. For augmentation, and in order not to distort the genetic composition of the population, it is important to maximize the number of individuals in the existing population used as a source of propagules. To the degree that individuals are successfully established, the donors will be more well represented in future generations than those naturally occurring individuals not sampled for propagules originally.

Pests, pathogens and weeds form a diffuse category of bad things to be considered in an introduction project. Given that many and diverse individuals, public and private organizations are likely to be involved in outplaning efforts, WADNR should give serious thought to developing and establishing a general set of phytosanitary protocols to be followed by any and all who might be involved in reintroduction efforts. The only one such set of protocols of which I am aware were developed for the US Army project to stabilize the many highly endangered plant species known to occur on the Makua Military Reservation, on Oahu, in Hawaii, by the Hawaii Natural Heritage Program on behalf of Wil Chee Planning, Inc. (1400 Rycroft Street, Suite 928. Honolulu, Hawaii, 96814). The protocols include consideration of both offsite propagation phase, and later monitoring of outplanting sites.

IV. Issues to consider in near-by introductions.

The primary, overarching issue in near-by introductions is to minimize the chance of doing harm to extant populations in our attempt to make things better. Potential threats include genetic contamination of extant populations with mal-adapted alleles or by disrupting co-adapted gene complexes. Bad genes are not the only things we want to avoid inflicting on extant populations. Outplanting any material propagated off site runs the risk of introducing pests and pathogens.

Issues of scale are also important to consider. What sorts of distances are likely to provide an 'adequate' buffer to prevent (or to minimize, to what degree?) genetic communication with extant populations? Should an adequate buffer be a single distance, applicable to all areas similarly, or can buffer distances differ according to circumstance? A single minimum buffer distance is easiest to apply, but would necessarily have to be set by conditions most favorable to gene flow by pollen or seed. A set standard minimum distance would thus potentially eliminate suitable habitat for introductions to be made in some areas. I defer to those with more experience with CALE to judge the actual distances appropriate, but it seems to me that pollinators are a more likely source of gene flow than dispersal by seed. Thus, and unless others with more knowledge disagree, minimum distances from extant populations should be less where intervening pollinator-friendly habitat exists than where it does not. Assuming the primary pollinators are bumble or other bees, relatively open areas with other nectar and pollen sources available probably represent less of a barrier than more heavily forested or open water areas.

V. Issues to consider for long distance introductions (i.e. Willamette and SW Washington).

There is greater latitude for potential actions in long distance introductions than there is for outplanting near extant populations.

A difference can be drawn between biological and programmatic or project goals. The biological goal for long distance introductions is to establish CALE in those portions of its historic range where it does not now exist. Project goals in this case also include learning as much as possible from the attempt regardless of whether or not it 'succeeds'.

A potential problem arises from the possibility, however, slight that those CALE populations have not been extirpated, and simply remain un-relocated. Even though the plant has not been found in SW Washington and the Willamette Valley for a very long time, several to many decades, it is close to if not impossible to prove a negative, so we cannot say for sure that CALE is no longer there. The implications of that possibility, however, may have consequences on any attempts to re-establish CALE in those portions of the range from which it has (apparently) become extirpated. *P*erhaps the most relevant to this discussion is whether to place a high prority on locating introduction sites as close known historical sites as possible (given current habitat and land ownership), or to avoid such sites. *A priori*, and all else being equal, it would seem the sites at which it was known to occur would be the place to begin looking for a place to reintroduce the species. In a sense, this is the most conservative approach, one that provides the greatest chance of locating suitable habitat. Alternatively, the implications of wanting to protect the as yet relocated populations from genetic contamination, by putting them 'off limits' to introduction attempts, would be to remove from consideration those areas most likely to provide suitable habitat. In my opinion, I think it best not to avoid those areas when looking for potential reintroduction sites, indeed, I would recommend beginning with those areas, if for no other reason than to begin to put together a 'search image' of appropriate habitat. That said, and allowing for the possibility of the historic populations surviving, any and all potential habitat within a reasonable distance from the reintroduction sites should be thoroughly searched in advance. The potential problem of genetic contamination of extant populations in SW Washington and the Willamette Valley is, of course, not limited to those sites from where it is known to have been, but to all potential habitat in the area. I would imagine that if any populations do exist in those areas that they are more likely to be populations that have never been found than to be those that have not been relocated in recent decades.

Given that there are no known extant populations, and thus no truly 'local' seed sources available for reintroduction in SW Washington and Oregon, the risk of genetic or other contamination of indigenous populations is so low as not to be an issue. Nevertheless, potential outbreeding depression may be an issue to consider. The issue of outbreeding depression is discussed in more detail below, in the section on long

Potential steps to take in the near-term (3-5 years) toward introduction/augmentationbased on the results of Godt's work. These steps could include appropriate experiments.

I. Collect Seeds.

Probably the first task will be to develop and implement a comprehensive collection strategy designed both to ensure the genetic information of each population is banked, and also to build up stocks for use in reintroduction/augmentation. Components of such a plan should contain the following elements:

a. A mechanism should be established that would oversee all collection activities to ensure that all populations are sampled and that none is subject to excessive collection. Recent computer modeling work by Menges *et al*. (2003) suggests that smaller, more frequent collections appear generally to have less of a demographic impact on sampled populations than larger, more infrequent collections.
b. In order to maximize their value for later reintroduction or other scientific work, seeds from each maternal line should be maintained separately for each collecting episode. In order to ensure continuity between years, it may be worth considering developing a mechanism (mapping, tagging?) that would allow collections in different years to sample different plants (or in some instances the same?) plants. Clearly pragmatic considerations will impact the level of detail that such a system could hope to achieve. In any case, some plan to maximize the numbers of individuals sampled over the years would be appropriate.
c. Samples should be stored in a proper facility that meets the standards of the Center for Plant Conservation. Two options exist, Berry Botanic Garden in Portland, Oregon, and the University of Washington's Center for Urban Horticulture's Miller Seed Vault, in Seattle. It is worth considering dividing the collection between both facilities to spread the risk of any one catastrophic event destroying what may well become irreplaceable germplasm.
d. A particular challenge will be to collect enough seed in the smallest, private, and potentially non-viable populations to build up stocks for introduction to a new site while not unduly impacting the sampled populations.

II. Designing Populations

With respect to designing/constructing/assembling the founders to be used for reintroduction/introduction/augmentation, understanding the genetic constitution of extant populations from which to draw founders is essential. Two critical questions must be addressed: 1.) is there genetic diversity within and among extant populations (and if so, how much, and how is it partitioned within and among populations?) and, 2.) are any genetic differences adaptive (as opposed to random)? We have both good and encouraging information about the first question, but not the second. I see an opportunity to use reintroduction attempts in the currently unoccupied part of the range as a vehicle for, in part, addressing the second question while at the same time attempting to re-occupy a portion of the range from which CALE has been extirpated. While available molecular genetic information provides critical insight into levels and patterns of genetic diversity within and among populations of CALE, they do not bear directly on essential questions of possible genetically based adaptive differences among populations. To establish whether or not genetically based adaptive differences exist, it will be necessary to conduct reciprocal transplant or other common garden experiments, ideally in multiple locations simultaneously.

Common garden experiments are required to address the question of whether the high genetic diversity revealed by Godt's electrophoretic study reflects adaptive differences among populations. Common garden experiments, where genetic material from diverse and documented parentage and provenance is grown in the same area and other than the same conditions, could also serve as a vehicle on which to design populations for reintroduction into currently unoccupied portions of the range. To realize the full potential (or at least get more information), it would also be desirable to find one or more locations toward the northern portion of the range with appropriate habitat and NO CALE in the vicinity where a set of replicates could also be established. For example, well designed experiments could reveal the existence of ecotypic or other less geographically structured adaptive differences among populations, while at the same time create new populations in previously unoccupied portions of the range. Nevertheless, using mixed-source populations does raise the possibility of creating populations that could suffer outbreeding depression, a factor that will have to be weighed.

Because it takes a number of generations to detect inbreeding or outbreeding depression, it is, I think, advisable to run a parallel series experiments designed specifically to look for such effects. Interpopulation hybrid seed of known parentage would be especially helpful in speeding up the process, relative to beginning with 'pure' seed. The experiments Dr. T. Kaye, of the Institute for Applied Ecology, in Corvallis, is conducting on CALE, lend themselves to such attempts, and I recommend he be encouraged to follow through on this aspect of his research.

More generally, I recommend that a common garden experimental framework be used as a vehicle for establishing populations in the southern part of the range, from which CALE has been extirpated. Available resources both biological and economic will, of course, greatly influence what can ultimately be done.

In order to determine if the generally high level of genetic differences described by Godt and Hamrick are indicative of large adaptive differences, replicate experimental populations could be set up at different sites at least, but ideally not only, at several sites in the extirpated portion of the range. These populations would, ideally, be identical to one another in terms of parentage, so any differences found would be due to differences in environment, and not genetic.

I recommend establishing a replicated series of experimental populations in at least three locations in what was the southern portion of the range. Ideally, they would be at or near to the sites of extirpated populations in Pierce and Clark (or possibly Skamania) Counties, in Washington, and Linn (or possibly Marion) County., Oregon. At each site, an 11 x 11 grid could be placed in suitable habitat (or 9 x 9, if the Canadian populations were not available for use), such that each 'cell' of the grid would consist of (for example)10 propagules each from 10 maternal lines from a single population. Thus, each population would be replicated either 9 or 11 times in the overall outplanting. Ideally, it would be best to follow the results for several generations, but there is a potential problem with interpopulation breeding if outbreeding depression were to become a significant problem. Nevertheless, if all seed capsules were collected each year and the seed saved for examination in greenhouse, or other controlled conditions, valuable data on relative survivorship, growth, fruit set, and seed production could be taken, and thus gain valuable insight into any adaptive differences that might exist among populations, as judged in three different environments. Ideally it would be desirable to have another test site in the northern part of the range, but the danger of genetic contamination might be sufficiently high as to make this imprudent. Nevertheless, it might be possible to set up a replicate at the Center for Urban Horticulture, in Seattle (King Co.), which, I would expect be sufficiently well isolated reproductively from extant populations as not to represent a significant threat.

Site	Protection status	Area (ac.)	Trend (fc)	5 year average pop size (FC0	Effective Pop. Size (G&H)	5 yr / Ne	Recommendations
Trial Island	Protected	1	s	2150	2150	1.0	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses
Alpha Islet	Protected	10?	s	876	877	1.0	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses
San Juan Valley	Private	2	I	4021	4021	1.0	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses
False Bay	Private	<1	I?	269	145	1.9	Collect approximately 10% of the seed from 50 individuals each year, rotating which plants are sampled if possible. Use this as a single source founder population to introduce a new population nearby.
Long Island	Private	<1	i	73	40	1.8	Collect approximately 10% of the seed from 50 individuals each year, rotating which plants are sampled if possible. Use this as a single source founder population to introduce a new population nearby.
West Beach	Private	<1	d	381	299	1.3	Collect approximately 10% of the seed from 50 individuals each year, rotating which plants are sampled if possible. Use this as a single source founder population to introduce a new population nearby.

Forbes Point	Protected	<1	d	1500	1282	1.2	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses
Ebey's Bluff	Protected	<1	s?	4353	1891	2.3	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses
Bocker	Private	1	d	187	135	1.4	Collect approximately 10% of the seed from 50 individuals each year, rotating which plants are sampled if possible. Use this as a single source founder population to introduce a new population nearby.
Ft. Casey	Protected	5?	d	170	170	1.0	Collect approximately 10% of the seed from 50 individuals each year, rotating which plants are sampled if possible. Use this as a single source founder population to introduce a new population nearby.
Rocky Prairie	Protected	ca. 30	s	5679	5672	1.0	Collect approximately 10% of the seed from 50 individuals as frequently as possible, rotating which plants are sampled if possible, until such time as sufficient seed is available in ex situ storage for all anticipated uses

References

Godt, M.J.W, and J.L. Hamrick. 2002 (December, but undated). Allozyme Diversity in the Federally Threatened Golden Paintbrush *Castilleja levisecta* (Scrophulariaceae). Unpublished report submitted to the Washington Department of Natural Resources. 28 pages + appendix.

Guerrant, E.O. Jr. 1996. Designing Populations: Demographic, Genetic and Horticultural Dimensions. Pages 171-207. In *Restoring Diversity: Strategies for Reintroduction of Endangered Plants*. Edited by DA Falk, CI Millar and M Olwell. Island Press, Covelo.

Hedrick, P. 1985.Genetics of Populations. Jones and Bartlett, Boston. 629 pp.

Menges, E.S., E.O Guerrant, and S. Hamze. 2003. What is the effect of seed collection on extinction risk of perennial plants? In: E. O. Guerrant Jr., K. Havens, and M. Maunder, editors. *Ex situ plant conservation: supporting species survival in the wild*. Island Press. Covelo, CA. 424 pp.

US Fish and Wildlife Service. 2000. Recovery Plan for the Golden Paintbrush (*Castilleja levisecta*). US Fish and Wildlife Service, Portland, Oregon. 51 pp.

Figure 1s. Graphical representation of genetic diversity (H_e) by population as a function of (1a) effective population size (N_e) or (1b) the 5 year average population size based on data in Godt and Hamrick (2002)

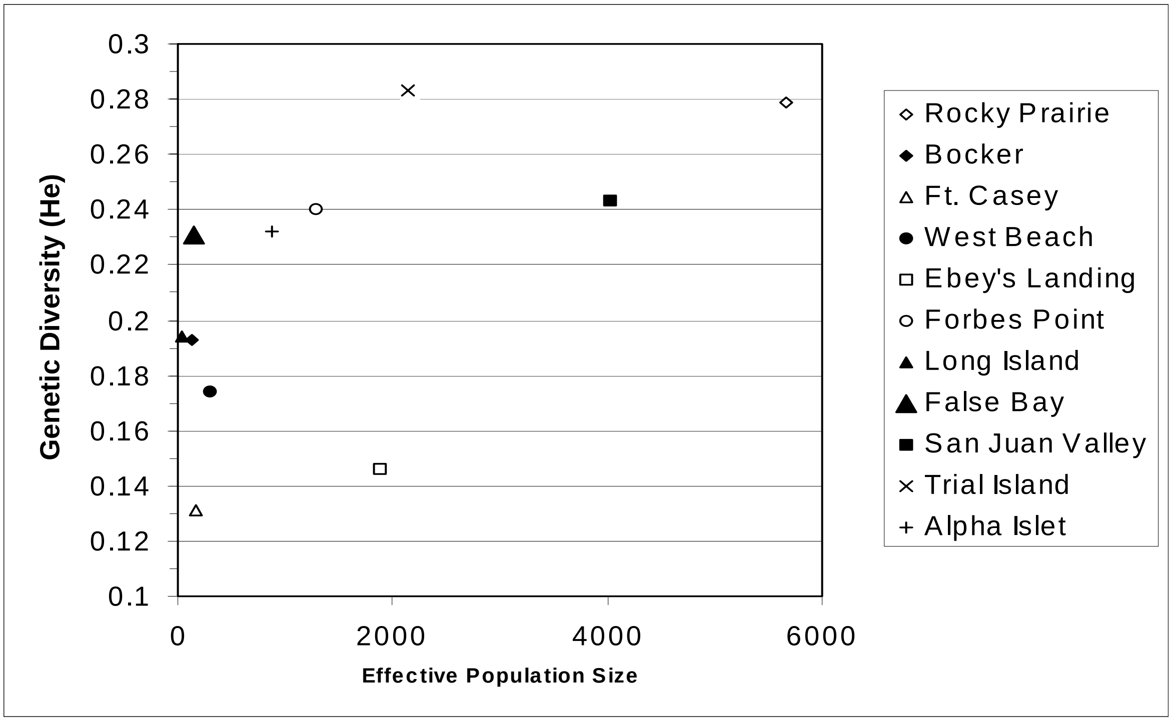


Figure 1b.

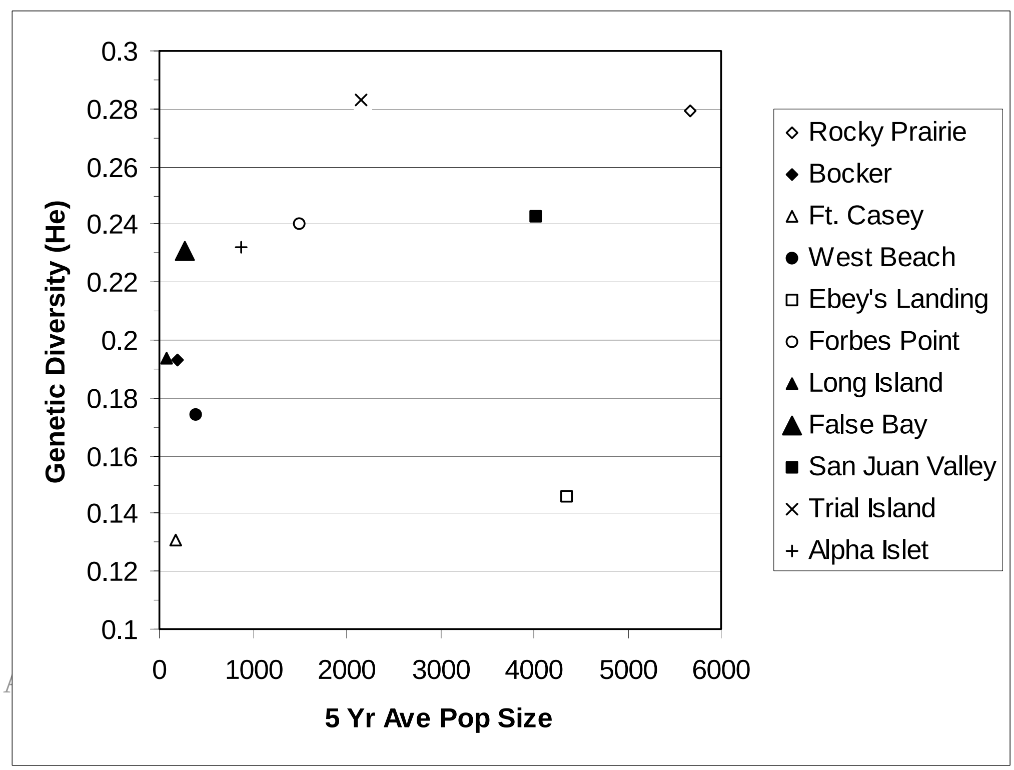
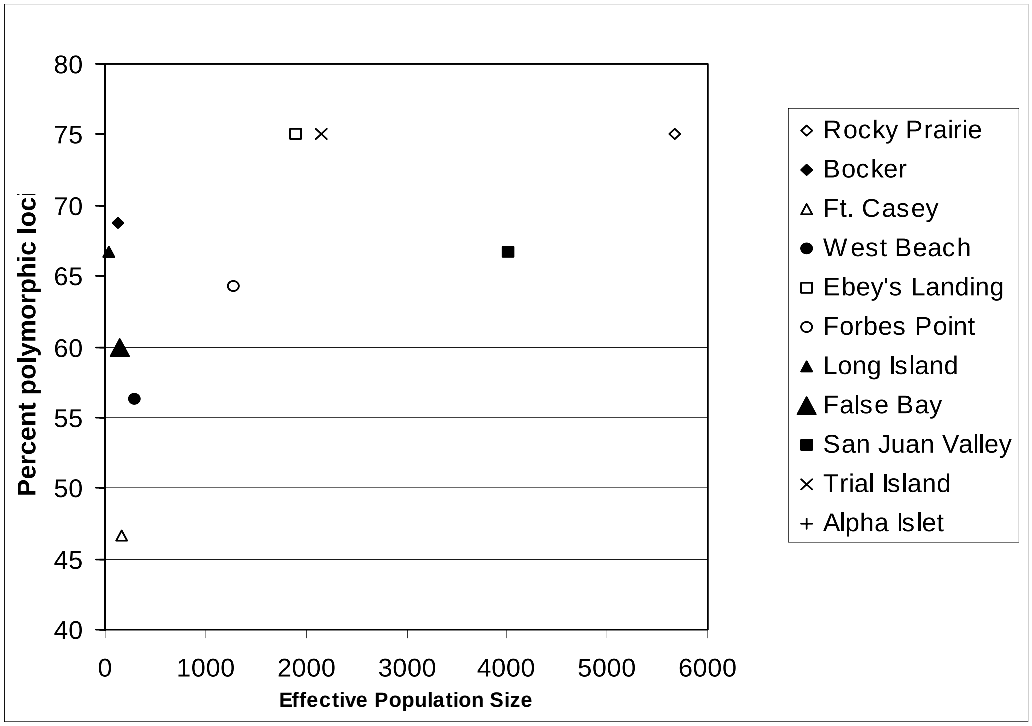


Figure 2. Graphical representation of percent polymorphic loci by population as a function of of (1a) effective population size (N_e) or (1b) the 5 year average population size based on data in Godt and Hamrick (2002)



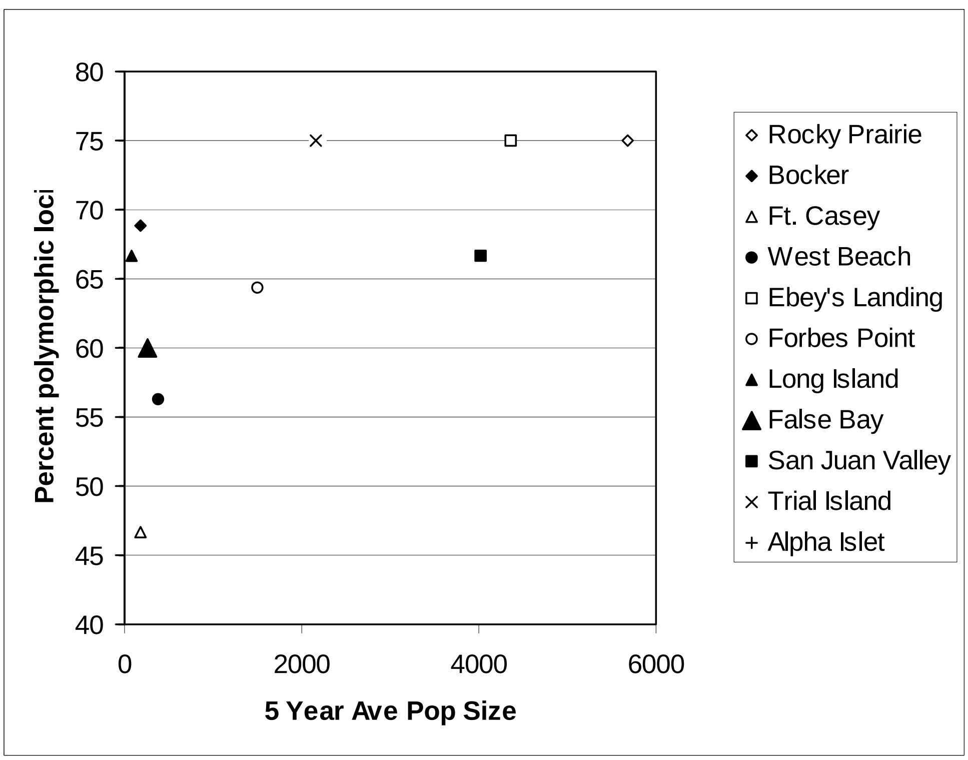
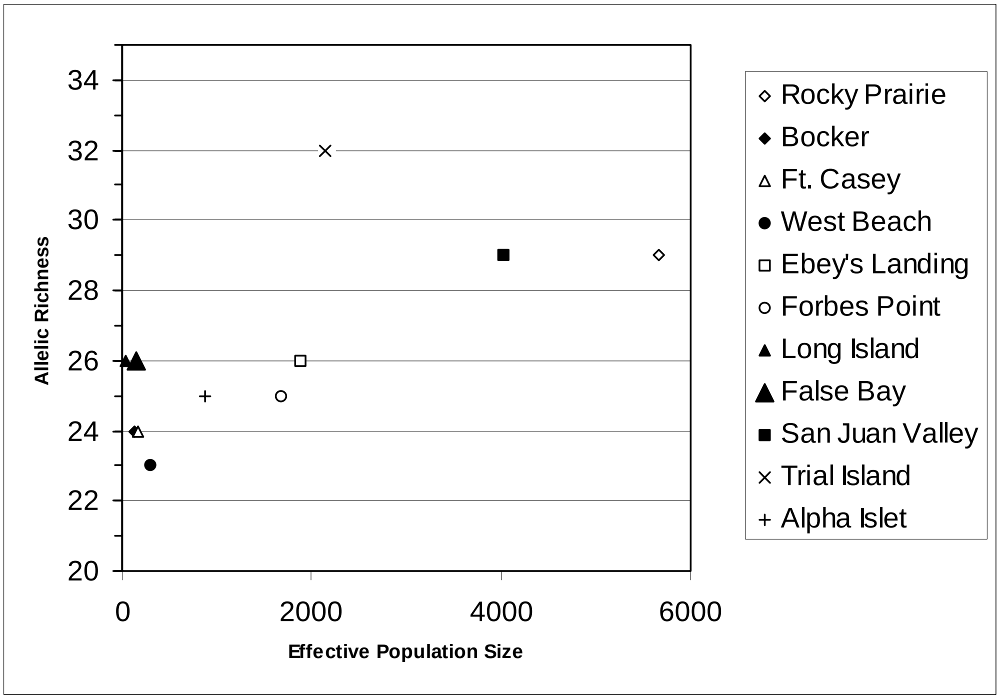


Figure 3. Graphical representation of allelic richness by population as a function of of (1a) effective population size (N_e) or (1b) the 5 year average population size based on data in Godt and Hamrick (2002)



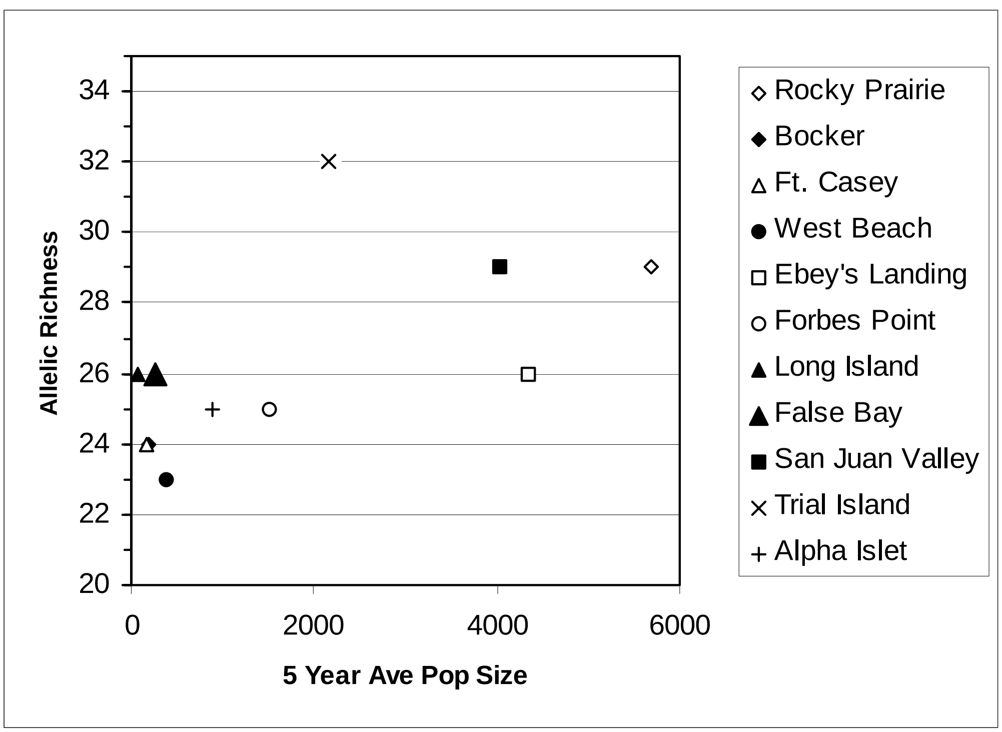
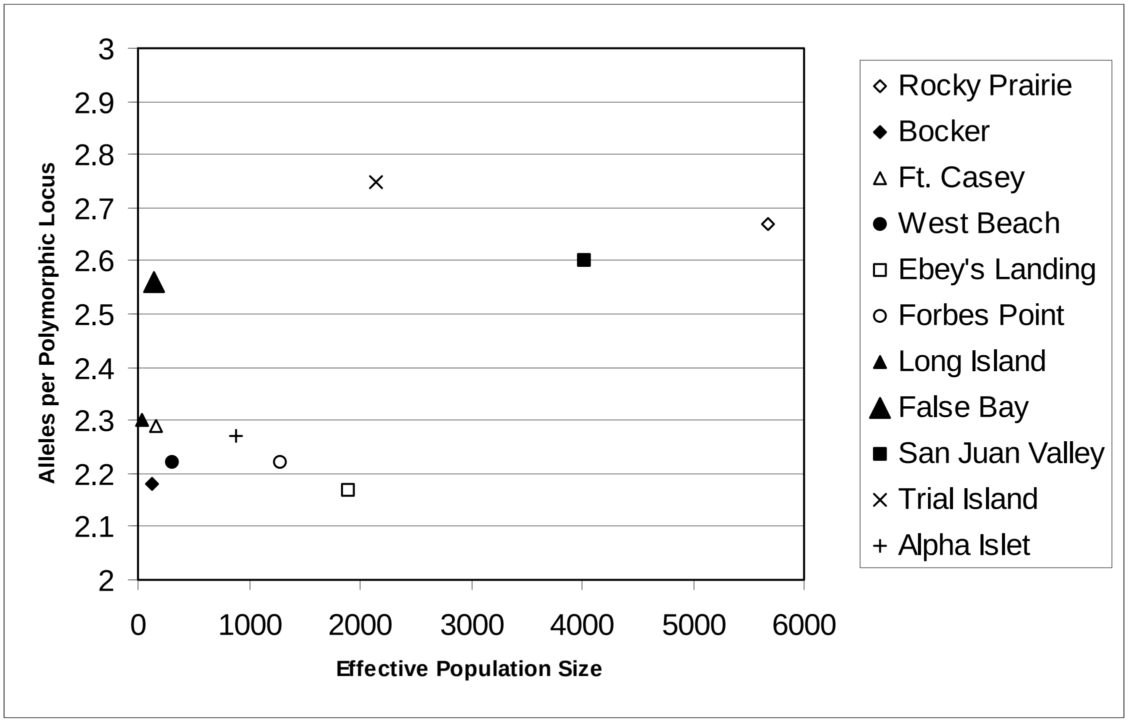


Figure 4. Graphical representation of alleles per polymorphic locus by population as a function of effective population size (N_e) based on data in Godt and Hamrick (2002)



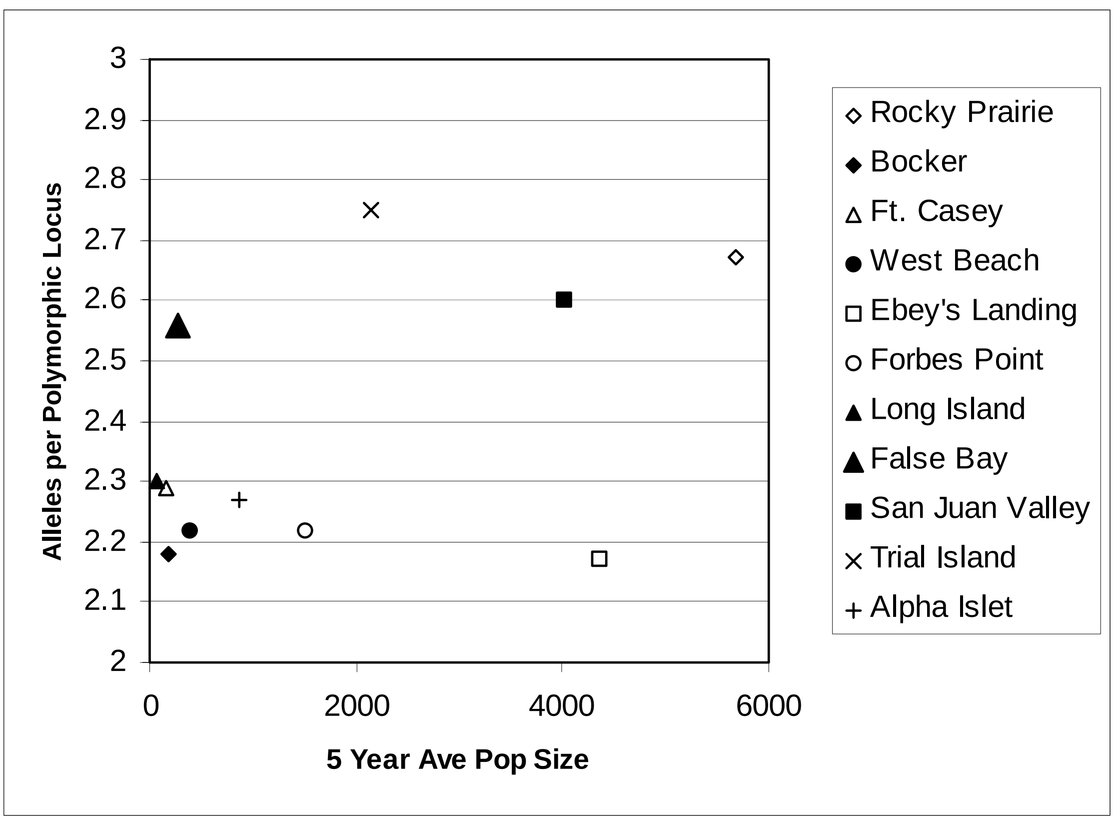


Figure 5. Graphical representation of effective number of alleles locus by population as a function of effective population size (N_e) based on data in Godt and Hamrick (2002)

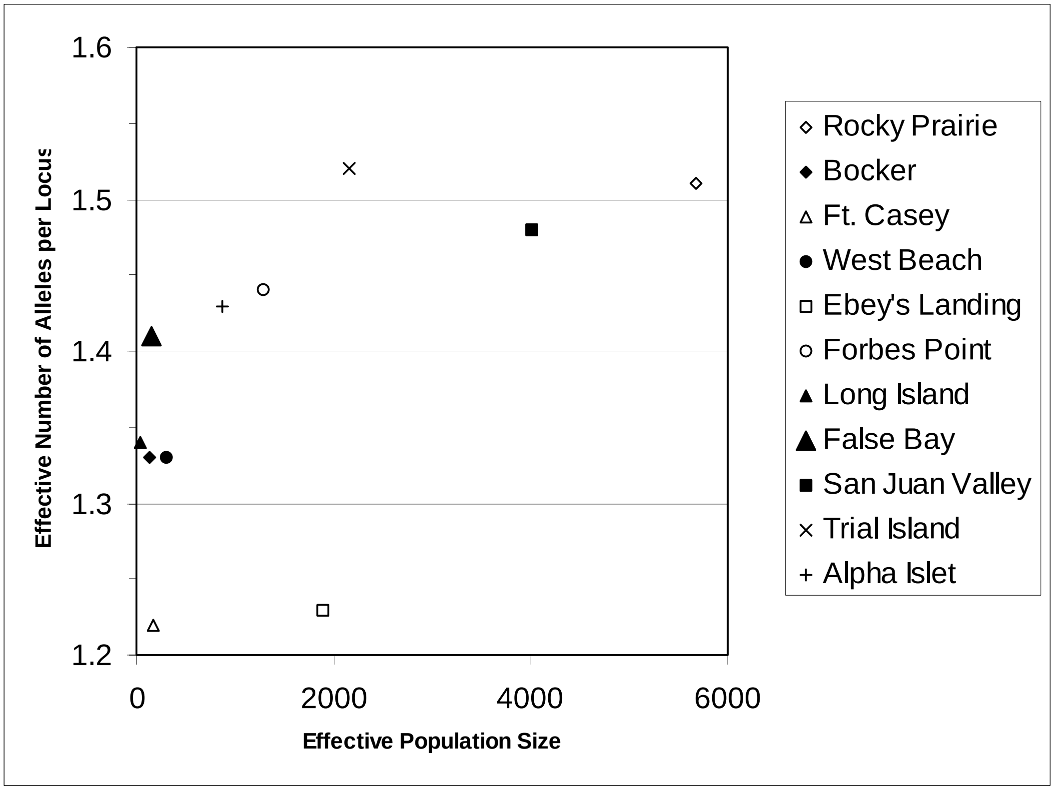


Figure 6. Relationship between Genetic Identity and Geographic Distance among *Castilleja levisecta* populations (data sources same as those in Table 1). Solid line indicates results of statistical regression of genetic identity as a function of geographic distance among all populations. Dashed line indicates same relationship excluding Rocky Prairie population. Note the considerable spread around the regression line, especially when Rocky Prairie is excluded from the analyses. See text for further explanation.

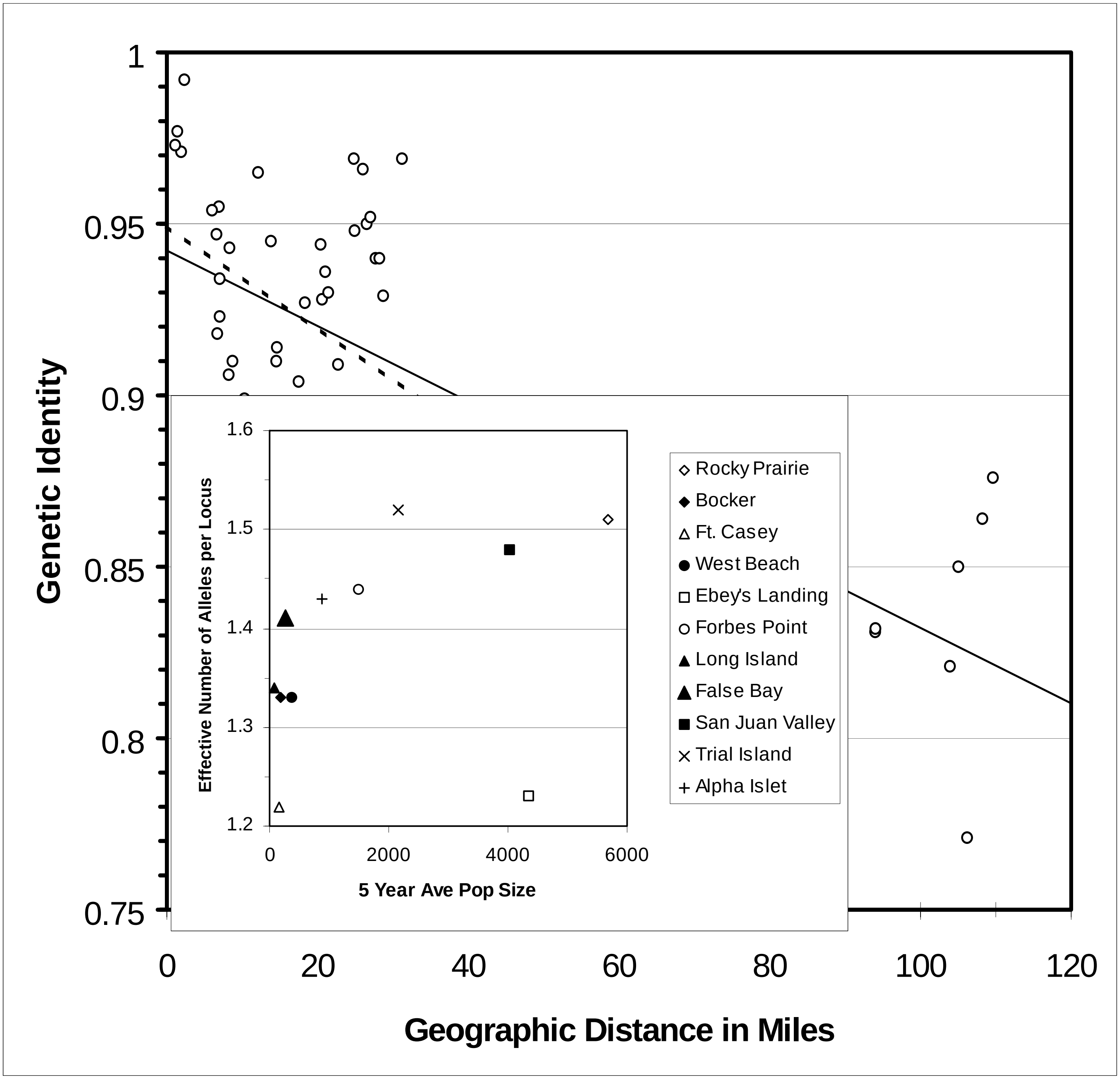


Table 1. All possible pairwise comparisons among possible populations of geographic distance (above diagonal, and in bold font) and genetic identity (below diagonal, and not in bold font). Data for geographic distances supplied by Ms. Florence Caplow, WADNR, and pairwise genetic identity values from Godt and Hamrick (2002.)

	R	FC	Bocker	Ebey's	WB	Forbes	LI	FB	SJ	A	T
	Rocky	Ft Casey	Bocker	Ebey's	West beach	Forbes	Long Island	False Bay	San Juan	Alpha	Trial
Rocky Prairie	Rocky	**86.1**	**87.02**	**88.3**	**94.03**	**94**	**105**	**108.2**	**109.6**	**106.2**	**103.9**
Ft Casey	0.827	Ft Casey	**1.4**	**2.3**	**8.3**	**8.2**	**22.7**	**28.2**	**28.7**	**32.4**	**32**
Bocker	0.828	0.977	Bocker	**1.12**	**7**	**7**	**21.4**	**27**	**27.7**	**31.3**	**30.92**
Ebey's Landing	0.851	0.992	0.973	Ebey's	**6**	**6.7**	**20.4**	**26**	**26.5**	**30.4**	**29.9**
West beach	0.832	0.943	0.923	0.954	West B.	**5.5**	**14.6**	**20.6**	**21**	**25.7**	**25.9**
Forbes Point	0.831	0.906	0.934	0.918	0.894	Forbes	**18.3**	**24.8**	**24.9**	**31**	**31.2**
Long Island	0.850	0.909	0.930	0.944	0.914	0.927	Long Isl.	**6.9**	**6.6**	**14.5**	**17.5**
False Bay	0.864	0.940	0.952	0.966	0.928	0.969	0.955	False Bay	**1.9**	**8.7**	**12.1**
San Juan	0.876	0.929	0.940	0.950	0.936	0.948	0.947	0.971	San Juan	**10.3**	**13.8**
Alpha Islet	0.771	0.855	0.875	0.873	0.849	0.889	0.910	0.910	0.899	**Alpha**	**3.7**
Trial Island	0.821	0.841	0.850	0.863	0.886	0.969	0.904	0.965	0.945	0.853	Trial
	R	FC	Bocker	Ebey's	WB	Forbes	LI	FB	SJ	A	T

Appendix C. Recommendations based on genetic analysis

Appendix D. Soils analysis of extant sites

In general, organic matter is very high, phosporus is low to very low, magnesium is high to very high, calcium is low to very low, pH is acidic, iron is very high, copper is very low, soluble salts are low, sand % is high, clay % is low.

Organic matter: percent
Phosphorus weak bray: ppm-P Rate
Phosphorus Olsen method: ppm-P Rate
Potassium: ppm-K Rate
Magnesium: ppm-Mg Rate
Calcium: ppm-C Rate
Sodium: ppm-S Rate
Hydrogen: Hmeq/100g
Nitrogen: ppm-N Rate
Sulfur: ppm-S Rate
Zinc: ppm-Zn Rate
Manganese: ppm-Mn Rate
Iron: ppm-Fe Rate
Copper: ppm-Cu Rate
Boron: ppm-B Rate
Soluble Salts: mmhos/cm Rate

Sample	sample number	organic matter	phosphorus weak bray	Phosphorus Olsen method	Potassium	Magnesium	Calcium
Ebey's Center	19	1.5	6	11	108	325	297
Ebey's West	14	1.6	17	20	98	425	503
Ebey's East	16	1.9	6	19	70	366	407
Forbes NW	18	4.3	17	19	145	258	751
West Beach	20	5.5	8	3	108	229	535
Forbes SE	10	5.5	7	8	189	538	1187
False-Schwartz	12	5.9	11	26	178	312	922
Bocker Central	8	7.4	7	6	38	253	720
False-Mar Vista	9	7.5	6	15	170	725	1110
Ft. Casey North	15	8.5	5	13	142	696	1215
Ft Casey	2	12.4	5	18	378	609	1564
Rocky Main Swale	5	12.9	12	26	63	54	560
San Juan-Pecan	3	14.7	6	10	125	420	1830
San Juan Ditch	13	15	4	35	44	445	1528
Rocky South	6	16.6	18	14	91	55	669
Rocky East	7	18.3	16	27	58	8	302
Long island C	17	18.7	9	27	75	299	807
Long Island B	4	21.2	6	10	162	596	2405
False-Newcom	1	22.8	5	22	108	311	3412
Long Island A	11	24.4	11	11	100	274	659

Sample	sample number	Sodium	Soil pH	buffer index	Hydrogen	Cation Exchange Capacity	%K	%Mg	%Ca
Ebey's Center	19	58	6.4	7.2	0.5	5.1	5.4	52	28.8
Ebey's West	14	87	6.4	6.9	0.7	7.3	3.5	47.9	34.4
Ebey's East	16	61	6.1	7	0.9	6.4	2.8	47.2	31.8
Forbes NW	18	52	5.7	6.6	1.7	8.2	4.5	25.9	45.8
West Beach	20	43	5.5	6.3	1.8	6.8	4.1	27.8	39.4
Forbes SE	10	87	6.1	6.6	1.8	13	3.7	33.9	45.4
False-Schwartz	12	52	5.6	6.2	2.4	10.3	4.4	25	44.8
Bocker Central	8	55	5.7	6.2	1.6	7.6	1.3	27.4	47.2
False-Mar Vista	9	131	6.1	6.7	2	14.5	3	41	38.1
Ft. Casey North	15	164	6.1	6.5	2.1	15	2.4	38.3	40.5
Ft Casey	2	122	6.1	6.4	2.3	16.6	5.8	30.1	46.9
Rocky Main Swale	5	27	5.5	5.7	1.2	4.7	3.4	9.3	58.8
San Juan-Pecan	3	53	5.6	6.1	4	17.2	1.9	20.1	53.2
San Juan Ditch	13	42	6.4	6.7	1.1	12.7	0.9	28.8	59
Rocky South	6	21	5.5	5.6	1.4	5.5	4.2	8.1	60.1
Rocky East	7	14	5.3	5.3	0.8	2.6	5.8	2.6	58.3
Long island C	17	84	5.3	5.9	3.2	10.2	1.9	24.1	39.5
Long Island B	4	180	6.1	6.2	2.9	21	2	233	57
False-Newcom	1	32	6.5	6.9	1.6	21.6	1.3	11.8	78.7
Long Island A	11	100	5.2	5.6	3.3	9.5	2.7	23.7	34.5

Sample	sample number	%H	%NA	Nitrogen	Sulphur	Zinc	manganese	Iron	Copper
Ebey's Center	19	9	4.9	6	19	0.4	1	21	0.3
Ebey's West	14	9	5.2	8	9	0.2	1	46	0.2
Ebey's East	16	14	4.2	27	1	0.7	2	40	0.3
Forbes NW	18	21	2.8	14	8	1	1	89	0.1
West Beach	20	26	2.8	7	9	1.2	2	93	0.1
Forbes SE	10	14	2.9	9	10	6.3	2	143	0.4
False-Schwartz	12	23.5	2.2	8	12	2.8	2	99	0.2
Bocker Central	8	21	3.1	4	19	1.5	2	92	0.1
False-Mar Vista	9	14	3.9	4	1	26.4	2	66	0.7
Ft. Casey North	15	14	4.8	4	17	3.5	5	110	0.4
Ft Casey	2	14	3.2	10	15	3.9	3	105	0.2
Rocky Main Swale	5	26	2.4	9	11	0.6	2	7	0.1
San Juan-Pecan	3	23.5	1.3	22	8	1.3	1	49	0.1
San Juan Ditch	13	9	1.4	5	17	0.6	1	98	0.9
Rocky South	6	26	1.6	9	19	1.5	5	26	0.4
Rocky East	7	31	2.1	5	20	1.9	2	10	0.5
Long island C	17	31	3.6	4	31	1.4	2	38	0.1
Long Island B	4	14	3.7	31	4	1.8	5	50	0.1
False-Newcom	1	7.5	0.6	29	18	1	1	36	0.4
Long Island A	11	34.5	4.6	10	7	9.7	6	80	0.2

Appendix D. Soils analysis of extant sites

Sample	sample number	Boron	Soluble Salts	%sand	%silt	%clay	texture
Ebey's Center	19	0.2	0.4	89	6	5	sand
Ebey's West	14	0.4	1	83	10	7	loamy sand
Ebey's East	16	0.3	0.5	91	4	5	sand
Forbes NW	18	0.5	0.6	61	24	15	sandy loam
West Beach	20	0.3	0.5	71	20	9	loamy sand
Forbes SE	10	0.4	0.4	43	30	27	clay loam
False-Schwartz	12	0.5	0.6	61	22	17	sandy loam
Bocker Central	8	0.7	0.6	69	22	9	sandy loam
False-Mar Vista	9	0.8	0.9	51	24	25	sandy clay loam
Ft. Casey North	15	1.2	0.7	65	24	11	sandy loam
Ft Casey	2	1.2	0.5	73	20	7	loamy sand
Rocky Main Swale	5	0.4	0.2	83	10	7	loamy sand
San Juan-Pecan	3	0.8	0.5	69	22	9	sandy loam
San Juan Ditch	13	0.7	0.6	59	24	17	sandy loam
Rocky South	6	0.6	0.6	77	16	7	loamy sand
Rocky East	7	0.5	0.6	77	16	7	loamy sand
Long island C	17	0.9	0.9	73	20	7	loamy sand
Long Island B	4	1.5	0.5	77	14	9	loamy sand
False-Newcom	1	1.5	0.6	74	18	8	loamy sand
Long Island A	11	0.8	0.8	69	18	13	sandy loam

Appendix E. Suggested research on *Castilleja levisecta* prior to reintroduction

STUDY	STATUS	PRIORITY
Seed germination *in situ*	Ongoing	1
Effects of burning and scarification on seed germination *in situ*	Ongoing	1
Effects of hosts on growth and survival.	Partially completed, ongoing.	1
Effect of legume hosts		2
Outplanting protocols	Begun	1
Pollinators of existing sites, and their presence and density at reintroduction sites		1
Detailed soil and hydrologic characteristics of existing sites	Begun	1
Garden experiments to determine appropriate seed sources for Willamette Valley and SW Washington reintroductions	Begun	1
Effects of mycorrhizal inoculation on growth and survival		2
Appropriate spatial distribution for outplanting		2
Effective means of controlling competing vegetation	Begun	2
Further studies on seed viability		2
Microsite effects on germination and survival	Begun	2
Studies of outbreeding depression on F_2 and F_3 generations		2